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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FLUTICASONE AND VILANTEROL BY RP-HPLC

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ABSTRACT

For the simultaneous estimation of the dosage type of Fluticasone & Vilanterol inhalation, a simple, accurate and precise method was developed. Chromatogram was run through ODS C18 150 x 4.6 mm. Mobile phase includes Buffer 0.01N KH_2PO_4: Methanol taken in 50:50 ratios and were pumped through column on a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 250nm. Retention time of Fluticasone & Vilanterol were found to be 2.184 min and 2.623min.%RSD of the Fluticasone & Vilanterol were and found to be 0.8 and 0.8 respectively. %Recovery was obtained as 99.73% and 99.88% for Fluticasone & Vilanterol respectively. LOD, LOQ values obtained from regression equations of Fluticasone & Vilanterol were 0.87, 2.64 and 0.23, 0.70 respectively. Regression equation of Fluticasone is y = 7267x + 11198, y = 8322.x + 3567 of Vilanterol. Retention times have been reduced and runtime has been reduced, so the system developed has been simple and economical that can be used in Industries' routine quality control tests.

INTRODUCTION

Fluticasone propionate is a medium strength synthetic corticosteroid that is topically used for the treatment of asthma and for intranasal treatment to alleviate inflammatory and pruritic symptoms of dermatoses and psoriasis and for allergic and non-allergic rhinitis. The proprionate of fluticasone is sold under various brands including Flonase ®. A combined product of azelastine hydrochloride and fluticasone propionate DymistaTM is also available as Fluticasone propionate. The symptom relief of seasonal allergic rhinitis in patients aged over 12 years is suggested for DymistaTM. Chemically it is known as (1R,2S,8S,10S,11S,13R,14R,15S,17S)1,8difluoro14{[(fluoromethyl)sulfanyl]carbonyl}-17-hydroxy-2,13,15-trimethyl-5oxotetracyclo[8.7.0.0^{2},^{7}.0^{11},^{15}]heptadeca3,6-dien-14-yl propanoate. Vilanterol is a selective long-acting beta2adrenergic agonist (LABA) with inherent 24-hour activity for once daily treatment of COPD and asthma. Vilanterol is approved for use in several combination products such as with fluticasone furoate under the trade name Breo Ellipta and in combination with umeclidinium bromide as Anoro Ellipta, chemically it is known as 4-[(1R)2[(6{2[(2,6dichlorophenyl)methoxy]teeth y}hexyl)amino]-1-hydroxyethyl]-2(hydroxymethyl)phenol.

MATERIALS AND METHODS:

Fluticasone& Vilanterol pure drugs (API), Combination Fluticasone & Vilanterol Breo Ellipta (Fluticasone 100 mcg /

Vilanterol -25 mcg) received from spectrum labs water, ACN, Phosphate buffer, CH3OH, KH_2PO_4 **buffer,** OPA.

Instruments:

- Electronics Balance-Denver
- p^H 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 absorbance's of Fluticasone & Vilanterol solutions.

Methods:

Diluent: Diluent, methanol and water in the 50:50:50 ratio were chosen based on the solubility of the products.

0.01N KH_2PO_4 Buffer: Precisely weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml volumetric flask add about 900ml of added milli-Q water and degas to sonicate and eventually make up the volume with water then PH balanced to 5.4 with dil. Solution of an orthophosphoric acid.

Preparation of Standard stock solutions: Accurately Weighed and transferred 25mg&6.25mg of Fluticasone and Vilanterol working Standards into a 25ml clean dry volumetric flask, add 25ml of diluent, sonicate 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 pipette out and taken into a 10ml volumetric flask and made up with diluents. (100µg/ml of Fluticasone and 25µg/ml of Vilanterol)

Preparation of Sample solutions: The contents of nasal spray delivered 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 pipette 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 254mg&6.25mg of Fluticasone 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 pipetted 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 Fluticasone 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) following stock solutions were prepared.

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 pipette 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 pipette 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 and 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 studies:

To 1 ml of stock solution of Fluticasone & Vilanterol, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60°C. 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 & Vilanterol, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60ºC. The resultant solution was diluted to be 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 & Vilanterol, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60°C. 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/mlFluticasone&250µg/ml Vilanterol solution to UV Light by keeping the beaker in UV Chamber for 1day or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was

RESULTS AND DISCUSSION:

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 60ºC. 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.

