"DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF FLUTICASONE FUROATE AND VILANTEROL IN PHARMACEUTICAL FORMULATIONS"

[1]Ashwini R. Pawar, [2]Sagar S. kale, [3]Sanket M. Patil, [4]Ranjeet P. Vibhute, [5]Kaufiya D. Sayyad

[1,3,4,5]M. pharmacy, [2]Assistant professor
Department of pharmaceutical chemistry
Sahyadri College Of pharmacy,
Methwade (Sangola), Solapur, Maharashtra, India

***Abstract*- The present study describes a new, simple, precise, accurate, reproducible, and efficient UV spectroscopic method that was developed and validated for simultaneous estimation of fluticasone furoate and Vilanterol in pure and pharmaceutical dosage form. The absorbance of fluticasone furoate and Vilanterol was found to be 242nm and 259nm, respectively. Calibration curves of fluticasone furoate and Vilanterol were found to be linear in the concentration ranges of 8-12µg/mL and 2-3µg/mL with their correlation coefficient values (R2) 0.9987 and 0.9992, respectively. LOD and LOQ were found to be 0.68µg/mL and 2.07µg/mL for Fluticasone Furoate and 0.13µg/mL and 0.40µg/mL for Vilanterol, respectively. In the precision study, the % RSD value was found within limits (RSD < 2%). The percentage recovery at various concentration levels varied from 99.73 to 100.60% for Fluticasone Furoate and 101.80 to 100.66% for Vilanterol, respectively. The proposed method can be applied successfully for the simultaneous estimation of fluticasone furoate and Vilanterol in pure and pharmaceutical dosage form. In this method simultaneous equation method was applied to find assay of both drugs in pharmaceutical dosage form. The methods were validated in accordance with ICH Q2 guidelines.[1]**

***Keywords*: Fluticasone furoate, Vilanterol, UV-visible Spectrometry.**

I.INTRODUCTION:

A series of lung conditions known as COPD (chronic obstructive pulmonary disease) make it difficult to breathe and progressively worsen over time [2].It is a preventable and treatable disease characterized by persistent respiratory symptoms and restricted airflow[3].Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide, causing 3.23 million deaths in 2019. Nearly 90% of COPD deaths in those under 70 years of age occur in low- and middle-income countries (LMIC).COPD is the seventh leading cause of poor health worldwide (measured by disability-adjusted life years)[4].A combination dosage form of fluticasone furoate and vilanterol, sold under the trade names BreoEllipta was approved by the USFDA in 2013 and is used to treat COPD, which includes chronic bronchitis, emphysema, and asthma. FFE is a synthetic trifluorinated corticosteroid with strong anti-inflammatory properties that is used to treat airflow obstruction in COPD patients with chronic bronchitis and emphysema over the long term. It is also licensed for the treatment of asthma and the symptoms of nasal allergies, such as runny nose, congestion, and sneezing. It functions by lessening inflammatory responses to allergens and irritants in the air that occur in the nasal airway. Asthma and COPD are treated with VTL, a selective long-acting beta2-adrenergic agonist, once per day [5].

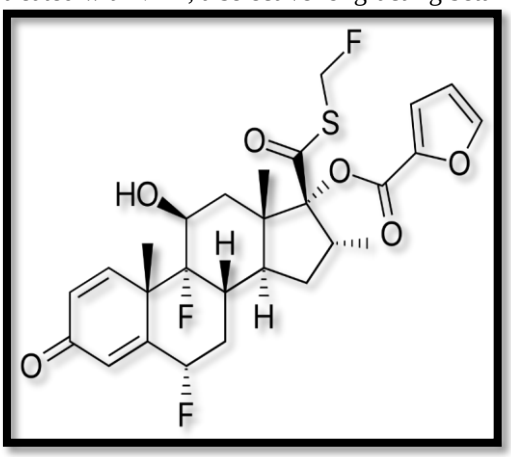


Fig 1: Chemical Structure of Fluticasone Furoate

Depending on the product, fluticasone furoate, an inhaled corticosteroid, can be used as a maintenance medication for asthma and/or chronic obstructive pulmonary disease (COPD). Additionally offered as a nasal spray inhaler to treat allergic rhinitis symptoms.For

a variety of inflammatory conditions, fluticasone furoate, a synthetic glucocorticoid.It was initially authorized in 2007. For the management of chronic obstructive pulmonary disease (COPD) and the treatment of asthma in patients under the age of 18, fluticasone furoate is available in two combination medications: one vilanterol-only and the other vilanterol and umeclidinium-only.For the symptomatic management of high fever and other upper respiratory allergens in patients under the age of two, fluticasone furoate is sold over-the-counter as a nasal spray. Fluticasone furoate stimulates glucocorticoid receptors systemically, inhibits nuclear factor kappa b, and prevents pulmonary eosinophilia in rats, according to in vitro research. Fluticasone furoate affects the activity of several cell types and inflammatory mediators via an unidentified method.

