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Development and Validation of Method for Estimation of Fluticasone and Salmeterol by RP-HPLC Method in Pharmaceutical Dosage Forms

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ABSTRACT

Analytical Method Development and Validation for Salmeterol and Fluticasone in Bulk and Combined Dosage Form by RP-HPLC, New method was established for simultaneous estimation of Salmeterol and Fluticasone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Salmeterol and Fluticasone by using Phenomenex Luna C18 (4.6mm×250mm, 5µm) particle size, the flow rate was 1.0 ml/min, mobile phase ratio was (40:60 v/v) Acetonitrile: TEA buffer pH-4.2 (pH was adjusted with orthophosphoric acid), detection wavelength was 220nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detectors. The retention times were found to be 2.246mins and 5.461mins respectively. The % purity of Salmeterol and Fluticasone was found to be 101.27% and 99.76% respectively. The system suitability parameters for Salmeterol and Fluticasone such as theoretical plates and tailing factor were found to be 5387, 0.97, and 5398 and 1.26, the resolution was found to be 2.97. The linearity study n Salmeterol and Fluticasone was found in the concentration range of 30µg-70µg and 60µg-140µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.14% and 100.56%, %RSD for repeatability was 0.1 and 0.5, % RSD for intermediate precision was 0.1 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 0.56 and 1.2, and the LOQ value was 1.7 and 3.6 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Salmeterol and Fluticasone in API and Pharmaceutical do forms.

INTRODUCTION

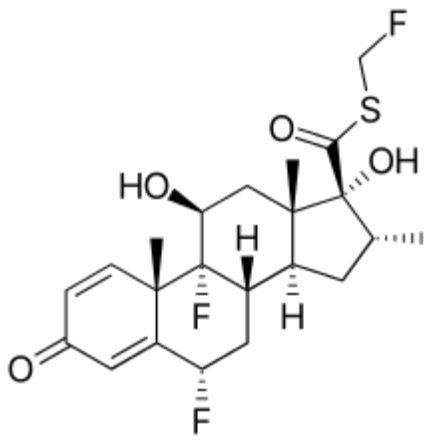

Fig:1 structure of fluticasone

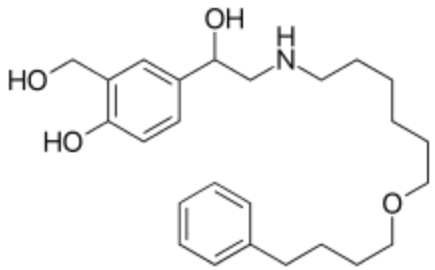

Fig: no 2 Structure of salmeterol

Fluticasone S-Fluoromethyl (6S,8S,9R,10S,11S,13S,14S,16R,17R)-6,9-difluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthrene-17-carbothioate is an extremely potent vasoconstrictor and anti-inflammatory agent. Its effectiveness in inhaled forms is due to its direct local effect. Binds to the glucocorticoid receptor. Unbound corticosteroids cross the membranes of cells such as mast cells and eosinophils, binding with high affinity to glucocorticoid receptors (GR). The results include alteration of transcription and protein synthesis, a decreased release of leukocytic acid hydrolases, reduction in fibroblast proliferation, prevention of macrophage accumulation at inflamed sites, reduction of collagen deposition, interference with leukocyte adhesion to the capillary wall, reduction of capillary membrane permeability and subsequent edema, reduction of complement components, inhibition of histamine and kinin release, and interference with the formation of scar tissue. In the management of asthma, the glucocorticoid receptor complexes down-regulates proinflammatory mediators such as interleukin-(IL)-1, 3, and 5, and up-regulate anti-inflammatory mediators such as IkappaB [inhibitory molecule for nuclear factor kappaB1], IL-10, and IL-12. The anti-inflammatory actions of corticosteroids are also thought to involve inhibition of cytosolic phospholipase A2 (through activation of lipocortin-1 (annexin)) which controls the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

