International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Fluticasone and Vilanterol by RP-HPLC Method in its Pharmaceutical Dosage Forms

S. Swapna[1], P. Sowjanya[1], Dr. Gampa Vijaya Kumar*[2]

[1]KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.
[2]Professor and Head, Department of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.

A B S T R A C T

A new method was established for simultaneous estimation of fluticasone and vilanterol by RP-HPLC method. The chromatographicconditionsweresuccessfullydevelopedfortheseparationoffluticasone and vilanterol by using inertsil C185µm (4.6*250mm) column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH4.6: CAN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. There tention times were found to be 2.399mins and 3.907mins. The % purity of fluticasone and vilanterol was found tobe100.7% and 101.4% respectively. The system suitability parameters for fluticasone and vilanterol such as the oretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 there solution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for fluticasone and vilanterol was found in concentration range of 100µg-500µg and 1µg-5µg and correlation coefficient(r2) was found to be 0.997 and 0.999, %mean recovery was found to be 100%, 100.5%,% RSD for repeatability was 0.2 and 0.4,% RSD for intermediate precision was0.5and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04 and LOQ value was 9.87and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of fluticasone and vilanterol in API and Pharmaceutical dosage form.

Keywords: Fluticasone, Vilanterol, RP-HPLC

A R T I C L E I N F O

CONTENTS

Article History: Received 05 September 2016, **Accepted** 09 October 2016, **Available Online** 10 December 2016

***Corresponding Author**
Dr. Gampa Vijaya Kumar
Professor and Head, Dept. of Pharmacy,
KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.
Manuscript ID: IJMPR3202

PAPER-QR CODE

Citation: Gampa Vijaya Kumar, *et al*. Analytical Method Development and Validation for the Simultaneous Estimation of Fluticasone and Vilanterol by RP-HPLC Method in its Pharmaceutical Dosage Forms. *Int. J. Med. Pharm. Res*., 2016, 4(6): 336-343.

1. Introduction

HPLC is probably the most universal type of analytical procedure. In addition HPLC also ranks as one of the most sensitive analytical procedures and is unique in that it easily copes with multi-component mixtures. Its application areas include quality control, process control, forensic analysis, environmental monitoring and clinical testing. It has achieved this position as a result of the constant evolution of the equipment used in LC to provide higher and higher efficiencies at faster and faster analysis times with a constant incorporation of new highly selective column packing.

Fluticasone, 6α, 9α-Difluoro-11β, 17α-dihydroxy-16α-methyl-21-thia-21-fluoromethylpregna-1,4-dien-3, 20-dione (Fig.1) is a synthetic glucocorticoid. [1] Both the furoate and propanoate esters, fluticasone furoate and fluticasone propionate, are used as topical anti-inflammatories [2] and inhaled corticosteroids.

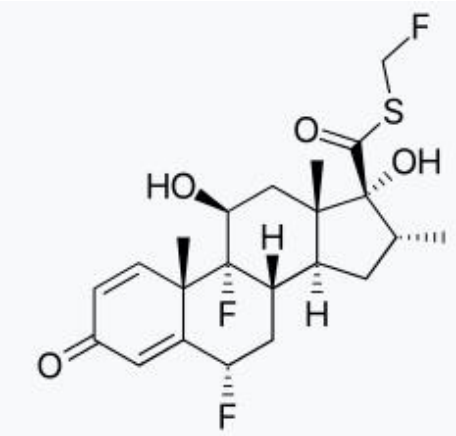

Figure 1: Fluticasone

Vilanterol, 4-{(1R)-2-[(6-{2-[(2,6-Dichlorobenzyl) oxy] ethoxy}hexyl)amino]-1-hydroxyethyl}-2-(hydroxyl methyl) phenol (Fig.2) is an ultra-long-acting β2 adrenoreceptor agonist (ultra-LABA), which was approved in May 2013 in combination with fluticasone furoate for sale as Breo Ellipta by GlaxoSmithKline for the treatment of chronic obstructive pulmonary disease (COPD).[1]