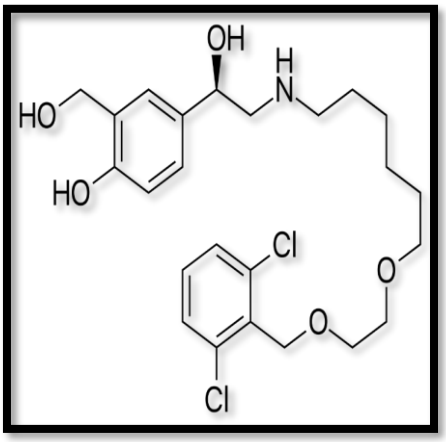


Fig 2: Chemical Structure of Vilanterol

Vilanterol is a long-acting Beta2-adrenergic agonist that is used in conjunction with other bronchodilators to treat COPD, which includes chronic bronchitis and/or emphysema. The stimulation of intracellular adenylyl cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3',5'-adenosine monophosphate (cAMP), is responsible for its pharmacological activity. Increases in cyclic AMP are linked to bronchial smooth muscle relaxation and the suppression of mast cell production of hypersensitivity mediators in the lungs[6].

II.MATERI L AND METHOD:

Instrument:

A Shimadzu1800UV/VIS double beam spectrophotometer with 1 cm matched quartz cells was used for different derivative spectral measurements. The UV spectra were recorded over the wavelength of 200-400nm. All the drugs and chemicals were weighed on Digital electronic balance citizen & Contact (CY220 &CY223) metter torledo model weighing balance.A gift sample of analytically pure Vilanterol and Fluticasone Furoate C was received from Aadhar Life Science Pvt Ltd. M. I. D. C, Solapur was used in the study. The solvent used was Methanol and Distilled water was used in the preparation of the mobile phase.

Chemicals and Reagents:

A gift sample of analytically pure Fluticasone Furoate and Vilanterol was received from Aadhar Life Science Pvt Ltd. M. I. D. C, Solapur was used in the study. The solvent Acetonitrile and Distilled water was used in the preparationof the mobilephase.

Selectionof Wavelength: Sample was scanned from 190-400 nm with UV Spectrophotometer. The Wavelength selected for simultaneous analysis of Fluticasone Furoate chosen was 242 nm and for Vilanterol chosen was 259 nm.

The sensitivity of UV spectrophotometric method depends on proper selection of wavelength.