Salmeterol(RS)-2-(hydroxymethyl)-4-{1-hydroxy-2-[6-(4-phenylbutoxy) hexylamino]ethyl}phenol is a long acting beta2-adrenoceptor agonist (LABA), usually only prescribed for severe persistent asthma following previous treatment with a short-acting beta

agonist such as salbutamol and is prescribed concurrently with a corticosteroid, such as beclometasone. The primary noticeable difference of salmeterol to salbutamol is that the duration of action lasts approximately 12 hours in comparison with 4-6 hours of salbutamol. When used regularly every day as prescribed, inhaled salmeterol decreases the number and severity of asthma attacks. However, it is not for use for relieving an asthma attack that has already started. Inhaled salmeterol works like other beta 2-agonists, causing bronchodilatation by relaxing the smooth muscle in the airwaytoo treat the exacerbation of asthma. Salmeterol is similar in action to formoterol, however formoterol has been demonstrated to have a faster onset of action than salmeterol as a result of a lower lipophilicity and has also been demonstrated to be more potent - a 12 µg dose of formoterol has been demonstrated to be equivalent to a 50 µg dose of salmeterol. Salmeterol's long, lipophilic side chain binds to exosites near beta(2)-receptors in the lungs and on bronchiolar smooth muscle, allowing the active portion of the molecule to remain at the receptor site, continually binding and releasing. Beta(2)-receptor stimulation in the lung causes relaxation of bronchial smooth muscle, bronchodilation, and increased bronchial airflow.

Table: 1 Marketed Formulation

Drug Name	Label Claim	Dosage	Manufacturer
Fluticasone And Salmeterol	125mg/25mg	Fluticasone +salmeterol cipla 125/25	Cipla Pvt Ltd

MATERIALS AND METHODS

Salmeterol provided by Sura labs, Fluticasone provided by Sura labs, Water and Methanol for HPLC LICHROSOLV (MERCK)m, Acetonitrile for HPLC Merck.

HPLC Method Development:

Trails

Method Development

A. **Selection of chromatographic methods:**

The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected.

B. **Optimization of Column:**

The method was performed with various columns like HypersilC_{18} column, X- bridge column and Symmetry C_{18} (4.6 x 150mm, 5µm), X-terra (4.6 ×150mm, 5µm particle size) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

C. **Mobile Phase Optimization**:

Initially, the mobile phase tried was Water: Methanol and Water: Acetonitrile with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetonitrile: Water in proportion (50:35:15%v/v) respectively.

Preparation of mobile phase: Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation:

Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase: Acetonitrile: TEA Buffer (pH-4.2) (40:60v/v)

