Advances in Bioresearch
Adv. Biores., Vol 17 (3) March 2026: 01-12

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.17.2.112

Advances in Bioresearch

ORIGINAL ARTICLE

Development & Validation of New Analytical Methods for Fluticasone Furoate and Vilanterol Trifenatate by RP-HPLC

[1]Kautik Dasharath Shirole*, [1]Ravindra Banilal Patil
[1]DCS's A. R. A. College of Pharmacy, Nagaon, Maharashtra, India
*Corresponding author

ABSTRACT
To develop and validate a simple, rapid, and selective RP-HPLC analytical method for the quantitative estimation of Fluticasone and Vilanterol Furoate applicable to both pure API and the marketed Vilor-F 100 (Fluticasone Furoate 100 mcg and Vilanterol 25 mcg Powder for Inhalation) formulation. Chromatographic separation was achieved using a methanol–water mobile phase adjusted to pH 3.0, with UV detection at 225 nm. Method validation included linearity, precision, accuracy, sensitivity, selectivity, recovery, system suitability, ruggedness following ICH recommendations. The developed method aimed to be a simple, sensitive, precise and accurate HPLC method for the simultaneous analysis of both FLU and VIL API and marketed formulation (Vilor F-100). The RP-HPLC method produced sharp, well-resolved peaks with consistent retention times. Excellent linearity was achieved through out the calibration range with acceptable correlation coefficients. Selectivity studies confirmed no placebo interference at the analyte retention time. Precision and accuracy values met acceptance criteria, with %RSD < 2% and recovery exceeding 99%. Ruggedness studies across different analysts and columns produced consistent results, confirming robustness. The validated RP-HPLC method is accurate, precise, sensitive, and robust for the quantitative estimation of Fluticasone and Vilanterol in both API and marketed formulation. Its specificity and reproducibility make it suitable for routine analysis.
Keywords; *Fluticasone Furoate, Vilanterol, RP-HPLC, Method Development, Method Validation, Pharmaceutical Analysis*

Received 12.02.2026 Revised 25.02.2026 Accepted 18.03.2026

How to cite this article:
Shirole K.D. & Patil R.B. Development & validation of new analytical methods for fluticasone furoate and vilanterol trifenatate by RP-HPLC. Adv. Biores., Vol 17 (3) March 2026: 01-12.

INTRODUCTION

The chemical name of vilanterol trifenatate (VIL), a LABA with the chemical name triphenylacetic acid-4-{(1R)-2-[(6-{2-[2,6-dicholorobenzyl)oxy]ethoxy}hexyl) amino]-1-hydroxyethyl}-2-(hydroxymethyl) phenol (1:1) and the following chemical structure as (figure 1) and fluticasone furoate (FLU) is a synthetic trifluorinated corticosteroid having the chemical name (6α,11β,16α,17α)-6,9-difluoro-17-{[(fluoro-methyl) thio]carbonyl}-11-hydroxy-16-methyl-3-oxoandrosta-1,4-dien-17-yl 2-furancarboxylate and the following chemical structure: (figure 2).

