WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 14, 600-620. Research Article ISSN 2277– 7105

SIMULTANEOUS ESTIMATION OF NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FLUTICASONE AND VILANTEROL BY RP-HPLC METHOD IN BULK AND MARKETED FORMULATION

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Article Received on
05 Sept. 2020,

Revised on 25 Sept. 2020,
Accepted on 15 October 2020

DOI: 10.20959/wjpr202014-18980

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of Fluticasone and Vilanterol inhalation dosage form. Chromatogram was run through STD Agilent C18 250 x 4.6 mm, 5□. Mobile phase containing Buffer 0.1% OPA: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.1%OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 255nm. Retention time of Vilanterol and Fluticasone were found to be 2.199 min and 3.087. %RSD of the Fluticasone and Vilanterol were and found to be 0.8 and 0.5 respectively. %Recovery was obtained as 99.61% and 100.23% for Fluticasone and Vilanterol respectively. LOD, LOQ values obtained from regression equations of Fluticasone and Vilanterol were 0.48, 1.45and 0.28, 0.34 respectively. Regression equation of Fluticasone was y = 1509.9x + 2896.9 and for Vilanterol was y = 19667x + 2677.3.

KEYWORDS: Method validation, Method development, RP-HPLC, Active pharmaceutical ingredient, Regression.

INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data.[1] Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.

The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively. While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.

The wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties. This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories. Different physicochemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photoolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Methods such as nuclear magnetic resonance (NMR) and para magnetic resonance (PMR) are becoming more and more popular. The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acidbase, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing. This requires new methods for controlling their quality.

MATERIALS AND METHODS

Materials

Fluticasone and Vilanterol pure drugs (API), Combination Fluticasone and Vilanterol BreoEllipta (Fluticasone 100 mcg / Vilanterol -25 mcg) received from reddy's lab, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Fluticasone and Vilanterol solutions.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Buffer 0.1%OPA: Accurately 1ml of OPA in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of Standard stock solutions: Accurately Weighed and transferred 25mg&6.25mg of Fluticasonee and Vilanterol working Standards into a 25ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution. (1000µg/ml of Fluticasone and 250µg/ml Vilanterol)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Fluticasone and 25µg/ml of Vilanterol)

Preparation of Sample solutions: The contents of nasal spray deliveried by 50 actuations (100&25 µg each) were collected in 10 ml volumetric flask. Then 8ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 5000&1250 µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF) 2ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Fluticasone and 25µg/ml of Vilanterol).

Validation

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Fluticasone (100ppm) and Vilanterol (25ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

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

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision

Preparation of Standard stock solutions: Accurately Weighed and transferred 25mg&6.25mg of Fluticasonee and Vilanterol working Standards into a 25ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution. (1000µg/ml of Fluticasone and 250µg/ml Vilanterol).

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Fluticasone and 25µg/ml of Vilanterol)

Linearity

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Fluticasone and 6.25µg/ml of Vilanterol)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of Fluticasone and 12.5µg/ml of Vilanterol)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Fluticasone and 18.75µg/ml of Vilanterol)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (100µg/ml of Fluticasone and 25µg/ml of Vilanterol)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (125µg/ml of Fluticasone and 31.25µg/ml of Vilanterol)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (150µg/ml of Fluticasone and 37.5µg/ml of Vilanterol)

Accuracy

Preparation of Standard stock solutions: Accurately Weighed and transferred 254mg&6.25mg of Fluticasonee and Vilanterol working Standards into a 25ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution. (1000µg/ml of Fluticasone and 250µg/ml Vilanterol).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance criteria

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was

maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Fluticasone, Vilanterol, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Fluticasone, Vilanterol, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies

Oxidation

To 1 ml of stock solution of Fluticasone and Vilanterol, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid degradation studies

To 1 ml of stock s solution Fluticasone and Vilanterol, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 100µg/ml&25µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies

To 1 ml of stock solution Fluticasone and Vilanterol, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 100µg/ml&25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation studies

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml&25µg/ml solution and10µl

were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo stability studies

The photochemical stability of the drug was also studied by exposing the 1000µg/ml Fluticasone &250µg/ml Vilanterol solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&25µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 100µg/ml&25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Trial 1:

Chromatographic conditions

Mobile phase	**:**	0.1%OPA and Methanol taken in the ratio 50:50
Flow rate	**:**	1 ml/min
Column	**:**	BDS C8 (4.6 x 150mm, 5µm)
Detector wave length:		255nm
Column temperature:		30°C
Injection volume	**:**	10□L
Run time	**:**	10 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50
Results	**:**	Both peaks was eluted but not eluted but peak shape Is not Good and less resolution so, further trail is carried out.

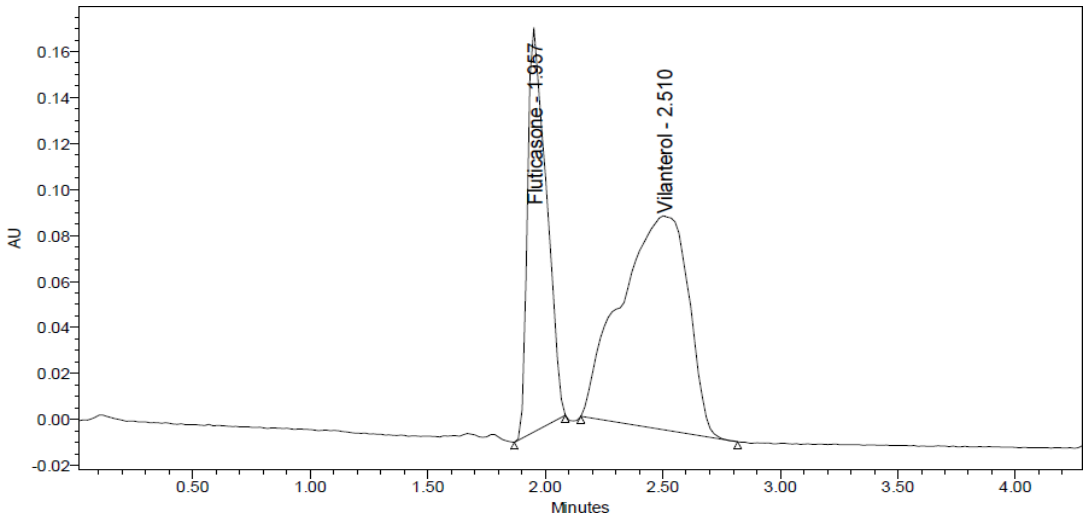


Fig. 7.1: Trial chromatogram 1.

Trial 2:

Chromatographic conditions

Mobile phase	**:**	Acetonitrile: 0.1% OPA (50:50)
Flow rate	**:**	1ml/min
Column	**:**	BDS C8 (4.6 x 150mm, 5µm)
Detector wave length:		255nm
Column temperature:		30°C
Injection volume	**:**	10□L
Run time	**:**	10 min
Diluent	**:**	Water and Acetonitrile in the ratio (50:50)
Results	**:**	Both peaks were eluted but peak shape was not good and less retention time so, Further trail is carried out.