Column: Phenomenex Luna C18 (4.6mm×250mm, 5µm) particle size

Flow rate: 1 ml/min

Wavelength: 220 nm

Column temp: Ambient

Injection Volume: 20 µl

Run: tes

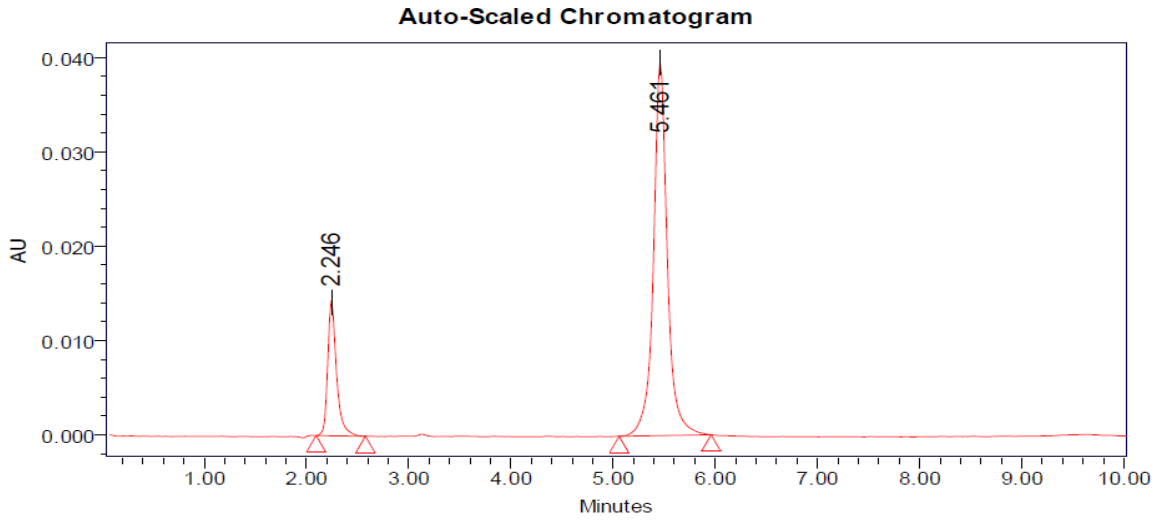

Fig-:3 Optimized Chromatogram

Table: 2 Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Salmeterol	2.246	765789	69584		0.97	5587.0
2	Fluticasone	5.461	2532158	190049	2.97	1.26	5398.0

The above trial has been accepted as Optimized since the theoretical plates are more than 2000, the tailing facistfacesss than 2 and the peaks are separate d with good resolution.

Method Validation: The newly proposed method was validated as per ICH guidelines.

System Suitability: It was performed by five replicate injections of sample and standard solutions and the parameters theoretical plates, tailing, were observed and tabulated.

Table-: 3 System Suitability results for Fluticasone and Salmeterol

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Salmeterol	2.256	759868	71255		1.7	5689	1
2	Fluticasone	5.427	2458754	215654	2.04	1.6	5362	1
3	Salmeterol	2.249	759458	72541		1.7	5748	2
4	Fluticasone	5.430	2465885	226565	2.00	1.6	5452	2
5	Salmeterol	2.248	759245	72584		1.7	5584	3
6	Fluticasone	5.443	2489578	221542	2.04	1.6	5456	3
7	Salmeterol	2.256	759868	71255		1.7	5689	4
8	Fluticasone	5.427	2458754	215654	2.04	1.6	5362	4
9	Salmeterol	2.248	759245	72584		1.7	5584	5
10	Fluticasone	5.443	2489578	221542	2.04	1.6	5456	5

% ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Salmeterol and Fluticasone in pharmaceutical dosage form was found to be 99.76 %.

Linearity: The linearity of the method was established by injecting five replicate injections of each sample of different concentrations. Peak areas at each concentration are recorded and a plot was constructed between concentrations and peak areas. The correlation coefficient was observed and reported.

Table: 4 Linearity data of Fluticasone and Salmeterol

Concentration µg/ml	Average Peak Area	Concentration µg/ml	Average Peak Area
30	51476	60	2286598
40	67598	80	3086587
50	84897	100	3867579
60	101114	120	4758517
70	119554	140	5604874