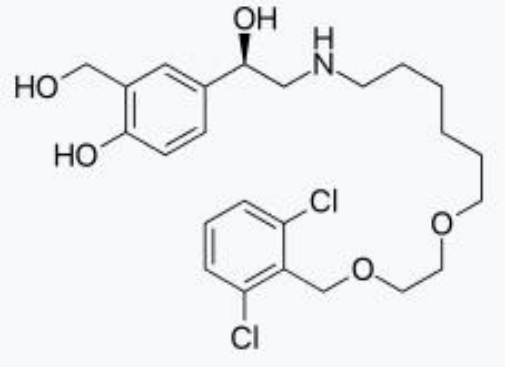

Figure 2: Vilanterol

2. Materials and Methods

Table 1: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module,
2	UV-spectrophotometer	Labindia UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Fluticasone	Mylon
2	Vilanterol	Cipla
3	KH_2PO_4	Finer chemical Lltd
4	Water and Methanol	Lichrosolv
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

Table 3: Solubility profile

Solubility	Fluticasone	Vilanterol
Water	Sligltly soluble	Insoluble
Methanol	soluble	Soluble
Acetonitril	soluble	Soluble
Chloroform	In soluble	Soluble

HPLC method development:

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

Wave length selection:

UV spectrum of 10 µg/ml Fluticasone and Vilanterolin diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb and inertsil ODS column. Inertsil ODS (4.6x150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

Preparation of Buffer and Mobile Phase:

Preparation of Phosphate buffer: Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask,

dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Preparation of the Fluticasone & Vilanterol Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Fluticasone and Vilanterol10mg of working standard into a 10Ml & 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Fluticasone and Vilanterol (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 1 ml of Fluticasonee and Vilanterol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Fluticasone and Vilanterol peaks and calculate the %Assay by using the formulae.

System Suitability:

Tailing factor for the peaks due to Fluticasone and Vilanterolin Standard solution. Should not be more than 2.0

Theoretical plates for the Fluticasone and Vilanterol peaks in Standard solution should not be less than 2000.

Calculation: (For Fluticasone)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim of Fluticasone mg/ml.

Calculation: (For Vilanterol)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt.}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim of Vilanterol mg/ml.

Method Validation Summary:

Precision:

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Fluticasone and Vilanterol working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 1 ml of Fluticasone & Vilanterol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%**.**

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method,

Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Fluticasone and 25mg of Vilanterol working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 1ml of Fluticasone & Vilanterol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

Accuracy:

Preparation of Standard stock solution:

Accurately weigh and transfer 100 mg of Fluticasone and 25mg Vilanterol of working standard into a 100ml & 100 ml clean dry volumetric flask add about 70mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 1 ml& 0.25 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 10mg of Fluticasone and 2.5mg of Vilanterol working standard into a 10mL and 10 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it

completely and make volume up to the mark with the same solvent.

Stock Solution

Further pipette 0.05 ml of Fluticasonee & 0.014 ml of Vilanterol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of Fluticasone and 10 mg of Vilanterol working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock Solution

Further pipette 0.1 ml of Fluticasone & 0.25 ml of Vilanterol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10mg of Fluticasone and 25mg of Vilanterol working standards into a 10mL and 100ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipette 0.15 ml of Fluticasone & 0.39 ml of Vilanterol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150%solutions. Calculate the Amount found and Amount added for Fluticasone & gemigliprin and calculate the individual recovery and mean recovery values.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Linearity [12,13]

Preparation of stock solution:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Fluticasone and Vilanterol (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution:

Preparation of Level–I (100ppm of Fluticasone &25ppm of Vilanterol): 1ml and 0.1 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level–II (200ppm of Fluticasone & 50ppm of Vilanterol): 2ml and 0.2 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level–III (300ppm of Fluticasone &75ppm of Vilanterol): 3ml and 0.3 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level–IV (400ppm of Fluticasone &100ppm of Vilanterol): 4ml and 0.4 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level–V (500ppm of Fluticasone &150ppm of Vilanterol): 5ml and 0.5 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Acceptance Criteria: Correlation coefficient should be not less than 0.999.

Limit of Detection: (for Fluticasone):

Preparation of 300µg/ml solution: Accurately weigh and transfer 10 mg of Fluticasone working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.12µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.4mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 52µV
Signal Obtained from LOD solution : 152 µV
S/N = 152/52 = 2.9

Acceptance Criteria: S/N Ratio value shall be 3 for LOD solution.