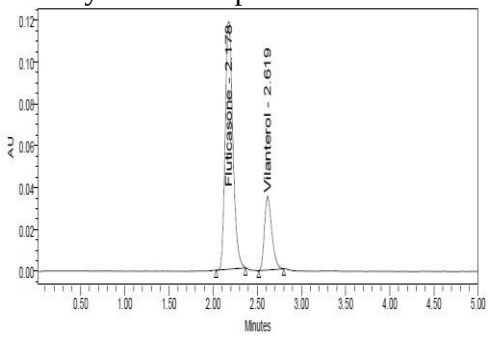


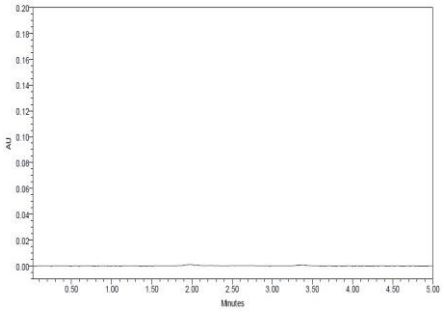
Figure 1: System suitability Chromatogram

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. Retention times of Fluticasone & Vilanterol were 2.184 min and 2.623 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 be specific. Six linear concentrations of Fluticasone (25-150µg/ml) and Vilanterol (6.2537.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Fluticasone was y = 7267x + 11198 and of Vilanterol was y y = 8322.x + 3567. Correlation coefficient obtained was 0.999 for the two drugs.

Table: 1 System suitability parameters for Fluticasone & Vilanterol

S no	Fluticasone			Vilanterol			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.165	3099	1.21	2.603	4556	1.32	2.8
2	2.177	3064	1.21	2.618	4632	1.26	2.8
3	2.178	3098	1.21	2.619	4572	1.26	2.8
4	2.184	3150	1.25	2.623	4678	1.37	2.7
5	2.190	3106	1.24	2.634	4653	1.26	2.8
6	2.192	3090	1.25	2.636	4637	1.27	2.8



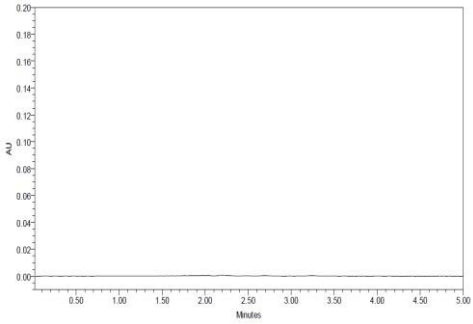


Figure 2: Specificity chromatograms of Blank and Placebo

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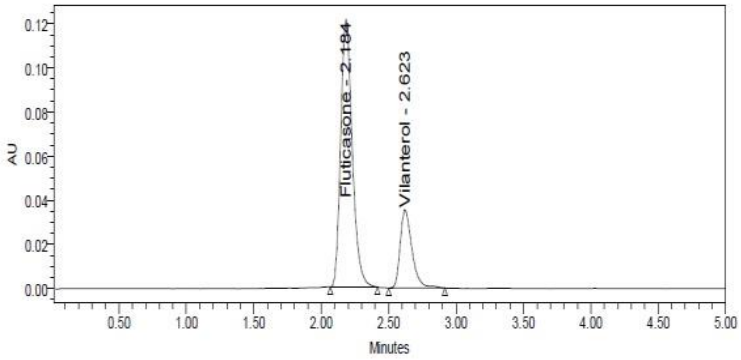

Figure 3:Chromatogram of Fluticasone and Vilanterol

Table 2: Linearity table of Fluticasone and Vilanterol

Fluticasone		Vilanterol	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
25	201382	6.25	57460
50	375364	12.5	106830
75	572898	18.75	163139
100	723072	25	213560
125	920568	31.25	264248
150	1100269	37.5	312027

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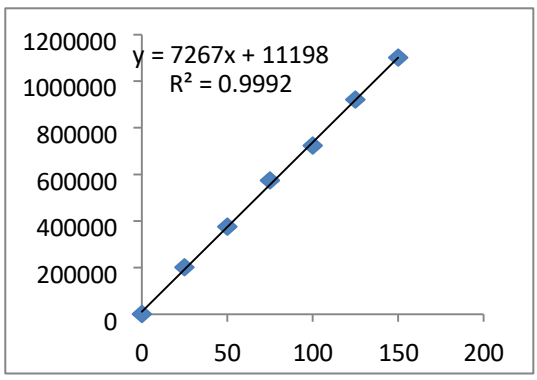