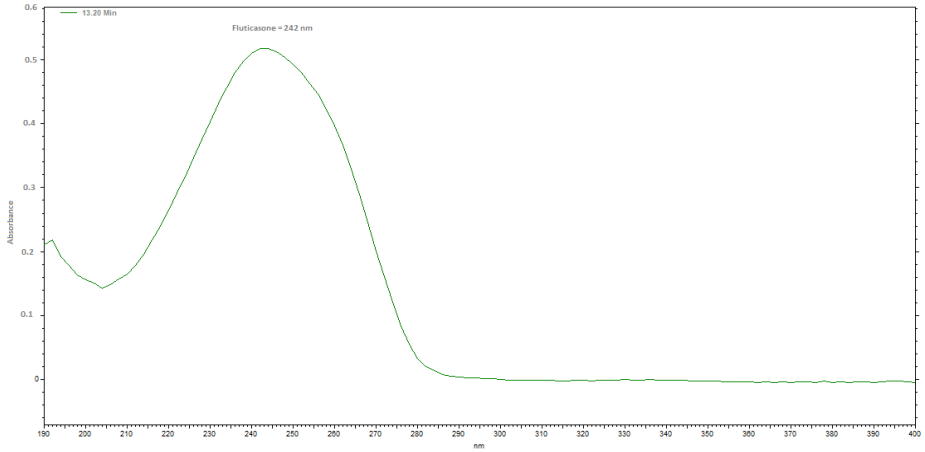


Fig 3:Individual spectra of Fluticasone furoate

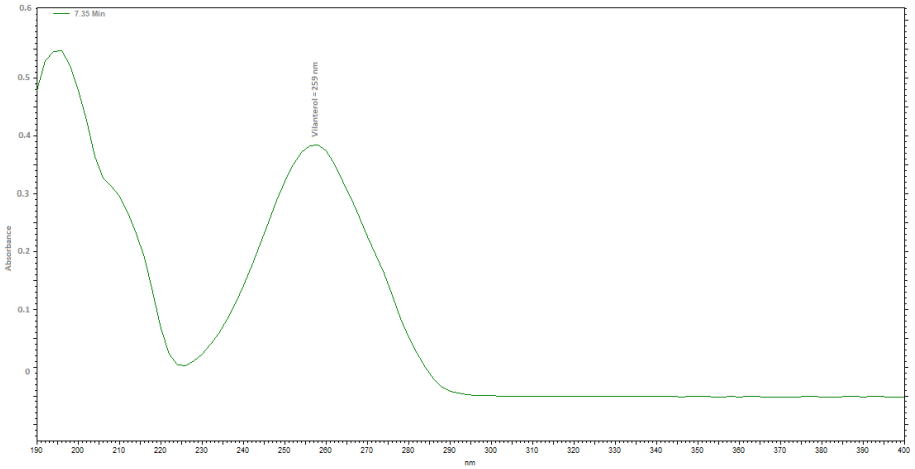


Fig 4: Individual spectra of Vilanterol

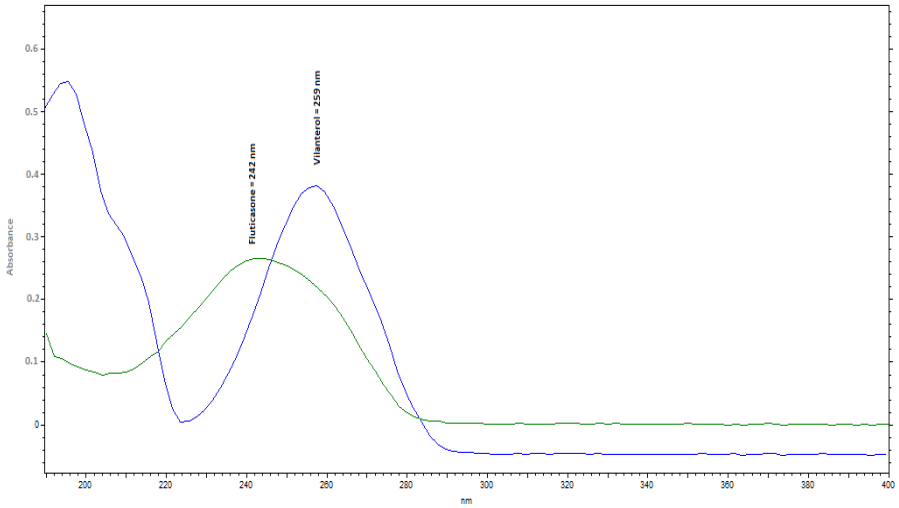


Fig 5: Isosbestic point of fluticasone furoate and vilanterol

- Isosbestic point of fluticasone furoate and vilanterol is observed at 246nm

tandard Preparation:

Preparation of Fluticasone furaote Standard Stock Solution-I (FSSS-I):

Initially Prepare a Standard Stock Solution (FSSS-I) of by adding 10 mg of Fluticasone furaote in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Fluticasone furaote = 1000 µg/ml).

Preparation of Vilanterol Standard Stock Solution-I (VSSS-I):

Then prepare a Standard Stock Solution (VSSS-I) of Vilanterol by adding 2.5 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent.(Conc. of Vilanterol = 250 µg/ml).

Then add 1.0 ml of FSSS-I & 1.0 ml VSSS-I in 100 ml volumetric flask and add 50 ml diluent and vortex and make up the volume with diluent. (Conc. of Fluticasone furaote =10 µg/ml & Vilanterol = 2.5 µg/ml).