Limit of Detection: (For Vilanterol)

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Vilanterol working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.015µg/ml solution: Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 52 µV
Signal Obtained from LOD solution: 156 µV
S/N = 156/52 = 3.0

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of Quantification:

Limit of Quantification (for Fluticasone HCL)

Preparation of 300µg/ml solution:

Accurately weigh and transfer 10 mg of Fluticasone working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.42µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 1.4 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 52 µV
Signal Obtained from LOQ solution : 522µV
S/N = 522/52 = 10.03

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Limit of Quantification: (for Vilanterol)

Preparation of 3µg/ml solution: Accurately weigh and transfer 10mg of Vilanterol working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.05µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.7mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 52 µV
Signal Obtained from LOQ solution: 524µV
S/N = 524/52 = 10.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.8 ml/min to 1.2ml/min.

Standard solution 300ppm of Fluticasone&3ppm ofVilanterol was prepared and analysed using the varied flow rates along with method flow rate.

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from 50% to 50%.

Standard solution 300 µg/ml of Setraline& 3µg/ml of Vilanterol was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%.Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10

*****Results for actual Mobile phase composition (55:45 Methanol: Buffer (ph-2.8) has been considered from Accuracy stand

3. Results and Discussions

Wavelength Detection:

The detection wavelength was selected by dissolving the drug in mobile phase togeta concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Fluticasone and Vilanterol was obtained and the isobestic point of Fluticasone and Vilanterol showed absorbance maximaat255 nm.

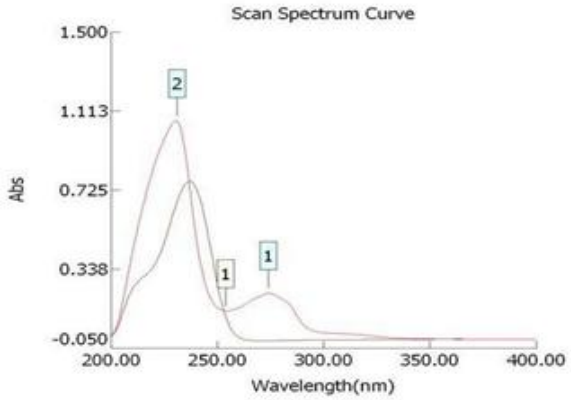

Figure 1: Overlay spectrum of Fluticasone and Vilanterol

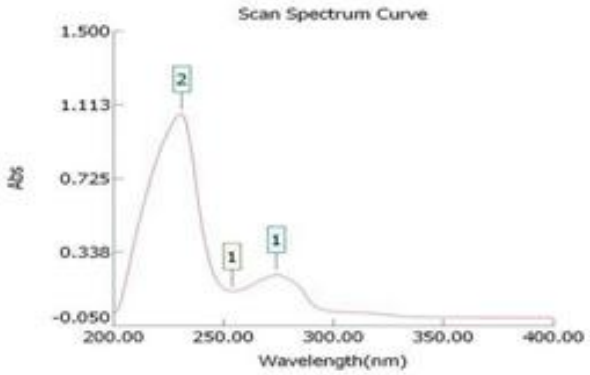

Figure 2: Spectrum of Fluticasone

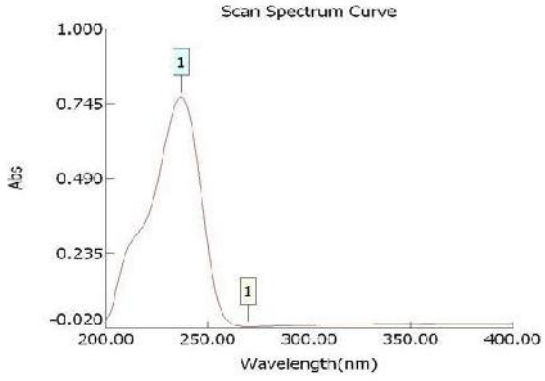

Figure 3: Spectrum of Vilanterol

Method development

Trial-1:

Chromatographic Conditions.