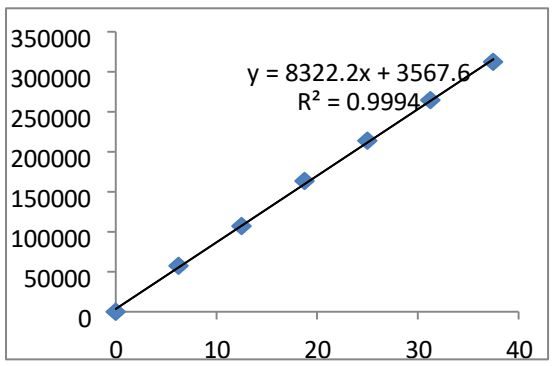


Figure 4:Calibration curve of Fluticasone **Figure 5: Calibration curve of Vilanterol**

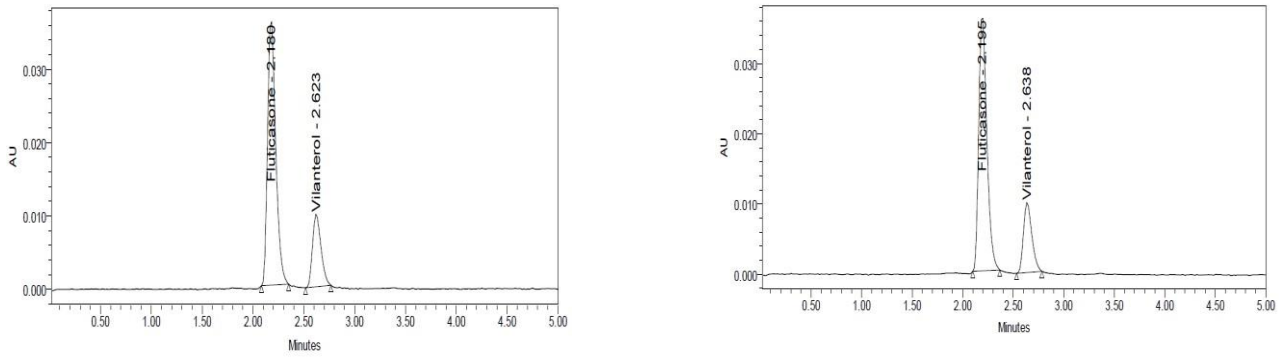


Figure 6:Linearity 25% Chromatogram of Fluticasone & Vilanterol

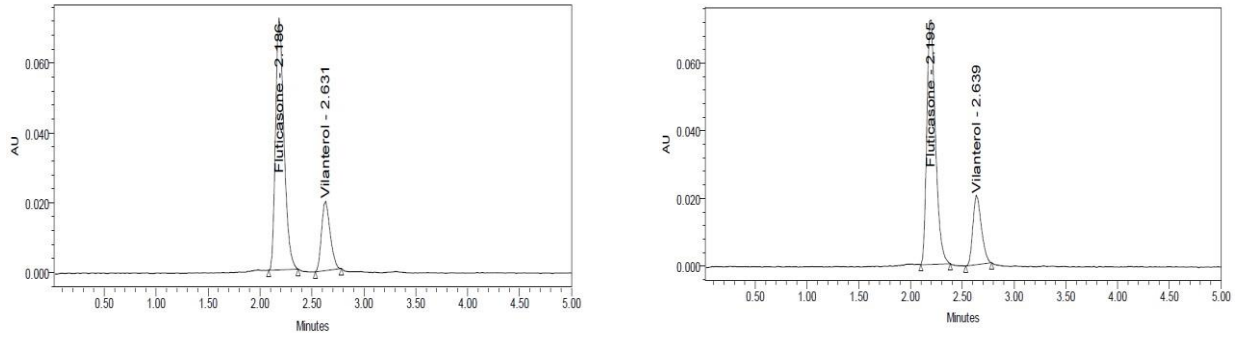


Figure 7: Linearity 50% Chromatogram of Fluticasone & Vilanterol

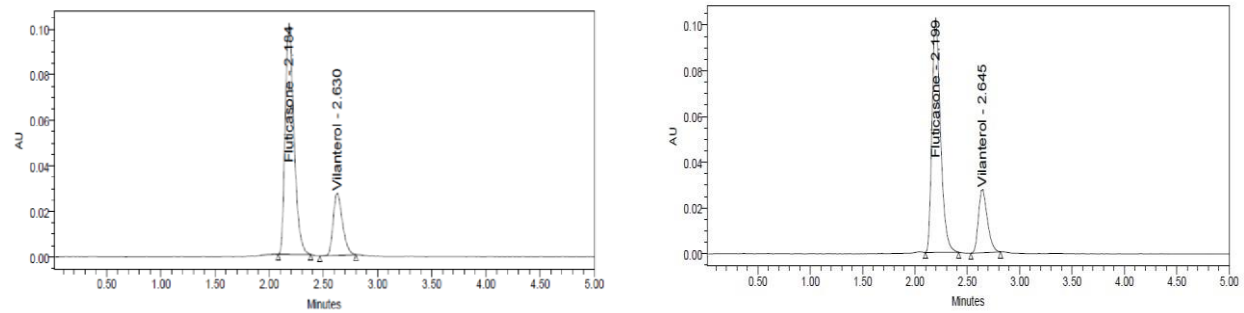


Figure 8: Linearity 75% Chromatogram of Fluticasone & Vilanterol

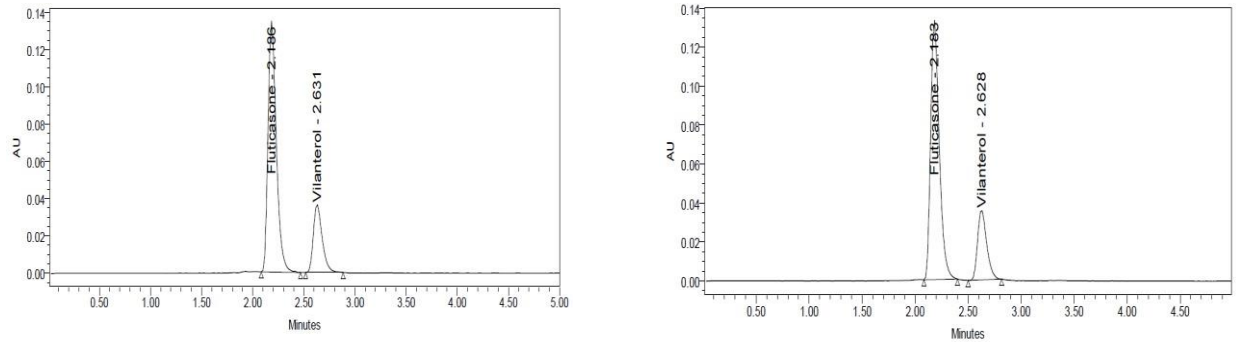


Figure 9: Linearity 100% Chromatogram of Fluticasone & Vilanterol

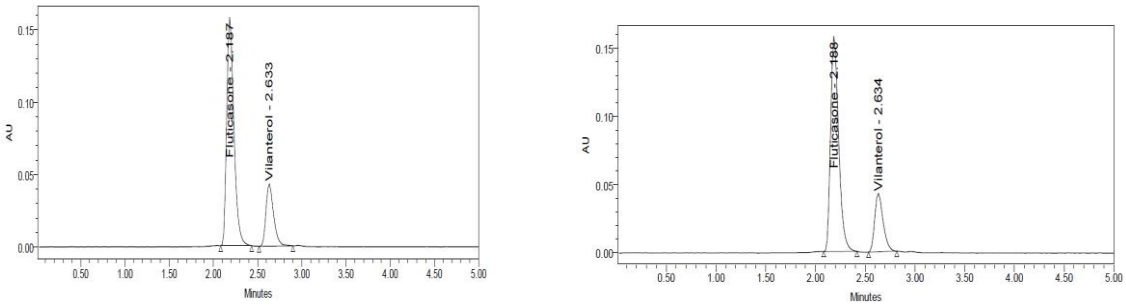


Figure 10: Linearity 125% Chromatogram of Fluticasone & Vilanterol

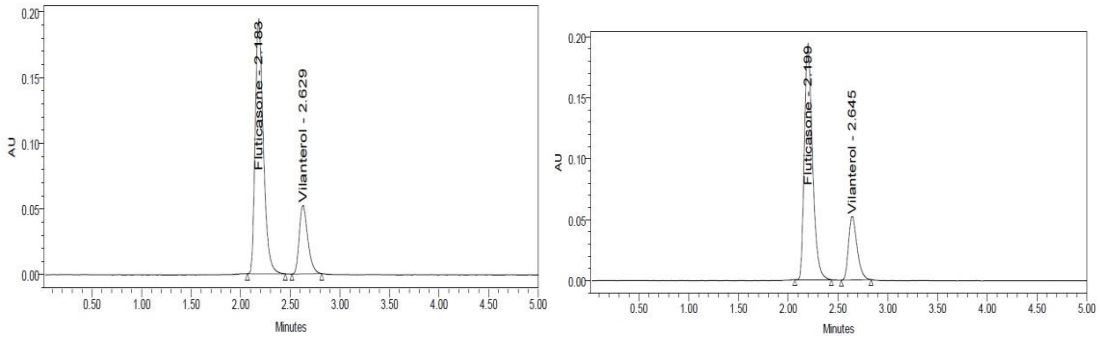


Fig No. 11: Linearity 150% Chromatogram of Fluticasone & Vilanterol

Table No 3: System precision table of Fluticasone & Vilanterol

S. No	Area of Fluticasone	Area of Vilanterol
1.	718892	203595