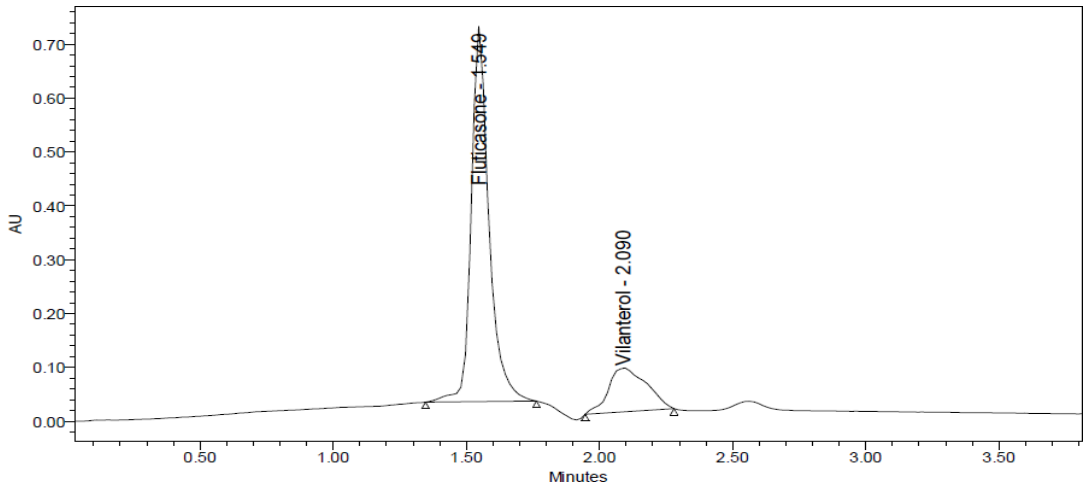


Fig. 7.2 Trial chromatogram 2.

Trial 3:

Chromatographic conditions

Mobile phase	**:**	0.1%
OPA	**:**	Acetonitrile (50:50)
Flow rate	**:**	1ml/min
Column	**:**	Kromasil C18 (4.6 x 150mm, 5µm)
Detector wave length:		255nm
Column temperature:		30°C
Injection volume	**:**	10□L
Run time	**:**	10 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50
Results	**:**	Vilanterol having fronting and fluticasone eluted at void time so, further process is carried out.

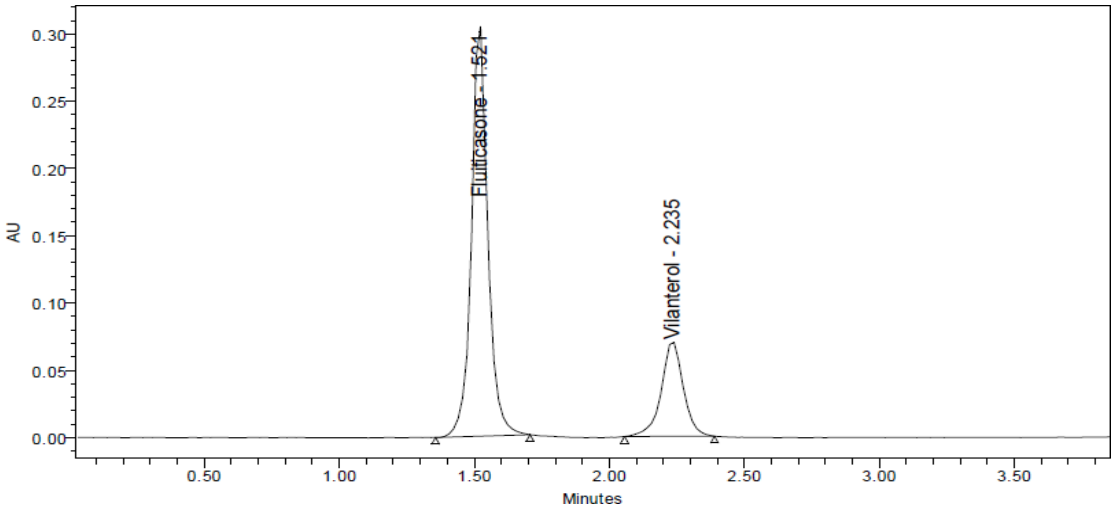


Fig. 7.3: Trial chromatogram.

Trial 4:

Chromatographic conditions

Mobile phase	**:**	60% (0.1%OPA): 40% Acetonitrile
Flow rate	**:**	1 ml/min
Column	**:**	Kromasil C18 (4.6 x 150mm, 5µm)
Detector wave length:		255nm
Column temperature:		30°C
Injection volume	**:**	10□L
Run time	**:**	8 min

Diluent : Water and Acetonitrile in the ratio 50:50

Results : Fluticasone &Vilanterol both peak are eluted but Fluticasone eluted at void time so, further process is carried out.

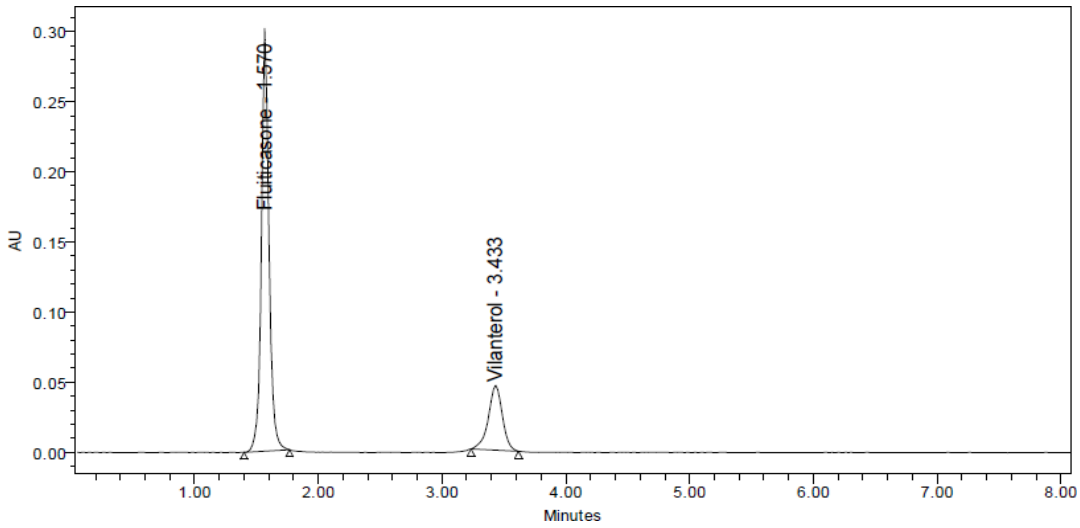


Fig. 7.4: Trial chromatogram.

Trial 5:

Chromatographic conditions

Mobile phase : 0.01N kh2po4: Acetonitrile (60:40)

Flow rate : 1 ml/min

Column : Agilent C18 (4.6 x250mm, 5µm)

Detector wave length: 255nm

Column temperature: 30°C

Injection volume : 10□L

Run time : 8 min

Diluent : Water and Acetonitrile in the ratio 50:50

Results : Fluticasone &Vilanterol both peak are eluted but Retention time so, further process is carried out.

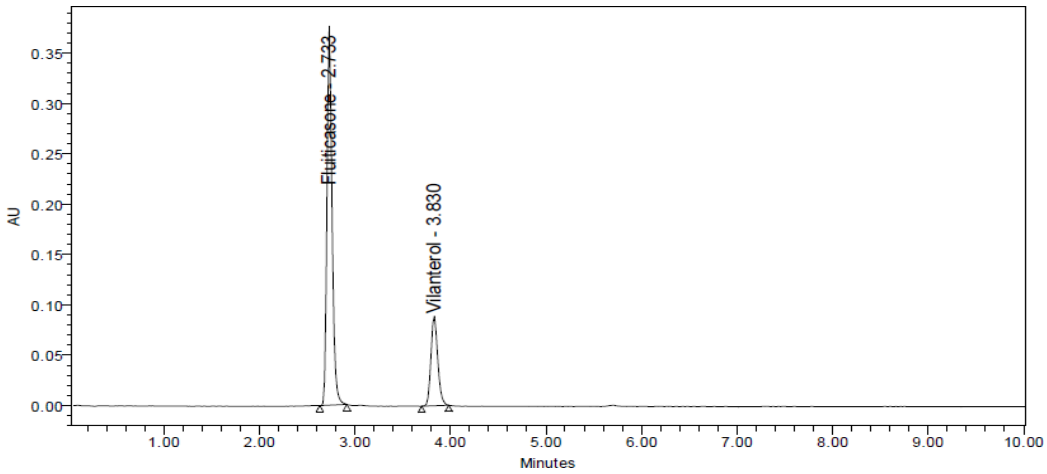


Fig. 7.5: Trial chromatogram.