Column: Agilent C18 (4.6*150mm) 5µm
Mobile phase ratio: Water: Methanol (40:60%v/v)
Detection wavelength : 255nm
Flow rate: 1ml/min
Injection volume : 10µl
Column temperature: Ambient
Auto sampler temperature: Ambient
RT : 2.551, 4.873

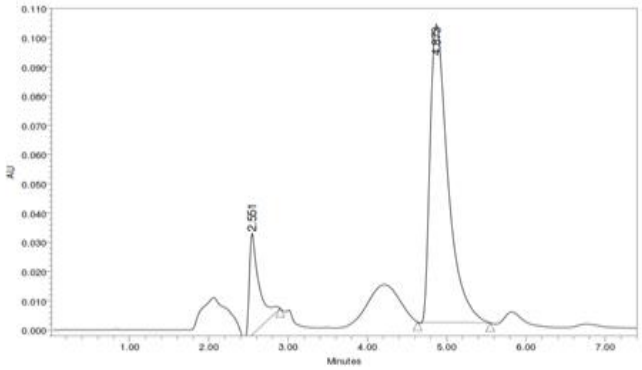

Figure 4: Chromatogram of Trial-1

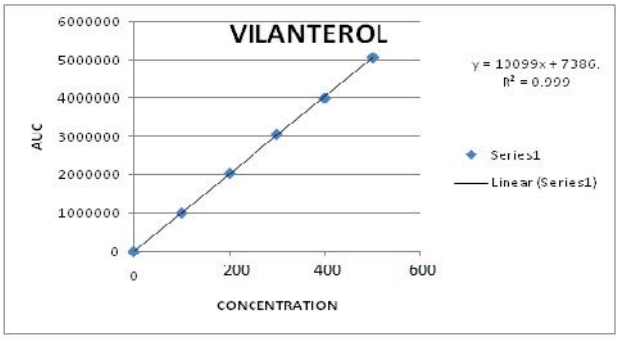

Figure 5: Calibration curve of Vilanterol

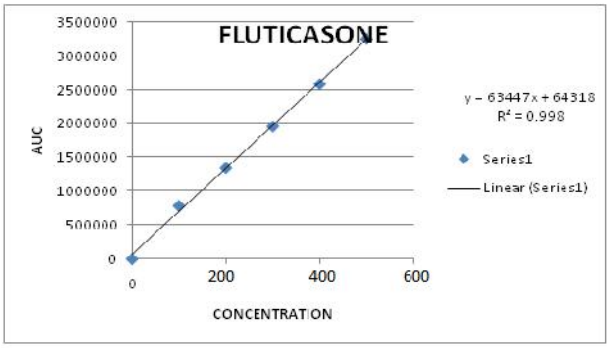

Figure 6: Calibration curve of Fluticasone

Table 1: Chromatogram showing o f system suitability

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Fluticasone	2.327	946124	155429	5105	1.3	8.1
2	Vilanterol	4.331	111541	13239	3788	1.4	

Table 2: The accuracy results for Vilanterol

% Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovey	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Table 3: Accuracy results of Fluticasone

% Concentration (at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	100.0%
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Table 4: Repeatability results of Fluticasone & Vilanterol

	Name	Rt	Area	Height
1	Vilanterol	4.304	1501417	100275
2	Vilanterol	4.300	1486940	100079
3	Vilanterol	4.308	1490656	98257
4	Vilanterol	4.310	1487329	98165
5	Vilanterol	4.314	1490384	98153
Mean			1491345	
Std .dev			5881.4	
% RSD			0.39	

Table 5: Repeatability results of Fluticasone

	Name	Rt	Area	Height
1	Fluticasone	2.321	2235319	196999
2	Fluticasone	2.317	2240678	198254
3	Fluticasone	2.323	2249490	195128
4	Fluticasone	2.322	2245822	196164
5	Fluticasone	2.324	2251694	195887
Mean			2244601	
Std .dev			6656.8	
% RSD			0.30	

Limit of Detection
Vilanterol
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank: 41µV
Signal
Obtained from LOD solution: 125µV
S/N= 125/41= 3.04
Acceptance Criteria:
S/NRatiovalueshallbe3forLODsolution
Fluticasone
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank: 41µV
Signal Obtained from LOD solution: 121µV
S/N= 121/41= 2.95
Acceptance Criteria:
S/N Ratio value shall be 3for LOD solution.
The LOD was performed for Fluticasone & Vilanterol was found to be2.95 and 3.04 respectively.
Limit of quantification:
Vilanterol
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank: 41µV
Signal Obtained from LOQ solution : 412µV
S/N = 412/41=10.0
Acceptance Criteria:
S/N Ratio value shall be10 for LOQ solution.
Fluticasone
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank: 41µV
Signal Obtained from LOQ solution : 405µV
S/N = 405/41=9.87
Acceptance criteria
S/N Ratio value shall be10 for LOQ solution.
The LOQ was performed for Fluticasone and Vilanterol was found to be 9.87and10 respectively**.**