2.	726046	204308
3.	713165	203084
4.	721349	204166
5.	728513	204279
6.	723723	203937
Mean	721948	203895
S.D	5475.1	477.7
%RSD	0.8	0.2

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.8 and 0.2% respectively for Fluticasone & Vilanterol .As the limit of Precision was less than "2" the system precision was passed in this method.

Table No 4:Repeatability table of Fluticasone & Vilanterol

S. No	Area of Fluticasone	Area of Vilanterol
1.	722664	202390
2.	725936	203648
3.	725290	203202
4.	728674	202965

5.	721229	206041
6.	714478	202197
Mean	723045	203407
S.D	4938.0	1273.6
%RSD	0.6829	0.6858

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% and 0.7% respectively for Fluticasone & Vilanterol. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy:

Table 5: Accuracy table of Fluticasone

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	50	50.09	100.18	99.73%
	50	50.24	100.49	
	50	50.09	100.18	
100%	100	100.39	100.39	
	100	99.01	99.01	
	100	98.22	98.22	
150%	150	149.69	99.79	
	150	149.75	99.84	
	150	149.17	99.45	

Table 6:Accuracy table of Vilanterol

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.51	100.12	99.68%
	12.5	12.48	99.81	
	12.5	12.47	99.80	
100%	25	24.94	99.77	
	25	25.15	100.60	
	25	24.90	99.59	
150%	37.5	37.79	100.78	
	37.5	37.15	99.08	
	37.5	37.28	99.40	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.73% and 99.68.

Table 7: Sensitivity table of Fluticasone & Vilanterol

Molecule	LOD	LOQ
Fluticasone	0.87	2.64
Vilanterol	0.23	0.70

Robustness:

Table 8:Robustness data for Fluticasone &Vilanterol.

S.no	Condition	%RSD of Fluticasone	%RSD of Vilanterol
1	Flow rate (-) 09ml/min	0.8	0.5
2	Flow rate (+) 1.1ml/min	1.3	1.4
3	Mobile phase (-) 55B:45A	1.1	0.6
4	Mobile phase (+) 45B:55A	1.2	1.7
5	Temperature (-) 25°C	0.6	0.2
6	Temperature (+) 35°C	0.6	0.6