Analysis Of Marketed Formulation

- 10 Capsules content were weighed and calculate average weight of 1 capsule content.
- Powder weight equivalent to 100 µg Fluticasone furaote and 25 µg of Vilanterol was weighed into 10 ml volumetric flask & add 5 ml diluent, Sonicate for 5 minutes and make the volume to 10 ml with diluent. (Conc. of Fluticasone furaote = 10 µg/ml and Vilanterol = 2.5 µg/ml)

Table.1 Analysis of marketed formulation

	Fluticasone Furoate			**Vilanterol**		
Sr No	**Absorbance**	**Amount Recovered in µg/ml**	**% Recovery**	**Absorbance**	**Amount Recovered in µg/ml**	**% Recovery**
1	0.529	10.02	99.97	0.329	2.51	100.36

2	0.527	9.98	100.25	0.328	2.50	100.05
3	0.529	10.02	99.87	0.327	2.49	99.75
Average	0.528	10.00	100.03	0.328	2.5	99.38
STDEV	0.001	0.021	0.218	0.001	0.007	0.305
RSD	0.189	0.21	0.217	0.304	0.28	0.306

Assay: Fluticasone & Vilanterol Working Standard of 10 µg/ml & 2.5 µg/ml were prepared and Capsule Sample was prepared and assay was calculated using following formula.

Formula: $C = A1\ ay2 - A2\ ay1/\ ax1ay2 - ax2ay1 \ldots\ldots Eq\ 1$

$Cy = A1\ ax2 - A2\ ax1/\ ay1ax2 - ay2ax1 \ldots\ldots Eq2$

Where,

A1= Absorbance of formulation at 242 nm

A2 = Absorbance of formulation at 259 nm

ax1 & ax2 = Absorptivity of Fluticasone at 242 nm & 259 nm

ay1 & ay2 = Absorptivity of at Vilanterol 242 nm & 259 nm

Cx = Concentration of Fluticasone

Cy = Concentration of Vilanterol

III.METHOD VALIDATION:

Method validation is a process used to establish that a particular analytical method is suitable for its intended purpose. Method validation is the process of evaluating and confirming the reliability, accuracy, and suitability of an analytical or measurement method.

Linearity-

Linearity was studied by plotting a graph of absorbance directly proportional to the concentration.A series of standard solutions of Fluticasone Furoate concentration range is 8µg/ml to 12 µg/ml and Vilanterol waspreparedintheconcentration range of about 2 µg/ml to 3 µg/ml is shown in below tables(1) & (2). The absorbance values for Fluticasone Furoate and Vilanterol were measured at respective wavelength for each drug separately.

Table.2 Linearity study of Fluticasone Furoate

Fluticasone Furoate		
% Level	**Conc (ug/ml)**	**Absorbance at 242 nm**
80	8	0.421
90	9	0.479
100	10	0.529
110	11	0.589
120	12	0.636

Figure No. 4:- Linearity Graph of Fluticasone Furoate

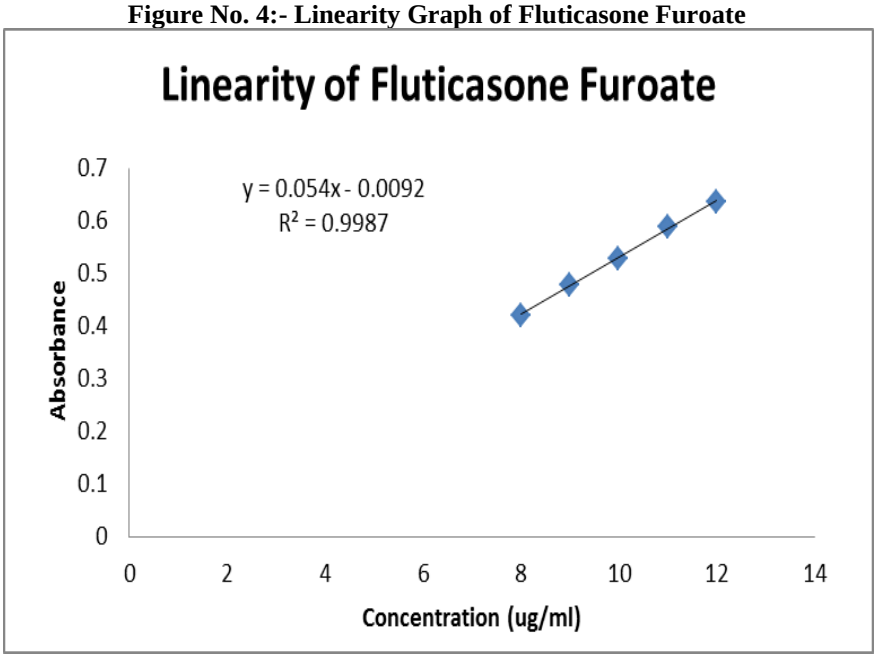
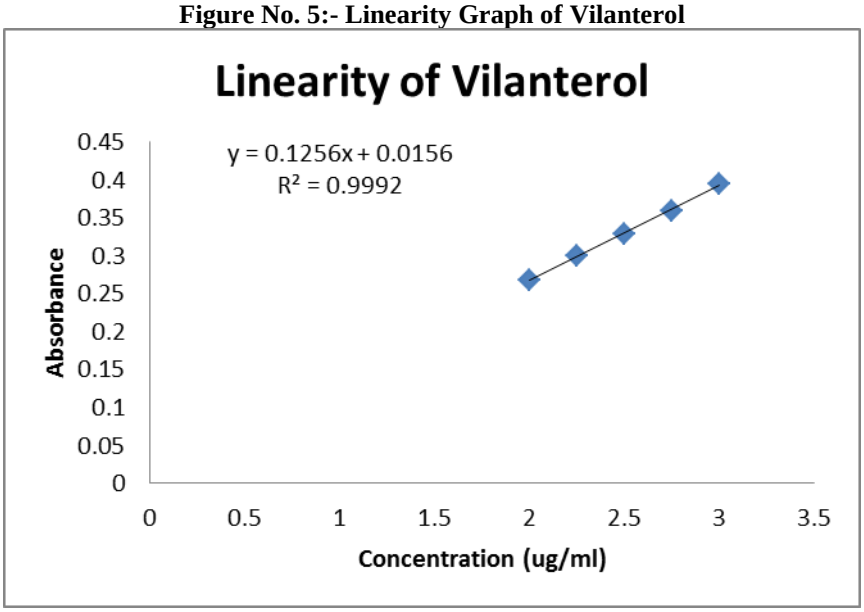