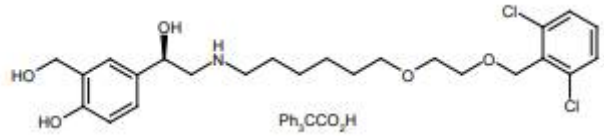

Figure 1: Vilanterol Trifenatate

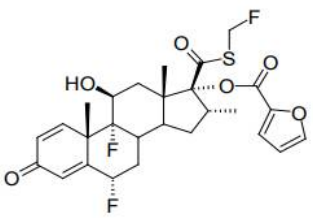

Figure 2: Fluticasone Furoate

Fixed dose combination of Fluticasone and VIL is approved by USFDA and DCGI. It is a prescription medicine used for maintenance and manage symptoms of asthma and chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. This combination is typically administered as a dry powder for inhalation once daily at the same time each day. [1]. Patients with COPD, particularly those with emphysema and chronic bronchitis, can benefit from long-term maintenance treatment by using Fluticasone Furoate, a synthetic trifluorinated corticosteroid with strong anti-inflammatory properties [2]. It is also approved for treatment of symptoms of nasal allergies, such as runny nose, congestion, itching, and sneezing [3,4]. Its mechanism of action is by suppressing the inflammatory responses that allergens and irritants in the air cause in the nasal passage. Once daily, VIL is a selective long-acting beta2-adrenergic agonist, which is used to treat asthma and COPD [5,6]. According to a review of the literature, a few high-performance liquid chromatographic methods have been established [7]. The aim of the present investigation is to develop an RP-HPLC method for the simultaneous estimation of FLP and VIL in pharmaceutical formulations, as there are limited analytical methods described for the analysis of these drugs [8,9].

MATERIALS AND METHODS

Chemicals and Reagents

FLU and VIL pure drug (API) was supplied as a gift sample by Glenmark Pharmaceuticals Ltd, Sinnar, Nashik, India. The marketed formulation, Vilor F 100 (FLU 100 mcg and VIL 25 mcg Powder for Inhalation), was sourced from the local market in Nashik through an authorized distributor. Orthophosphoric acid of analytical grade was obtained from S.D. Fine Chemicals Ltd., Mumbai, India. HPLC grade methanol and acetonitrile was purchased from Merck (India) Ltd., Mumbai, India. HPLC grade water was collected from Milli Q water purification system.

Method, Chromatography optimization

The chromatographic separation was achieved on RP-HPLC binary gradient system model HPLC 3000 Series from Analytical Technologies Ltd consisting of P-3000-M Reciprocating (40MPa) pump, UV detector and thermostat column compartment connected to HPLC solutions software.

A 20µL Rheodyne syringe was used for injecting the samples. Data was analyzed by using HPLC solutions software. Shimadzu Double beam -UV 1900I, UV-Visible spectrophotometer was used for spectral studies. Data was analyzed by using UV Probe software. Degassing of the mobile phase was done by using a Wenser Ultra Sonicator (WUC-4L). A Wenser High Precision Balance (model- PGB 100) was used for weighing the materials. The analysis of the drug was carried out on Cosmosil C18 column (250 mm x 4.6 mm ID; particle sizer 5 µm). UV spectra recorded between 190–400 nm identified 225 nm as the optimal detection wavelength for accurate and sensitive method development. A C18 column was used under isocratic conditions with a mobile phase consisting of methanol and water (pH 3.0, adjusted with ortho phosphoric acid) in a 70:30 v/v ratio was optimized to achieve clear separation of FLU and VIL API in bulk drug and dry powder formulation. The mobile phase was filtered through a 0.45 µm membrane and degassed prior to use. The analysis was carried out at a flow rate of 1.0 mL/min, with an injection volume of 20 µL, detection wavelength of 225 nm, column ambient temperature, and a total run time of 10 minutes [10].

Preparation of mobile phase

Methanol and water (70:30) was used as mobile phase. o-phosphoric acid is used for 3 pH adjustment. 0.45-micron PTFE (poly-tetra-fluoro ethanol) filter was used to filter resultant mixture. Filtration was conducted using vacuum pump. Degassing was performed using sonication for 10 min.

Preparation of standard stock solution

FLU **API standard stock solution**

Accurately weighed quantity 10 mg of FLU was dissolved in methanol and volume was made up to 100 ml mark (100 µg/ml). The stock standard solution was diluted further with mobile phase to get various concentrations.

VIL API standard stock solution

Accurately weighed quantity 25 mg of VIL was dissolved in methanol and volume was made up to 100 ml mark (250 µg/ml). 10 ml from above solution was further diluted to 100 ml with mobile phase to prepare 25 µg/ml standard stock solution. The stock standard solution was diluted further with mobile phase to get various concentrations.

Preparation of working standard solution

The series of working standard concentration from 10-50µg/ml for FLU and 2.5-12.5 µg/ml for VIL drug solutions were prepared by serially diluting the stock solution to obtain concentrations.