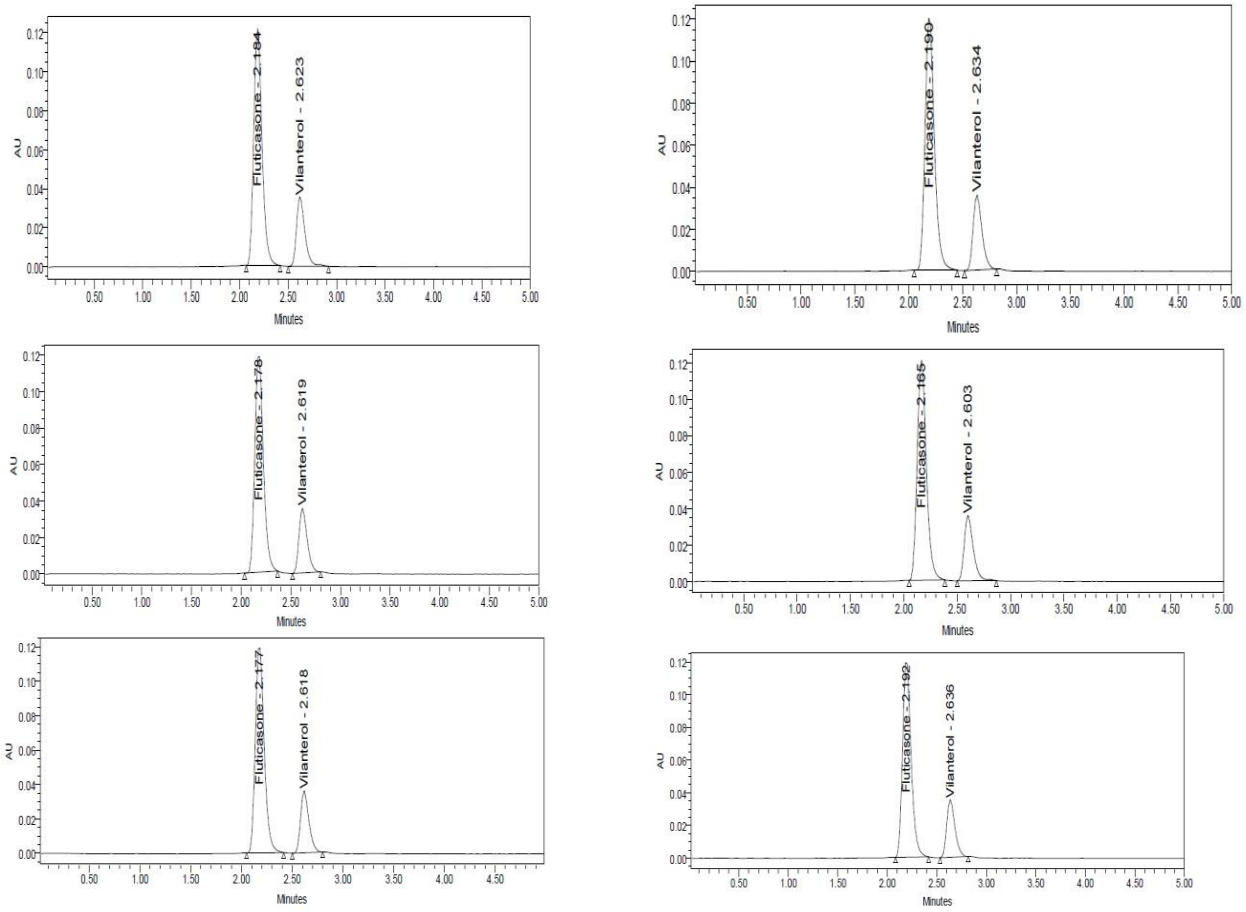
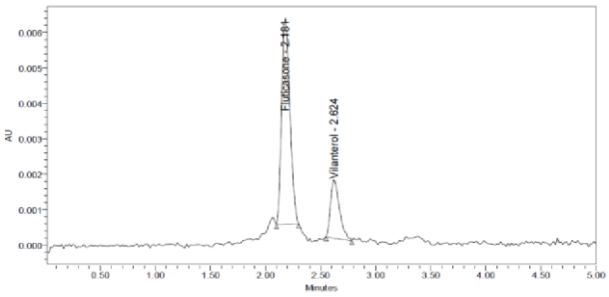


Fig No. 12: Repeatability chromatogram



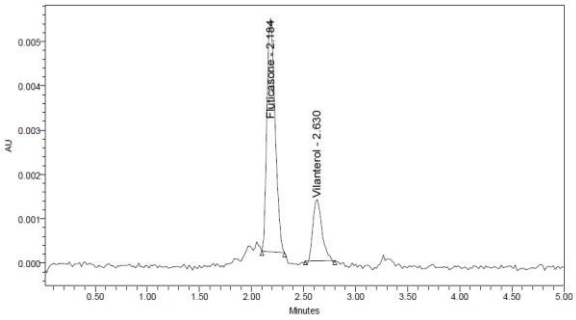


Figure 13: LOD Chromatogram of StandardFigure 14: LOQ Chromatogram of Standard

Table 9:Assay Data of Fluticasone

S.no	Standard Area	Sample area	% Assay
1	718892	722664	100.00
2	726046	725936	100.45
3	713165	725290	100.36
4	721349	728674	100.83
5	728513	721229	99.80
6	723723	714478	98.87
Avg	721948	723045	100.05
SD	5475.1	4938.0	0.68
%RSD	0.8	0.7	0.68