Trial 6:

Optimized method:

Chromatographic conditions:

Mobile phase	**:**	60% (0.1%OPA): 40% Acetonitrile
Flow rate	**:**	1.0 ml/min
Column	**:**	Agilent C18 (4.6 x250mm, 5µm)
Detector wave length	**:**	255nm
Column temperature	**:**	30°C
Injection volume	**:**	10□L
Run time	**:**	6 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50
Results	**:**	Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

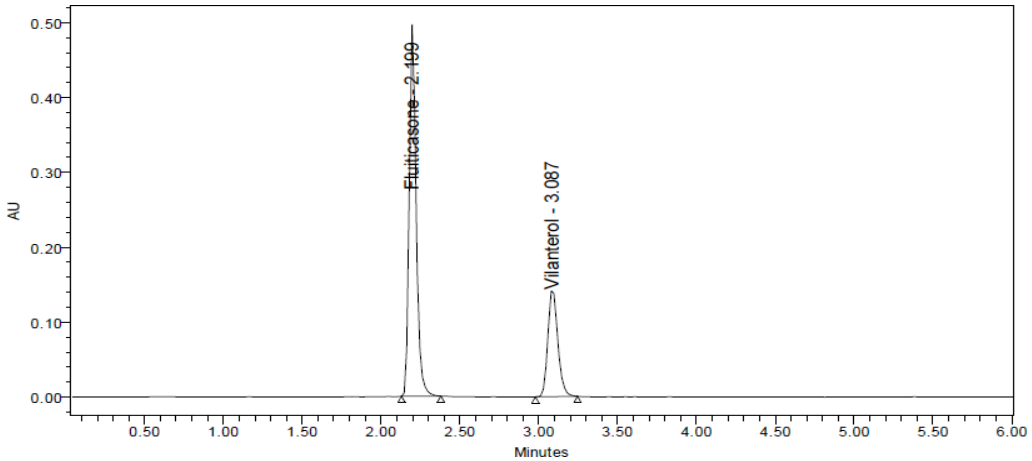


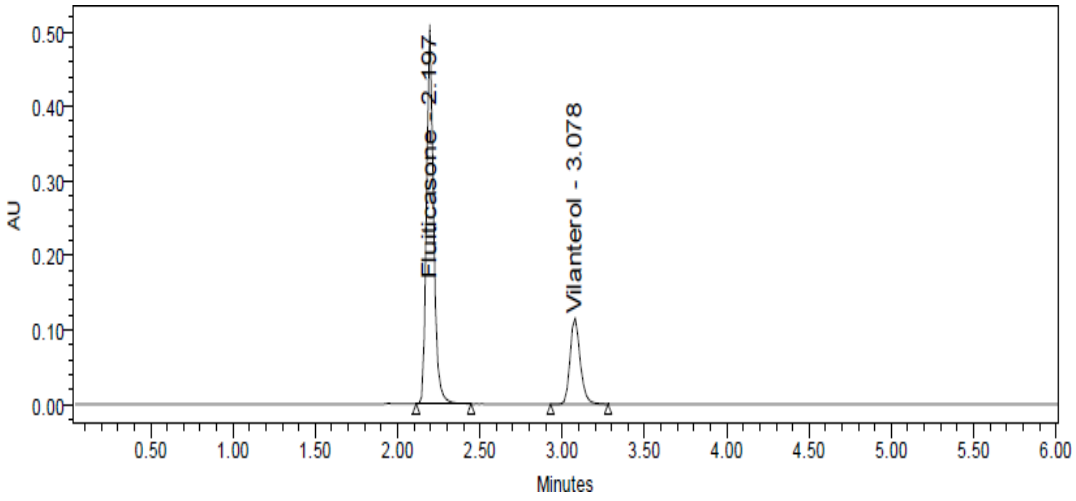
Fig. 7.6: Optimized Chromatogram.

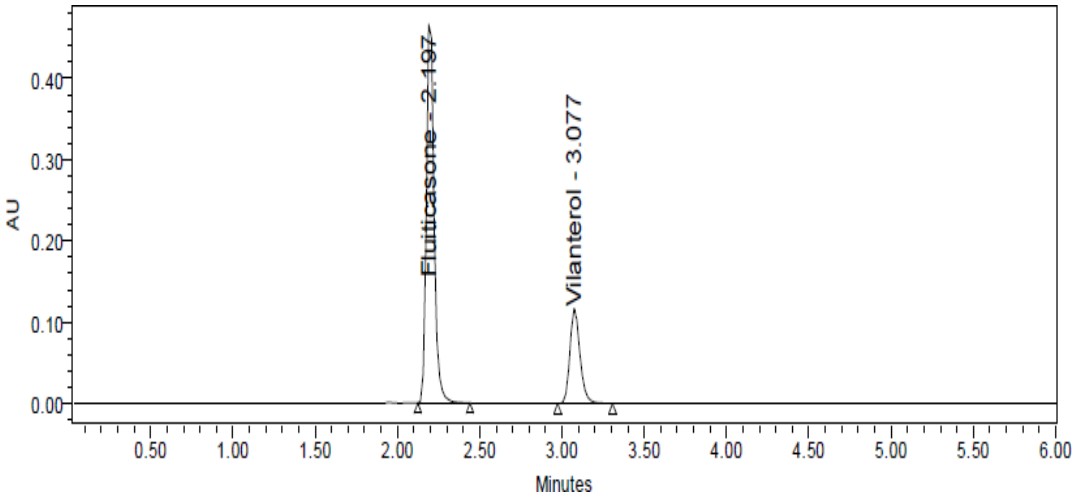
Observation: Fluticasone and Vilanterol were eluted at 2.199 min and 3.087 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