Preparation of sample solution

Commercial Vilor-F 100 was purchased from the Local market. The twenty capsules weighed, and then average weight was determined. Powder from each capsule was collected and mixed on butter paper. The weight of the powder equivalent to 10 mg of FLU and 2.5 mg of VIL were transferred into a 100 mL standard volumetric flask. Added 50 mL of methanol sonicated for 10 min and diluted to 100 mL with methanol. Then the mixture was filtered through 0.45µ membrane filter. The above solution was further diluted with mobile phase to 10 ml to get the concentration of 50 µg/ml of FLU and 12.5 µg/ml of VIL.

Equal volume (20 µL) of standard solution and test solution was then injected into the column. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated.

Procedure for preparation of the calibration curves:

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 10-50 µg/ml for FLU and 10-50 µg/ml of VIL solutions were injected and peak area was recorded. The graph plotted as the concentration of the drug Vs peak area depicted in Figure 3 and 4 [11].

Parameters for method validation

The analytical method was validated in accordance with ICH guidelines by assessing key parameters including accuracy, linearity, range, system suitability, specificity, sensitivity, precision (repeatability and reproducibility), recovery, ruggedness.

Accuracy

The results of recovery studies and statistical data are recorded in Table No.2 and 3. The deviation between the mean value found and the true value is accuracy. Accuracy was ascertained on the basis of recovery studies performed by standard addition method by recovery experiments. A known amount of standard drug was added to the fixed amount of pre-analyzed dry powder solution. % recovery was calculated by area comparison before and after the addition of the standard drug. The standard addition method was performed at three levels. Triplicate analysis was performed at each level as per the proposed method. The percent recovery and %RSD at each level was calculated. Satisfactory recoveries indicates that the proposed method was accurate.

Linearity and range:

Linearity was assessed using least squares regression analysis of five-point calibration curves prepared in Microsoft Excel. Both calibration curves demonstrated excellent linearity across the concentration range of 80-120% of Label claim.Linearity was performed by preparing mixed standard solutions of FLU and VIL at different concentration levels including working concentration mentioned in experimental condition 10-50 µg/mL for FLU and 2.5 -12.5 µg/mL for VIL. Twenty microliters of each concentration was injected into the HPLC system. The response was read at 225 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. From the stock solutions of FLU and VIL 1ml, 2ml, 3ml, 4ml, and 5ml were taken in five different 10 ml volumetric flasks and diluted with the mobile phase to the give the concentrations as FLU: 10µg/ml, 20µg/ml, 30µg/ml, 40 µg/ml, 50 µg/ml and VIL: 2.5µg/mL, 5µg/ml, 7.5µg/ml, 10.0µg/ml and 12.5µg/ml. These Solutions were injected into the chromatographic system and response were recorded. The calibration graph was plotted with mean peak area on Y axis and concentration of standard solution on X axis. The degree of linearity was estimated by calculating the correlation coefficient. Y- Intercept, slope of the regression line. Beer's law was found to be obeyed over this concentration range. The correlation coefficient shall not be less than 0.998. Linearity results were presented in Table 4.

Dry powder from capsules equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. FLU and VIL marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug. The plot showing linearity and range study for FLU and VIL is shown in the Figure 5 and 6 [12].

System suitability:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions (Table 5).

Specificity and sensitivity:

The specificity of RP-HPLC method was determined by complete separation of FLU and VIL. The tailing factor for peaks of FLU and VIL was less than 2% and resolution was satisfactory. The average retention time for FLU and VIL were found to be 6.339 and 4.509 respectively, for five replicates. The peaks

obtained for FLU and VIL were sharp and have clear baseline separation. Analysis was also performed for active FLU and VIL, as well as blank/mobile phase sample at different conditions. The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix also after analysis it was found that there is no interference of peak in the FLU and VIL region for the blank solution & active sample. Hence the developed method was specific for the analysis of this product (Figure 7, 8 and 9).

Sensitivity: LOD was determined as lowest concentration giving response and LOQ was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The LOD and LOQ were found to be 0.41µg/mL and 0.1.25µg/mL for FLP and 0.3245µg/mL and 0.98µg/mL for VIL. The LOD and LOQ showed that the method is sensitive for FLP and VIL [13].