Table.3 Linearity study of Vilanterol

Vilanterol		
% Level	**Conc (ug/ml)**	**Absorbance at 259 nm**
80	2	0.267
90	2.25	0.299
100	2.5	0.329
110	2.75	0.359
120	3	0.394

Figure No. 5:- Linearity Graph of Vilanterol



LOD/ LOQ-

The lowest concentration of analyte that can be reliably detected and quantified by the method is determined.It was calculated for both drugs by using ANOVA technique.

Formula:

$$LOD = \frac{3.3 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

$$LOQ = \frac{10 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

Table. 4 Result of LOD and LOQ

Sr no	**Nameofdrugs**	**LOD (µg/ml)**	**LOQ(µg/ml)**
1	Fluticasone Furoate	0.68	2.07
2	Vilanterol	0.13	0.40

Repeatability-

Table. 5 Repeatability study of Vilanterol and Fluticasone furoate

	Vilanterol	**Fluticasone Furoate**
	Absorbance at 259 nm	**Absorbance at 242 nm**
Rep 1	0.329	0.529
Rep 2	0.328	0.527
Rep 3	0.327	0.529
Rep 4	0.326	0.528
Rep 5	0.328	0.526
Rep 6	0.329	0.527
Average	0.328	0.528
STDEV	0.001169045	0.00121106
RSD	0.36	0.23

Accuracy-

The closeness of the measured values to the true values is determined by comparing the results with a reference or known values.

Table. 6 Recovery Study of Fluticasone Furoate

Sample ID	**Reps**	**Spiked Conc. (ug/ml)**	**Absorbance at 242 nm**	**Amt Recovered (ug/ml)**	**% Recovery**	**Average**	**STDEV**	**RSD**
80%	Rep 1	8.00	0.421	7.98	99.73	99.97	0.236892	0.24
	Rep 2	8.00	0.423	8.01	100.21			
	Rep 3	8.00	0.422	8.00	99.97			
100%	Rep 1	10.00	0.529	10.02	100.25	100.13	0.218831	0.22
	Rep 2	10.00	0.527	9.98	99.87			
	Rep 3	10.00	0.529	10.02	100.25			
120%	Rep 1	12.00	0.636	12.05	100.44	100.44	0.157928	0.16
	Rep 2	12.00	0.635	12.03	100.28			
	Rep 3	12.00	0.637	12.07	100.60			

Table.7 Recovery Study of Vilanterol

Sample ID	**Reps**	**Spiked Conc. (ug/ml)**	**Absorbance at 259 nm**	**Amt Recovered (ug/ml)**	**% Recovery**	**Average**	**STDEV**	**RSD**
80%	Rep 1	2.00	0.267	2.04	101.80	101.17	0.582432	0.58
	Rep 2	2.00	0.264	2.01	100.66			

	Rep 3	2.00	0.265	2.02	101.04			
100%	Rep 1	2.50	0.329	2.51	100.36	100.05	0.305033	0.30
	Rep 2	2.50	0.328	2.50	100.05			
	Rep 3	2.50	0.327	2.49	99.75			
120%	Rep 1	3.00	0.394	3.00	100.15	100.24	0.388288	0.39
	Rep 2	3.00	0.393	3.00	99.90			
	Rep 3	3.00	0.396	3.02	100.66			

Precision-The repeatability and reproducibility of the method are assessed by performing multiple measurements on the same sample or under different conditions.