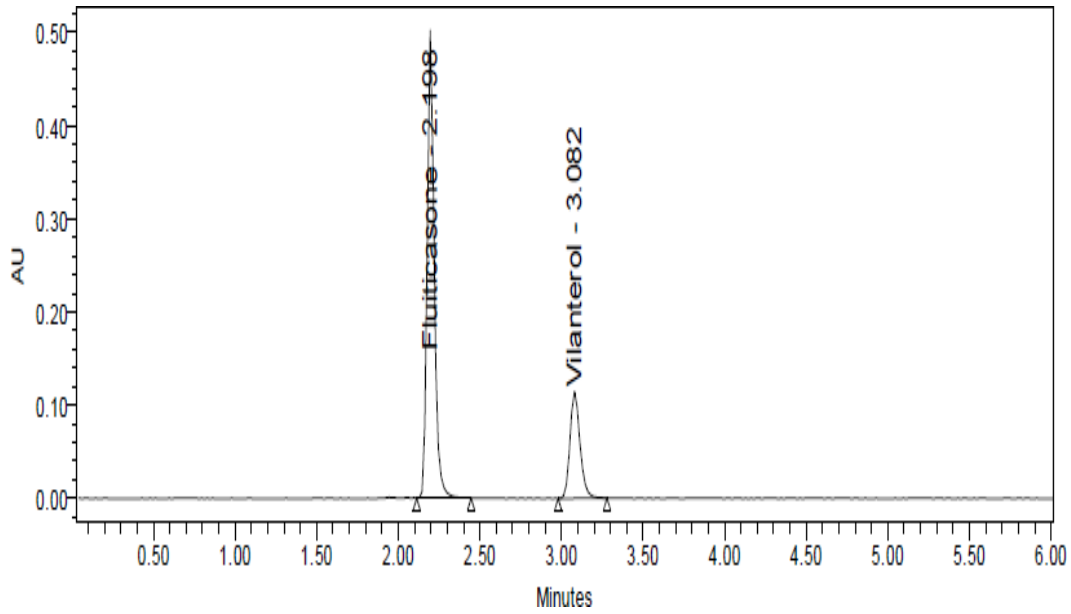
System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

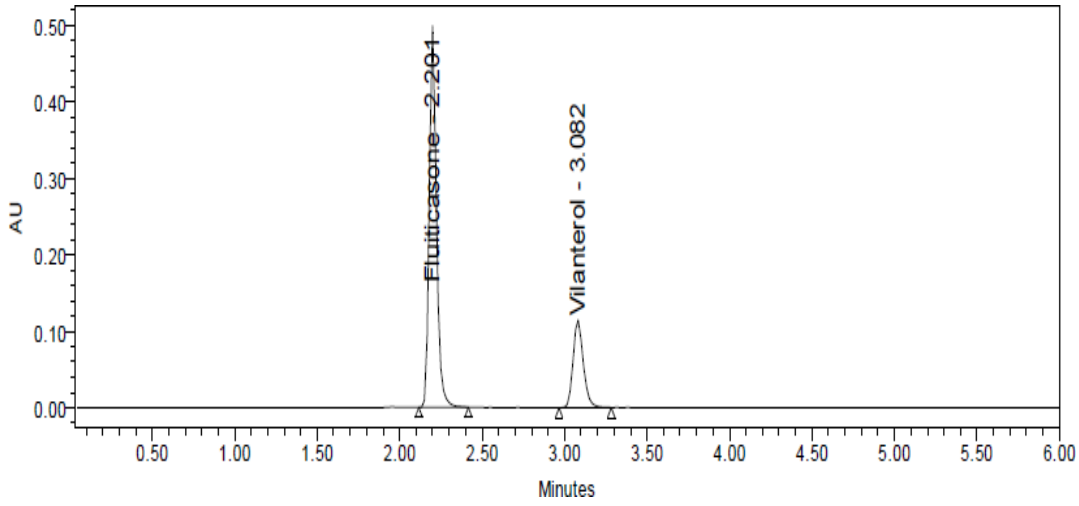
Table 7.1: System suitability parameters for Fluticasone and Vilanterol.

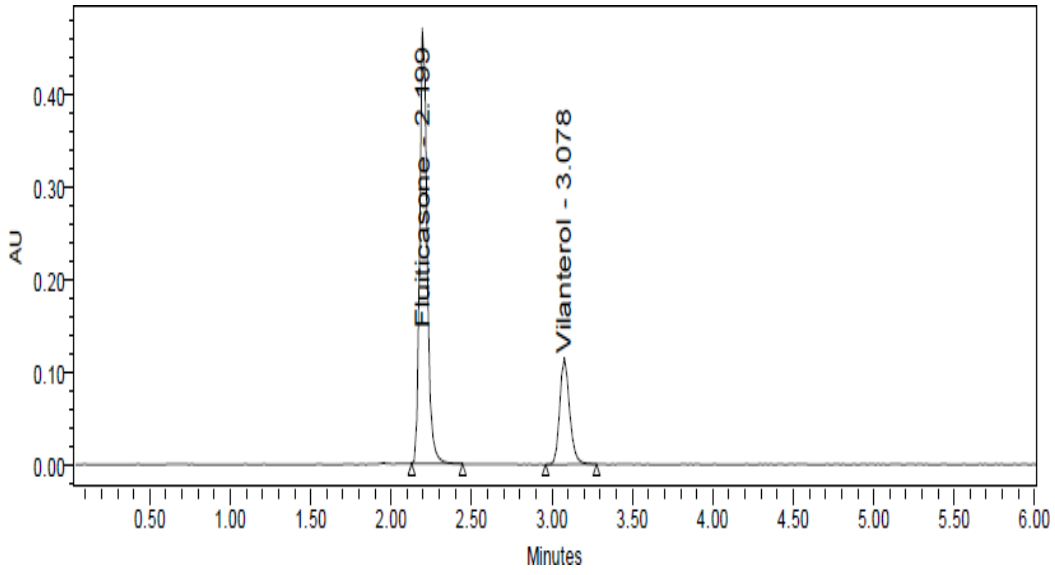
S. no.	Fluticasone			Vilanterol			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.197	12486	1.24	3.077	12106	1.19	9.0
2	2.197	10906	1.17	3.078	12214	1.17	8.9
3	2.198	10578	1.17	3.078	12070	1.16	8.9
4	2.199	11793	1.21	3.082	12218	1.17	8.8
5	2.201	10829	1.17	3.082	11960	1.17	8.8
6	2.201	10501	1.17	3.087	11619	1.19	8.8






Fluticasone - 2.198
Vilanterol - 3.082
AU
Minutes


Fluticasone - 2.201
Vilanterol - 3.082
AU
Minutes


Fluticasone - 2.199
Vilanterol - 3.078
AU
Minutes

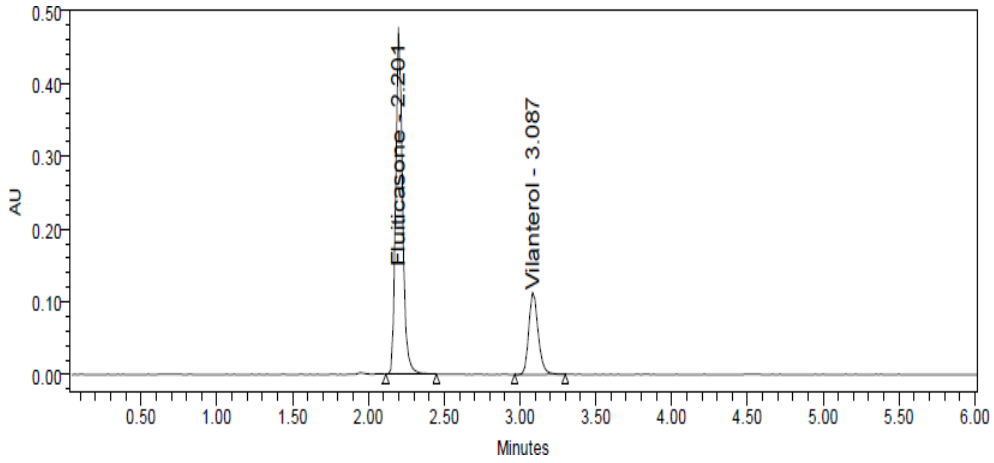
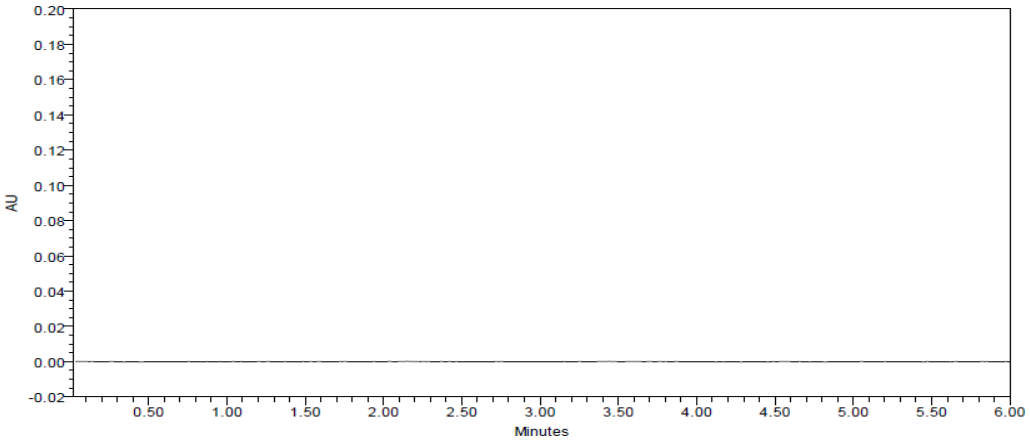