Precision

The precision of an analytical procedure indicates the closeness of repeated measurements between a series of measurements obtained from multiple sampling of the sample under the prescribed conditions. Precision of the method was performed as system precision, method precision and intermediate precision. Precision of an analytical method is expressed as S.D or R.S.D of series of measurements (Table 7). It was ascertained by replicate estimation of the drugs by proposed method.

Ruggedness:

The studies of ruggedness were carried out under two different conditions like different days and by different analysts

i) Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 8

ii) Intraday:

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. Interval within a day. The percent label claim was calculated using formula & Result and statistical data are shown in Table No. 9

iii) Different analyst:

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation (Table 10).

Robustness and ruggedness:

The ability of an analytical method to remain unaffected by small variations in method parameters and influential environmental factors and characterize its reliability during normal usage. No change of the detected amount of the analyte in a certain sample in spite of the variation of the method parameter. The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust. Method's reproducibility under varying conditions, with results expressed as % RSD and the results are shown in table no 11 & 12

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value (Table 13) [14].

RESULT

Calibration curves:

The calibration data for FLU and VIL at different concentration levels are presented in Table No. 1. A proportional increase in peak area was observed with increasing concentrations for both drugs, indicating a direct relationship between concentration and detector response. The results demonstrate good linearity of the method over the selected concentration range. This confirms the suitability of the developed RP-HPLC method for quantitative analysis of FLU and VIL.

Table 1: Observation of standard curves of FLU and VIL

Conc. (µg/ml) FLU	Conc. (µg/ml) VIL	Peak Area	
		FLU	VIL
10	2.5	460608	75746
20	5	540062	126424
30	7.5	631034	182764
40	10	720087	230556
50	12.5	810228	285145