Table No.8:- Intra-day precision of Fluticasone Furoate

Fluticasone Furoate						
Conc(**µg/ml**)	**Absorbance**			**Avg**	**STDEV**	**RSD**
	Trial 1	**Trial 2**	**Trial 3**			
8.00	0.423	0.433	0.432	0.429	0.005	1.282
10.00	0.527	0.52	0.511	0.519	0.008	1.544
12.00	0.635	0.625	0.632	0.630	0.005	0.813

Table No. 9:-Inter-day precision of Fluticasone Furoate

Fluticasone Furoate						
Conc(**µg/ml**)	**Absorbance**			**Avg**	**STDEV**	**RSD**
	Trial 1	**Trial 2**	**Trial 3**			
8.00	0.421	0.418	0.428	0.422	0.005	1.215
10.00	0.525	0.529	0.533	0.529	0.004	0.756
12.00	0.636	0.63	0.64	0.635	0.005	0.792

Table No.10:-Intra-day precision of Vilanterol

Vilanterol						
Conc(**µg/ml**)	**Absorbance**			**Avg**	**STDEV**	**RSD**
	Trial 1	**Trial 2**	**Trial 3**			
2.00	0.268	0.27	0.278	0.272	0.005	1.945
2.50	0.327	0.32	0.33	0.325	0.005	1.575
3.00	0.323	0.32	0.319	0.320	0.002	0.649

Table No.11:-Inter-day precision of Vilanterol

Vilanterol						
Conc(**µg/ml**)	**Absorbance**			**Avg**	**STDEV**	**RSD**
	Trial 1	**Trial 2**	**Trial 3**			
2.00	0.254	0.258	0.264	0.258	0.005	1.945
2.50	0.323	0.318	0.329	0.323	0.005	1.703
3.00	0.358	0.365	0.363	0.362	0.003	0.996

Robustness-

The method's ability to remain unaffected by small, deliberate variations in method parameters, such as temperature, pH, or sample preparation, is evaluated.

Table.12 Robustness study Vilanterol and Fluticasone furoate

Diluent ratio			
Condition	**Sample**	**Vilanterol**	**Fluticasone Furoate**
		Assay	**Assay**
52A-48W	**DP**	99.48	99.62

50A-50W	**DP**	99.53	99.64
48A-52W	**DP**	99.56	99.57
	Average	99.52	99.61
	STDEV	0.0404145	0.036055513
	RSD	0.040	0.036

IV.RESULT AND DISCUSSION:

The proposed method is based on spectrophotometric simultaneous estimation of Fluticasone Furoate and Vilanterol in this method Acetonitrile and distilled water is used as solvent.

Linearity

Linear regression data for the calibration plots revealed good linear relationship between absorbance and concentration over the ranges 8µg/ml to 12 µg/ml of Fluticasone Furoate and 2µg/ml to 3µg/ml of Vilanterol . The linear equation for the calibration plots were y = 0.054x -0.0092 and y = 0.1256x +0.0156with Regression(R2) being 0.9987 and 0.9992 for Fluticasone Furoate and Vilanterol, respectively.(Figure4 and 5) (Table 2 and 3)

Accuracy

When the method was used for accuracy and subsequent analysis of both the drugs from the pharmaceutical dosage form and spiked with 80,100, 120% of additional pure drug, the recovery was found to be99.97% and 100.44% for Fluticasone furoate and 101.17% and 100.24% for
Vilanterol.(Table No.6 and 7)

LOD and LOQ

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 0.68µg/ml and 2.07 µg/ml for Fluticasone Furoate and 0.13 µg/ml and 0.40 µg/ml for Vilanterol.(Table No.4)

5. CONCLUSION

The proposed method was developed for the determination of of Fluticasone Furoate and Vilanterol in the presence of each other.Methods was validated and found to be simple,rapid, sensitive, accurate and precise. The shortchromatographic time makes this method suitable for processing of multiple samples in short time.The method shows no interference by the excipients. This method can be useful and suitable for the estimation of of Fluticasone Furoate and Vilanterol and pharmaceutical dosage form.

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