Table 10:Assay Data of Vilanterol

S.no	Standard Area	Sample area	% Assay
1	203595	202390	99.16
2	204308	203648	99.78
3	203084	203202	99.56
4	204166	202965	99.44
5	204279	206041	100.95
6	203937	202197	99.07
Avg	135801	203407	99.66
SD	477.7	1395.1	0.68
%RSD	0.4	0.7	0.7

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45M), mobile phase plus (45B:55M), 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.

Assay: Breo Ellipta,bearing the label claim Fluticasone 100mcg, Vilanterol 25mcg. Assay performed with above formulation. Average % Assay for Fluticasone & Vilanterol obtained was 100.05% & 99.66% respectively

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 11 Degradation Data of Fluticasone

S.NO	Degradation Condition	Area	% Recovery	% Drug Degraded
1	**Acid**	673862	93.25	6.75
2	**Alkali**	685030	94.79	5.21
3	**Oxidation**	693486	95.96	4.04
4	**Thermal**	702959	97.27	2.73
5	**UV**	713467	98.73	1.27
6	**Water**	720832	98.73	1.27

Table `12: Degradation Data of Vilanterol

S.NO	Degradation Condition	Area	% Recovery	% Drug Degraded
1	**Acid**	187581	91.91	8.09
2	**Alkali**	189481	92.84	7.16
3	**Oxidation**	190855	93.51	6.49
4	**Thermal**	193471	94.79	5.21
5	**UV**	195435	95.76	4.24
6	**Water**	203210	99.56	0.44