Fig. 7.7: System suitability Chromatogram.

DISCUSSION

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation



Specificity

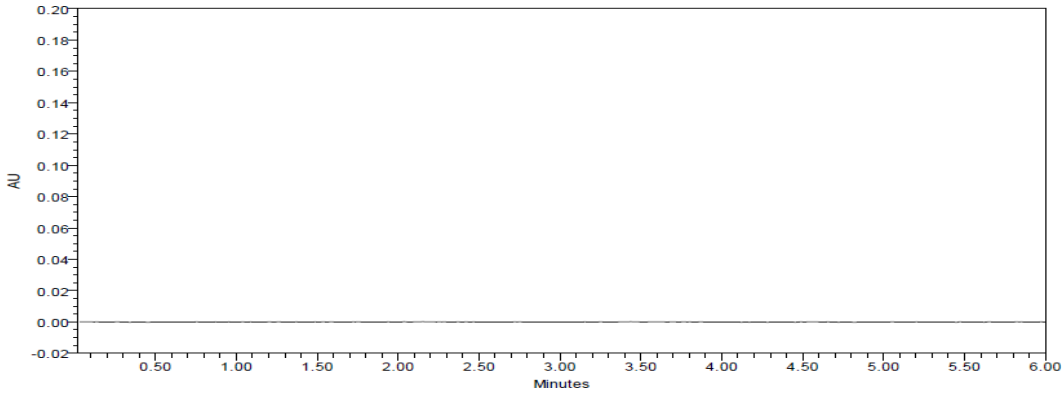


Figure no. 7.8: Chromatogram of blank.

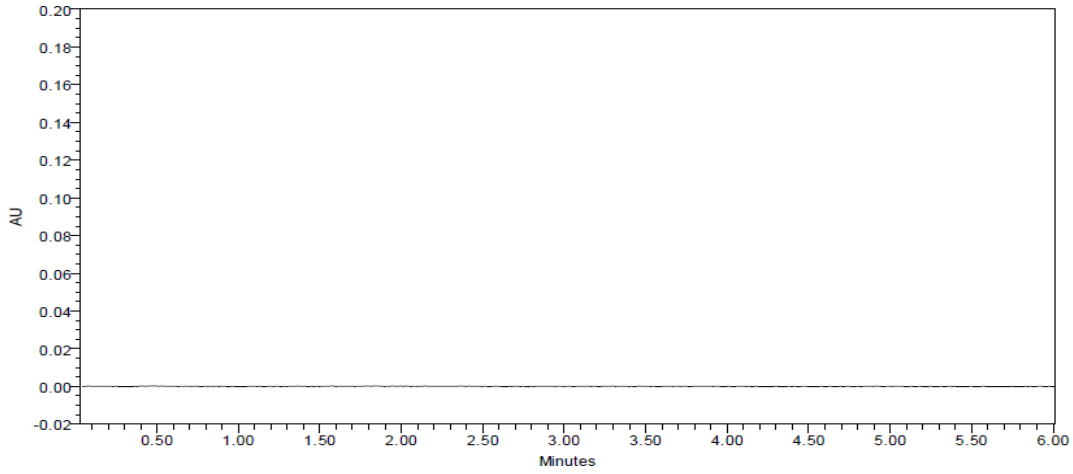


Figure no. 7.9: Chromatogram of placebo.

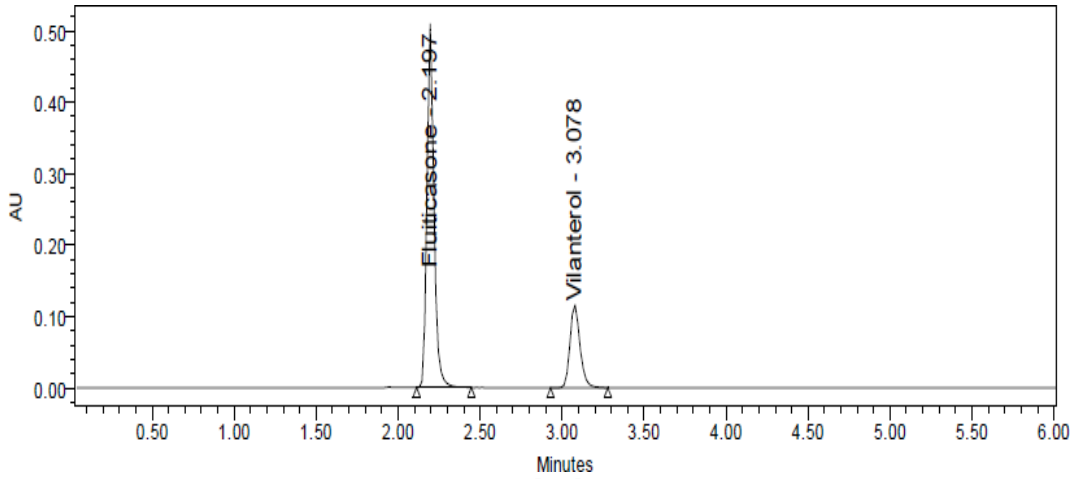


Fig. 7.10: Typical Chromatogram.

DISCUSSION

Retention times of Fluticasone and Vilanterol were 2.197 minand 3.078 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to bespecific.

Linearity

Table 7.2: Linearity table for Fluticasone and Vilanterol.

Fluticasone		Vilanterol	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
25	388386	6.25	126460
50	730840	12.5	245563
75	1158532	18.75	378441
100	1521797	25	495191
125	1882031	31.25	618223
150	2262098	37.5	736216

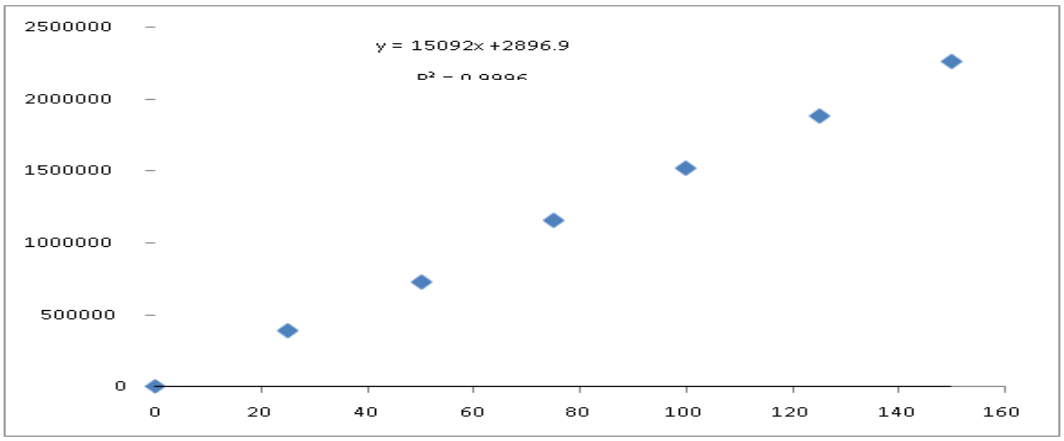


Fig. no. 7.11: Calibration curve of fluticasone.