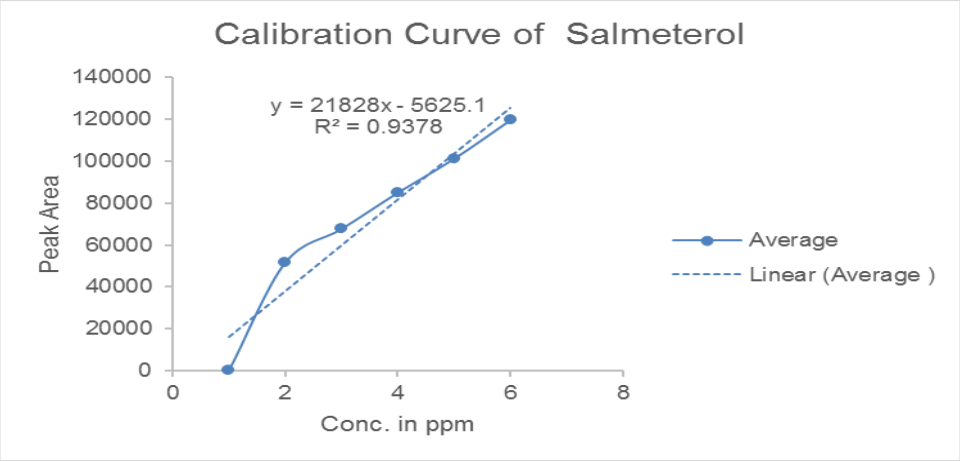

Figure: 4 Calibration graph for Salmeterol

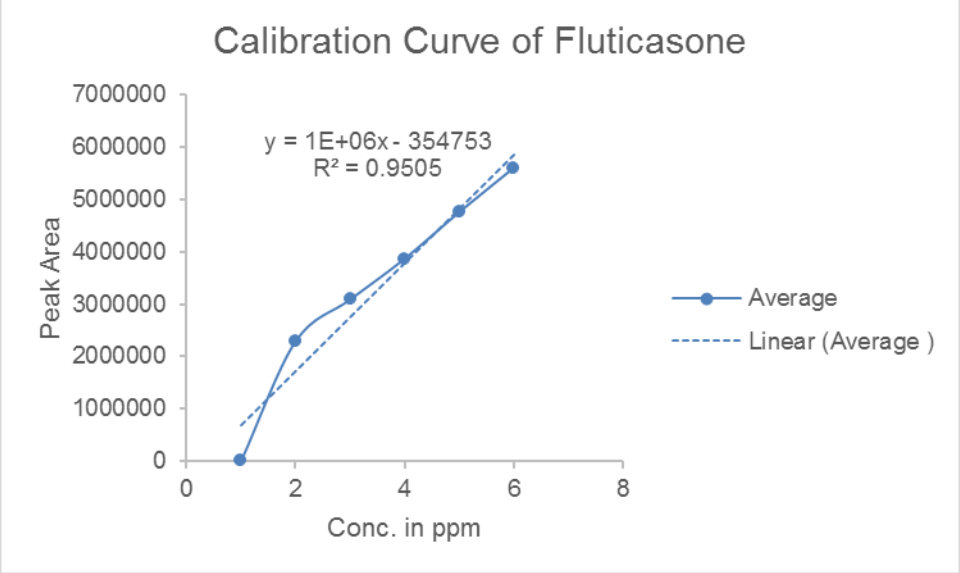

Figure:5 Calibration Graph for Fluticasone

The method was identified to be linear in the range 30-70 µof g/ml and 60-140µg/ml for fluticasone and salmeterol respectively.

Precision: It was executed by repeatability and intermediate precision. Repeatability was evaluated by five replicate injections on the same day and % RSD of the peak areas was observed and reported. Intermediate precision was determined by six replicate injections of the same on two different days.

REPEATABILITY

Table-:5 Results of Repeatability for Salmeterol and Fluticasone

S.No.	Peak Name	Area	Peak Name	Area
1	Salmeterol	766854	Fluticasone	2569865
2	Salmeterol	765884	Fluticasone	2578474
3	Salmeterol	765842	Fluticasone	2568985
4	Salmeterol	768985	Fluticasone	2586845
5	Salmeterol	765845	Fluticasone	2545898
Mean		766682		2570013
S D		1357.973		15309.45
% RSD		0.177123		0.595695

Table-:6 Results of Intermediate precision for Salmeterol and Fluticasone Day-1

S.No.	Peak Name	Peak Area	Peak Name	Peak Area
1	Salmeterol	758955	Fluticasone	2659852
2	Salmeterol	759869	Fluticasone	2648574
3	Salmeterol	758985	Fluticasone	2659865
4	Salmeterol	756894	Fluticasone	2658547
5	Salmeterol	759854	Fluticasone	2648981
6	Salmeterol	756985	Fluticasone	2654652
Mean		758590.3		2655079
Std. Dev		1339.793		5242.086
% RSD		0.176616		0.197436