4. Conclusion

A new method was established for simultaneous estimation of fluticasone and vilanterol by RP-HPLC method. The chromatographic conditions were successfully developed for these paration of fluticasone and vilanterol by using inertsil ODSC185µm (4.6*250mm) column, flowrate was1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH4.6: CAN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wavelength was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996 and Empower-software version-2. The retention times were found to be 2.399 mins and 3.907mins. The %purity of fluticasone and vilanterol was found to be 100.7%, 101.4% respectively. The system suitability parameters for Fluticasone and Vilanterol such as the oretical plates and tailing factor were foundtobe1.3, 5117.5and1.4, 3877.3 there solution was foundtobe8.0. The linearity study for Fluticasone and Vilanterol was found in concentration range of 100µg-500µg and 1µg-5µg and correlation coefficient (r2) was found to be 0.997and 0.999,%mean recovery was foundtobe100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5and 0.1 respectively. The precision study was precise, robust, and repeatable. LODvaluewas2.95and3.04, and LOQ value was 9.87and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of fluticasone and vilanterol in API and Pharmaceutical dosage form.

5. References

[1] Beckett A.H and Stenlake J.B;text book of pharmaceutical chemistry 4th Edn,-part 2 CBS publishers and Distriburots,New Delhi,1998:278,307
[2] Douglas Skoog A., James Hollar F. and Timothy Nieman. A Principles of Instrumental Analysis. 5thed., Thomson Learning Inc. Singapore, 1998; 110 ,300
[3] Sethi, P.D., Quantitative Analysis of Drugs in Pharamceutical formulation,3rded.,CBS Publishers and Distributors, 1997; 1-29 ,50-64
[4] Mendham, R.C., Denny, J.D., Barnis , M. and Thomas, J.K., Vogel"s Text Book of Quantitative Chemical Analysis, 6thed., Pearson Education,2003; 1, 676
[5] Sharma, B.K., Instrumental method of Chemical Analysis, 24th ed., GOEL Publishing House, Meerut,2005; 46, 68.
[6] Chatwal G.R and Anand K.S; Instrumental methods of chemical analysis,5th Edn Himalaya publishing House,mumbai, 2002,2-149
[7] Munson J.W:Modern Methods of Pharmaceutical Analysis, Medical book distributors, Mumbai, 2001, 17-54.
[8] Willard H.H,Merritt L.L,Dean J.A. and settle F.A:Instrumental Methods of analysis,7th Edn,CBS Publishers and Distributors, New Delhi 1988,436-439.

[9] Synder K.L,Kriklad J.J and Glajch J.L:Practical HPLC Method Development 2nd Edn,Wiley-Interscience Publication, USA, 1983,1-10.

[10] Bently and Drivers:text book of pharmaceutical chemistry,8th Edn,O'Brein,oxford university press,1985,1-3.International conference on harmonization "Validation of analytical procedures Methodology",14,Federal Register Nov.1996,1-8.

[11] Jitamanyu Chakrabarty et al , Development and validation of a stability-indicating NP-HPLC method for simultaneous determination of betamethasone dipropionate and calcipotriene in topical dosage form Applied Science Research, 2013, 5 (2):15-24

[12] Freddy H. Havaldar, DharmendraL.Vairal. Simultaneous estimation of metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride in the tablets dosage form. Int. J. Appl .Bio. Pharm. Tec. 2010; 1(3): 1000-1005.

[13] AbuRuz, S., Millership J, McElnay J. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. J Chromatogr. B, 2005; 817(2): 277-286.

[14] Jain D, Jain S,Jain D ,Maulik A. Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by RP-HPLC in Tablet Formulation. Journal of Chromatogr. Sci. 2008; 46:501-504.

[15] Efstratios Vakirlis. Calcipotriol / betamethasone dipropionate in the treatment of psoriasis vulgaris, Asian Journal of Chemistry; 2014, 26(19): 6465-6468