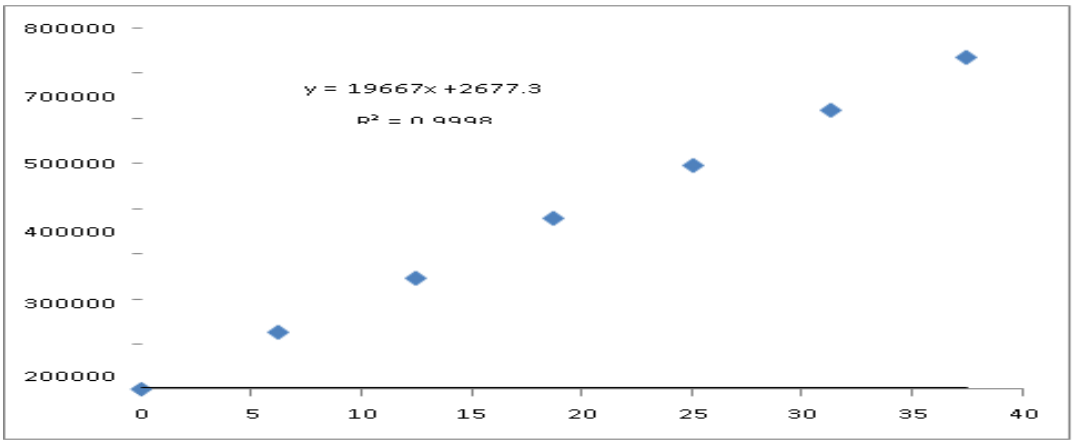
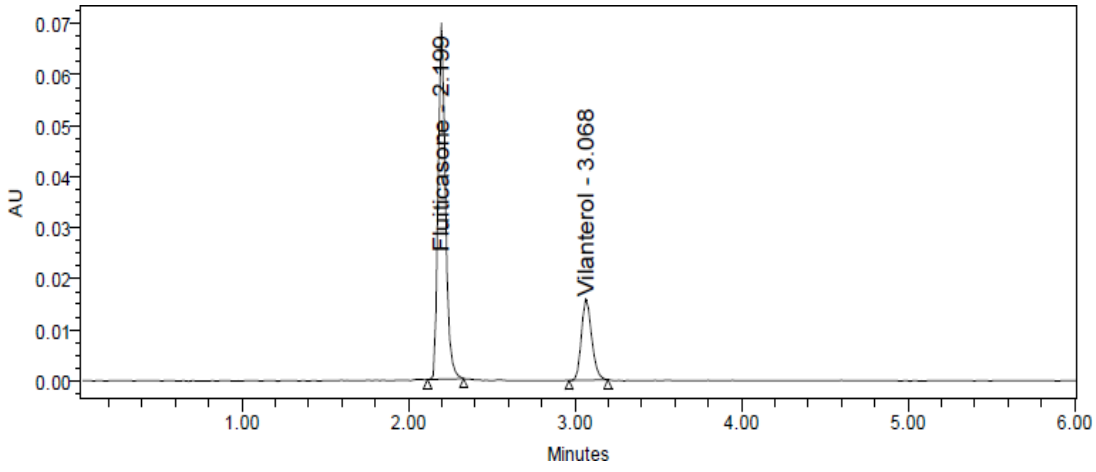


Fig. no. 7.12: Calibration curve of Vilanterol.

DISCUSSION

Six linear concentrations of Fluticasone (25-150µg/ml) and Vilanterol (6.25- 37.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Fluticasone was y = 15092x + 2896.9 and of Vilanterol was y = 19667x + 2677.3 Correlation coefficient obtained was 0.999 for the two drugs.



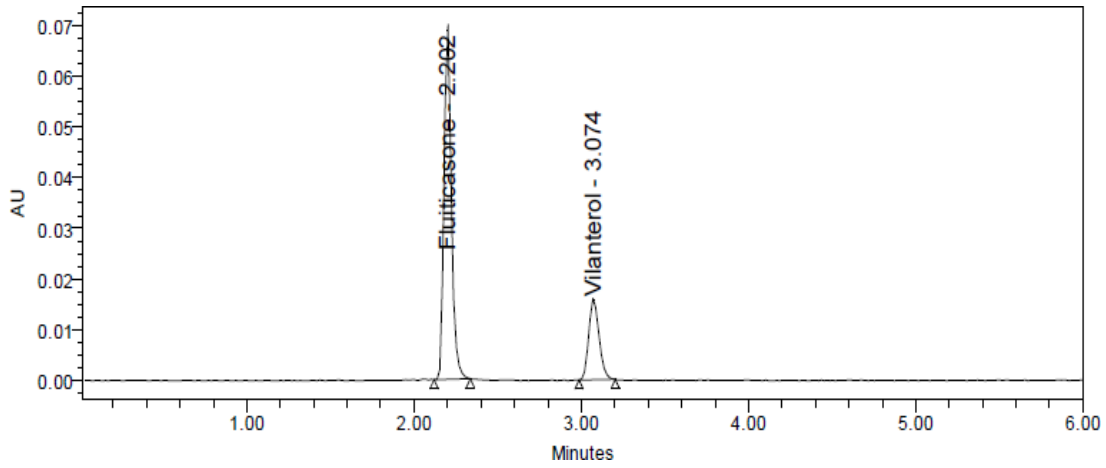
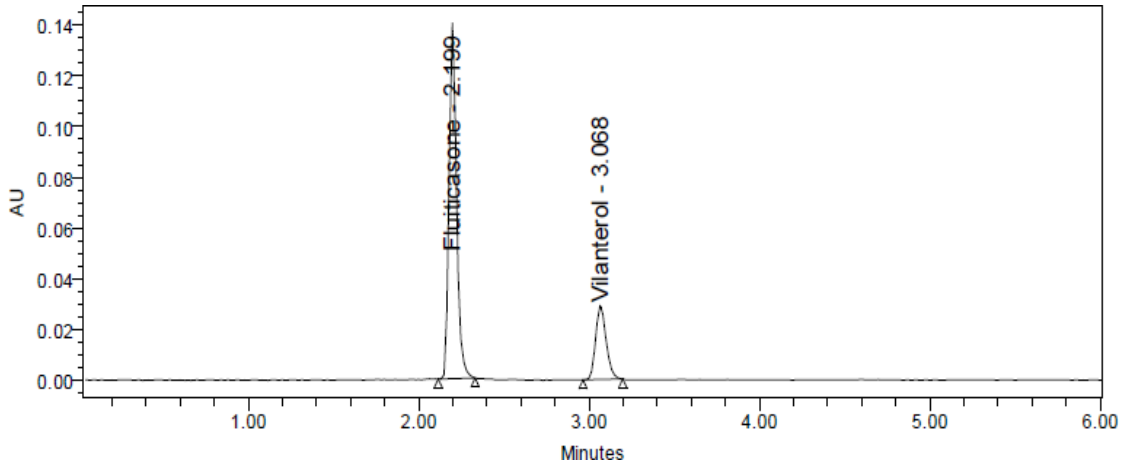


Fig. No. 7.13: Linearity 25% Chromatogram of Fluticasone and Vilanterol.