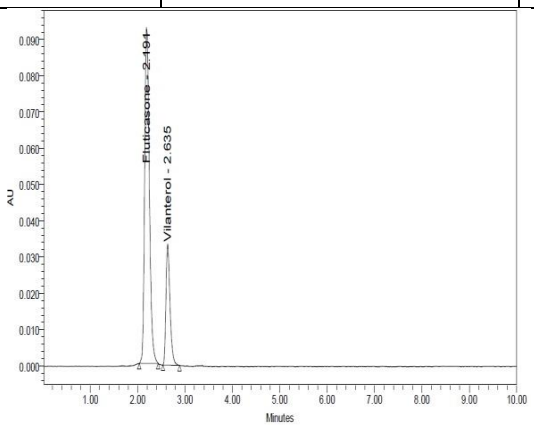


Figure 15: Acid chromatogram of Fluticasone & Vilanterol

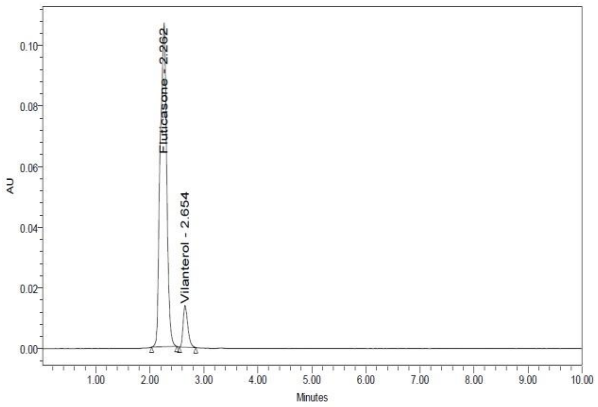


Figure 16:Peroxide chromatogram of Fluticasone & Vilanterol

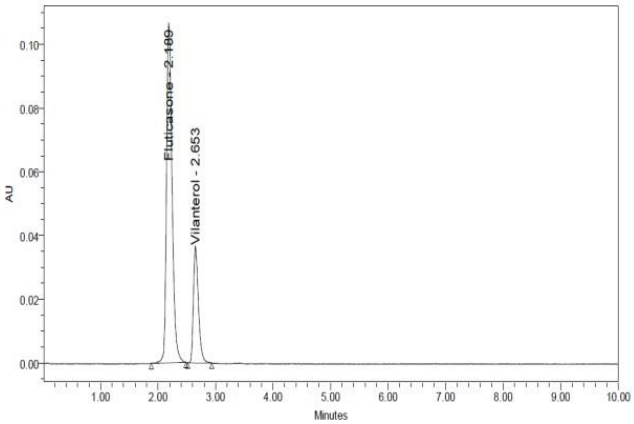


Figure 17: Base chromatogram of Fluticasone & Vilanterol

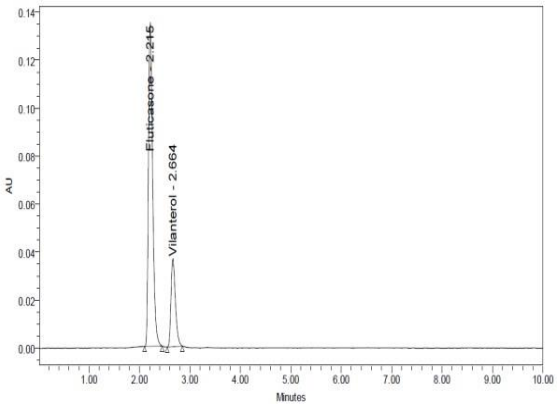


Figure 18: Thermal chromatogram of Fluticasone & Vilanterol

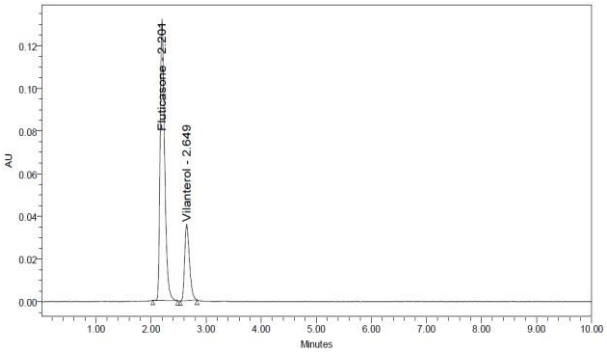


Figure 19: UV chromatogram of Fluticasone & Vilanterol

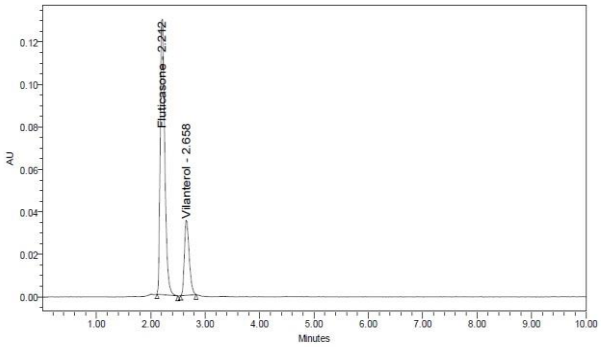


Figure 20:Water chromatogramof Fluticasone & Vilanterol

Discussion: Regarding the pHadjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time.

SUMMARY AND CONCLUSION

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)		7267	8322	
Intercept(c)		11198	3567	
Regression equation (Y=mx+c)		y = 7267x + 11198	y = 8322.x + 3567	
Assay (% mean assay)		100.05%	99.66%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.8	0.2	NMT 2.0%
Method precision %RSD		0.7	0.7	NMT 2.0%
Accuracy %recovery		99.73%	99.88%	98-102%
LOD		0.87	0.23	NMT 3
LOQ		2.64	0.70	NMT 10
Robustness	**FM**	0.8	0.5	%RSD NMT 2.0
	FP	1.3	1.4	
	MM	1.1	0.6	
	MP	1.2	1.7	
	TM	0.6	0.2	
	TP	0.6	0.6	

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Fluticasone & Vilanterol in Tablet dosage form. Retention time of Fluticasone & Vilanterol were found to be 2.184 min and 2.623 min.. %RSD of the Vilanterol and Fluticasone were and found to be 0.2 and 0.8 respectively. %Recovery was obtained as 99.88% and 99.73% for Vilanterol and Fluticasone respectively. LOD, LOQ values obtained from regression equations of Vilanterol and Fluticasone were 0.23, 0.70 and 0.87, 2.64 respectively. Regression equation of Fluticasone is y = 8322.x + 3567. y = 7267x + 11198 of Vilanterol.

Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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