Table-: 7 Results of Intermediate precision for Salmeterol and Fluticasone Day-2

S.No.	Peak Name	Peak Area	Peak Name	Peak Area
1	Salmeterol	766895	Fluticasone	2653254
2	Salmeterol	765988	Fluticasone	2648985
3	Salmeterol	766532	Fluticasone	2658213
4	Salmeterol	766214	Fluticasone	2653652
5	Salmeterol	765897	Fluticasone	2648978
6	Salmeterol	765245	Fluticasone	2658985
Mean		766128.5		2653678
Std. Dev		567.7234		4313.355
% RSD		0.074103		0.162543

The proposed method was found to be precise an the %RSD values of peak areas for repeatability and intermediate precision which were assessed on two different days was found to be less than two.

Accuracy: The accuracy of the method was determined by recovery studies. Three different concentrations were prepared and injected in triplicates. %recovery at each level was identified from which mean recovery was obtained**.**

Table-:8 The accuracy results for Salmeterol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	% Mean Recovery
50%	42594.67	25	25.070	100.280%	100.14%
100%	84867	50	49.965	99.930%	
150%	127654	75	75.164	100.218%	

Table-:9 the accuracy results for Fluticasone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	% Mean Recovery
50%	2079124	50	50.445	100.890%	100.56%
100%	4082412	100	100.571	100.571%	
150%	6070195	150	150.309	100.206%	

The method was accurate as the mean recovery values were 100.14 and 100.34 for salmeterol and fluticasone respectively. The values indicate the accuracy.

Robustness: The robustness was determined to ascertain the usage of the method on some unexpected changes. It is evaluated by deliberately changing the conditions like flow rate and organic phase composition.

Table:10 Robustness results of Salmeterol:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	The actual
The actual Flow rate of 1.0 mL/min	765789	2.246	5387.0	0.97
Less Flow rate of 0.9 mL/min	758698	2.505	5458	0.96
More Flow rate of 1.1 mL/min	7689584	2.046	5696	0.94
Less organic phase	758412	2.505	5586	0.92
More organic phase	769852	2.046	5355	0.95

Table: 11 Robustness results of Fluticasone:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	5.461	5398	1.26
Less Flow rate of 0.9 mL/min	2458692	5.599	5329	1.25
More Flow rate of 1.1 mL/min	2658642	4.576	5256	1.24
Less organic phase	2452148	5.599	5214	1.23
More organic phase	2653894	4.576	5524	1.22

The robustness of the method was established as there were no considerable deviations observed in parameters like retention time, tailing factor and theoretical plates.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Salmeterol and Fulticasone in bulk drug and pharmaceutical dosage forms. The method was optimized for the separation of Salmeterol and Fluticasone by using Phenomenex Luna C18 column, flow rate 1.0 ml/min, mobile phase ratio was (40:60 v/v) Acetonitrile: TEA buffer pH-4.2 and detection wavelength was 220nm. The retention times were found to be 2.246mins and 5.461mins for salmeterol and fluticasone respectively. The % purity of Salmeterol and Fluticasone was found to be 101.27% and 99.76% respectively. The linearity s Salmeterol and Fluticasone was established in concentration range of 30µg-70µg and 60µg-140µg for salmeterol and fluticasone respectively. % recovery was found to be 100.14% and 100.56% for salmeterol and fluticasone respectively. The method was precise as the %RSD values were less than 2. The LOD was 0.56 and 1.2, for salmeterol and fluticasone respectively and LOQ values were 1.7 and 3.6 respectively. This method can be used for the routine determination of Salmeterol and Fulticasone in bulk drug and in Pharmaceutical dosage forms.

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