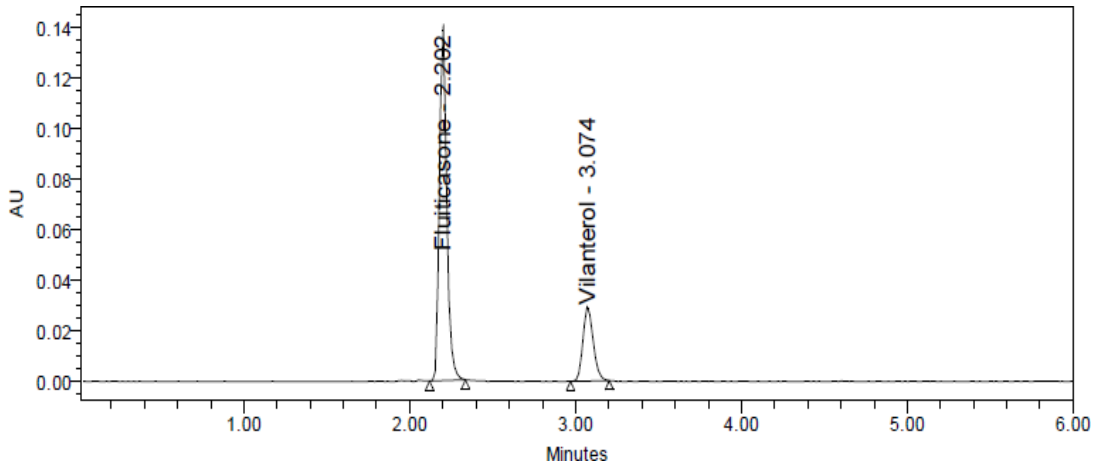


Fig. no. 7.14: Linearity 50% Chromatogram of Fluticasone and Vilanterol.

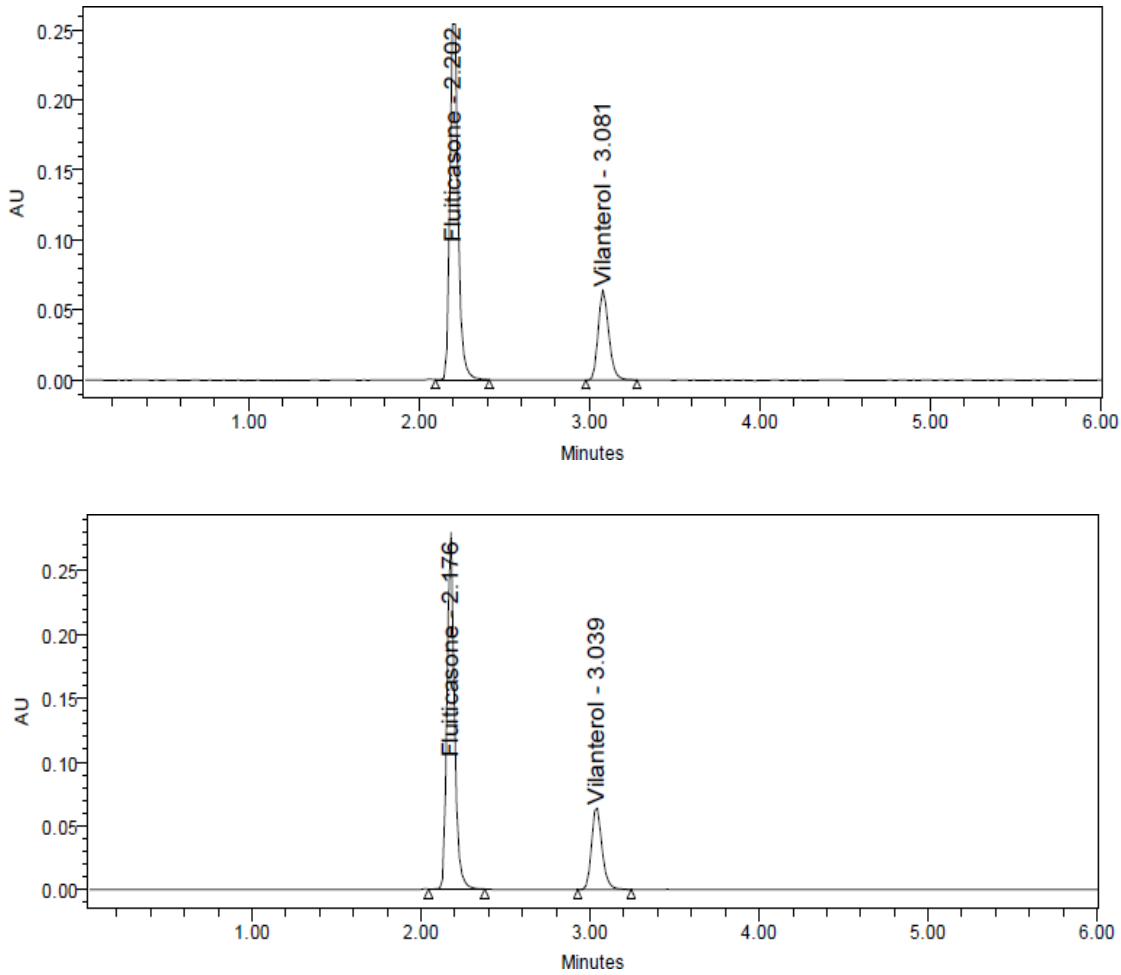


Fig. no. 7.15: Linearity 75% Chromatogram of Fluticasone and Vilanterol.

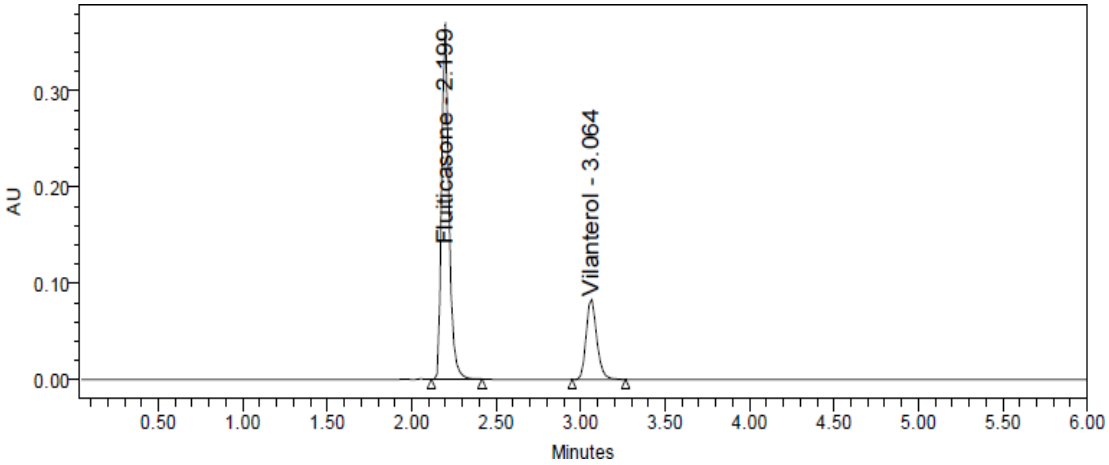


Fig. no. 7.16: Linearity 100% Chromatogram of Fluticasone and Vilantero.

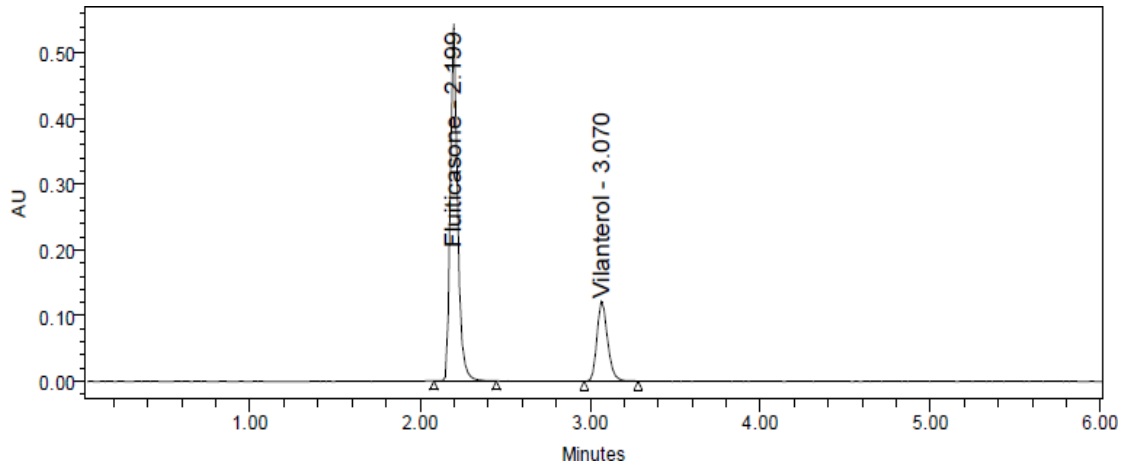


Fig. no. 7.17: Linearity 125% Chromatogram of Fluticasone and Vilanterol.

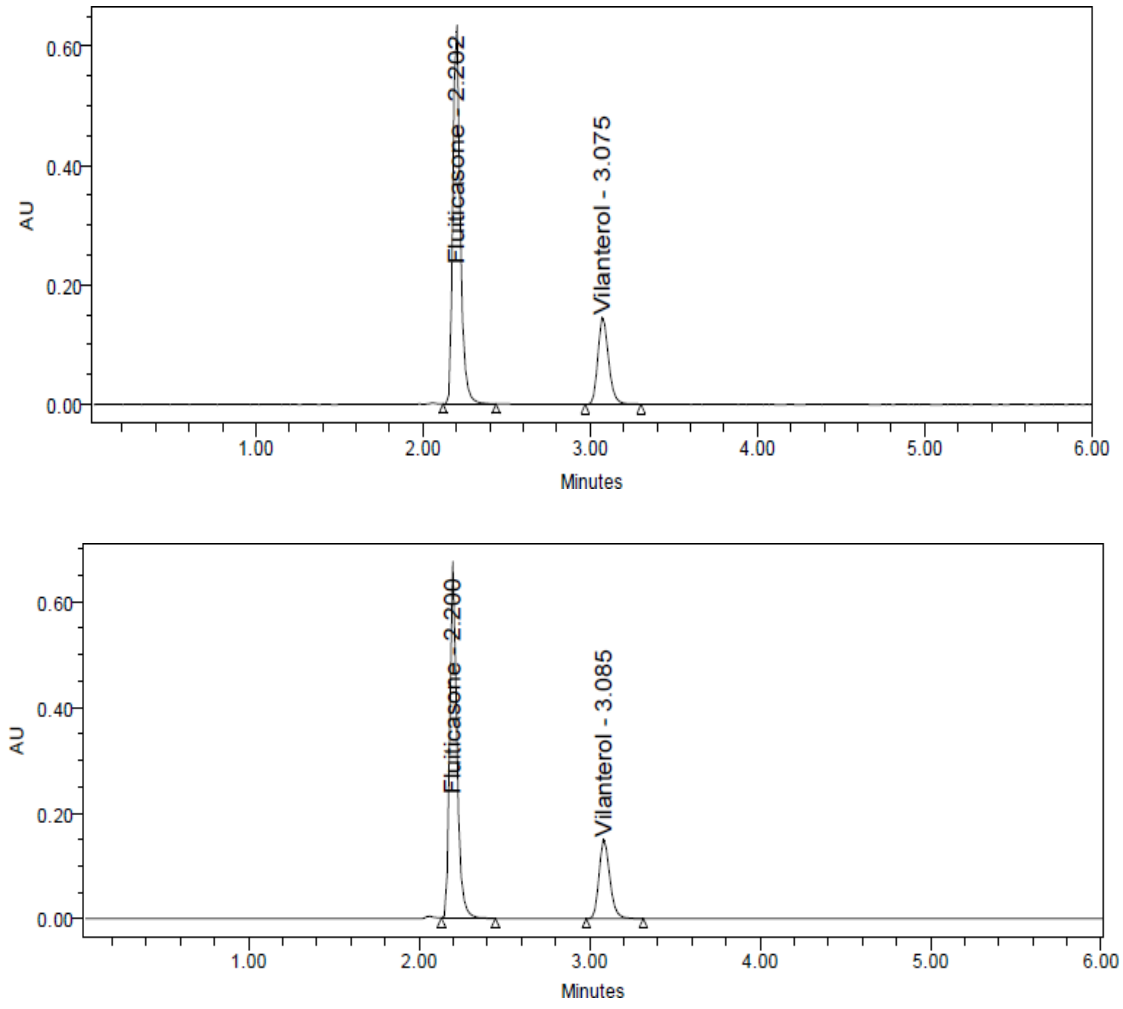


Fig. no. 7.18: Linearity 150% Chromatogram of Fluticasone and Vilanterol.

Summary table

Parameters		Fluticasone	Vilanterol	LIMIT
Linearity Range (µg/ml)		25-150 µg/ml	6.25-37.5µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope(m)		15092	19667	
Intercept(c)		2896.9	2677.3	
Regression equation (Y=mx+c)		y = 15092x +2896.9	y = 13542x +2677.3.	
Assay (% mean assay)		100.73%	100.28%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.8	0.5	NMT 2.0%
Method precision %RSD		0.5	0.2	NMT 2.0%
Accuracy %recovery		99.72-99.40%	100.33-98.98%	98-102%
LOD		0.48	0.28	NMT 3
LOQ		1.45	0.83	NMT 10
Robustness	FM	0.7	0.6	%RSDNMT 2.0
	FP	1.5	1.2	
	MM	1	0.7	
	MP	0.7	0.7	
	TM	1.2	1.3	
	TP	0.7	0.6	

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of Fluticasone and Vilanterol inhalation dosage form. Chromatogram was run through STD Agilent C18 250 x 4.6 mm, 5□. Mobile phase containing Buffer 0.1% OPA: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.1%OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 255nm. Retention time of Vilanterol and Fluticasone were found to be 2.199 min and 3.087. %RSD of the Fluticasone and Vilanterol were and found to be 0.8 and 0.5 respectively. %Recovery was obtained as 99.61% and 100.23% for Fluticasone and Vilanterol respectively. LOD, LOQ values obtained from regression equations of Fluticasone and Vilanterol were 0.48, 1.45and 0.28, 0.34 respectively. Regression equation of Fluticasone was y = 1509.9x + 2896.9 and for Vilanterol was y = 19667x + 2677.3. Retention times and that run time were decreased, so the method developed was simple and economical that can be adopted in regular Quality control test inIndustries.

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