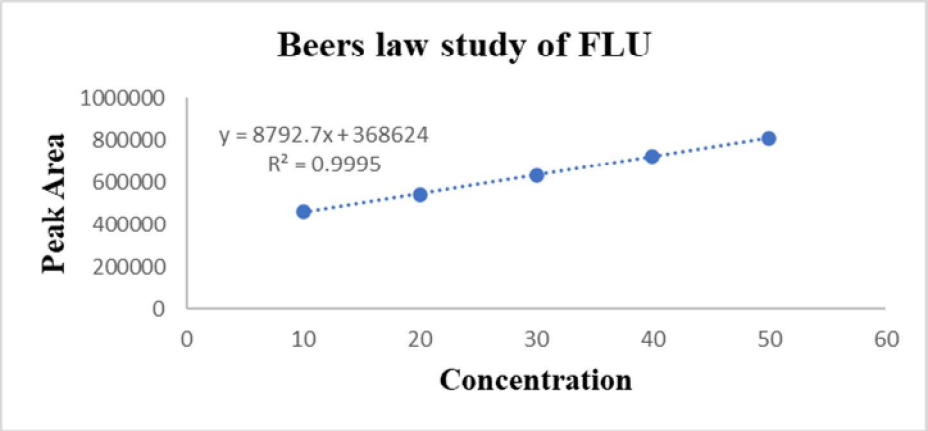

Figure 3: Standard calibration curve for FLU

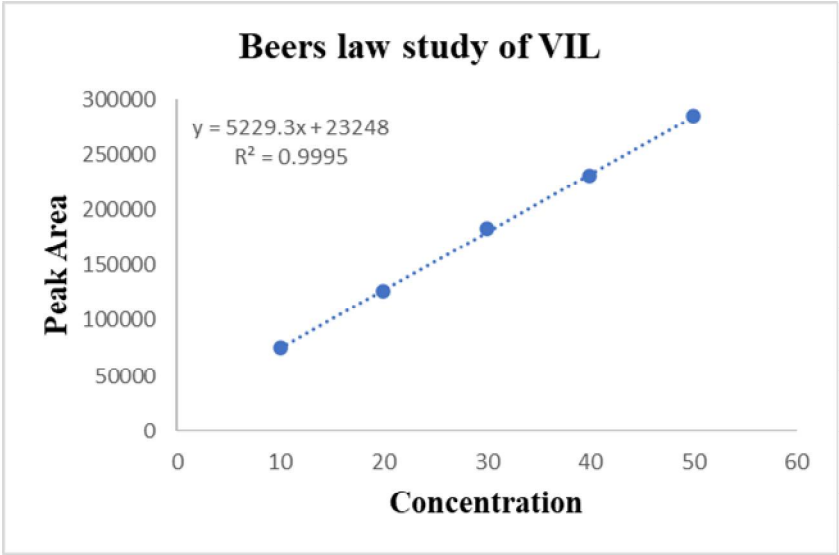

Figure 4: Standard calibration curve for VIL

Parameters for method validation:

Accuracy:

The accuracy of the method for FLU was evaluated by recovery studies at three concentration levels, as shown in Table No. 2. The results demonstrated consistent recovery with low %RSD values, indicating minimal variation between replicate measurements. The close agreement between observed and expected values confirms the reliability of the method. These findings establish that the proposed RP-HPLC method is accurate for the estimation of FLU.

Table 2: Results and statistical data for Recovery study of FLU

Conc.	Area	Mean	%RSD
10	460608	461859.00	0.1535
	462256		
	462713		
30	631034	631832.33	
	635462		
	629001		
50	810228	814754.66	
	816578		
	817458		

The accuracy of the method for VIL was assessed through recovery studies at three concentration levels, as presented in Table No. 3. The obtained results showed consistent recovery with low %RSD values, indicating good precision and minimal variability. The closeness of the mean values to the expected concentrations confirms the reliability of the method. Hence, the developed RP-HPLC method is accurate for the estimation of VIL.

Table 3: Results and statistical data for Recovery study of VIL

Conc.	Area	Mean	%RSD
2.5	75746	75440.66	0.1544
	74847		
	75729		
7.5	182764	181609.66	
	181137		
	180928		
12.5	285145	285671.00	
	286901		
	284967		

Linearity and range:

The linearity and range of the developed method for FLU and VIL were evaluated over 80–120% of the label claim, as shown in Table No. 4. A consistent increase in peak area with increasing concentration was observed for both drugs, indicating a strong linear relationship. The results demonstrate that the method is linear within the studied range. This confirms the suitability of the method for quantitative analysis of FLU and VIL in pharmaceutical formulations.

Table 4: Observations of Linearity and range study for FLU and VIL

%Label claim	Peak área	
	FLU	VIL
80	460608	75746
90	540062	126424
100	631034	182764
110	720087	230556
120	810228	285145

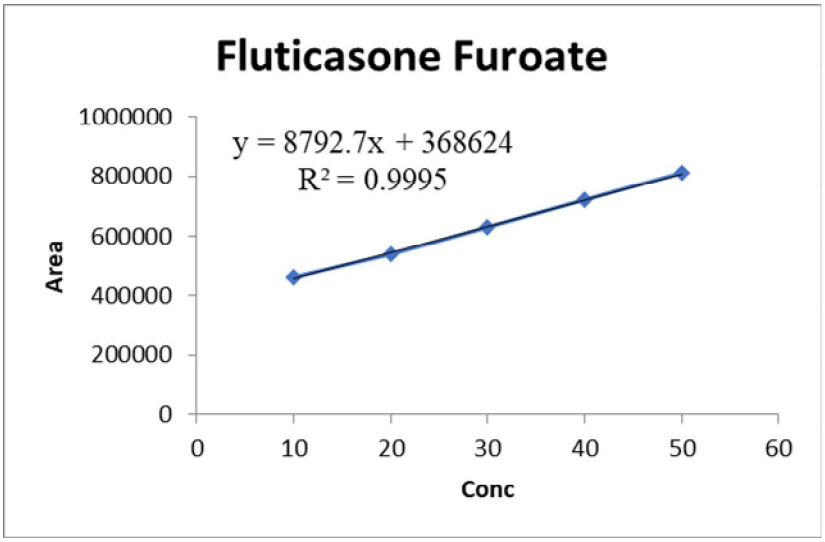

Figure 5: -Plot of linearity and range study for FLU

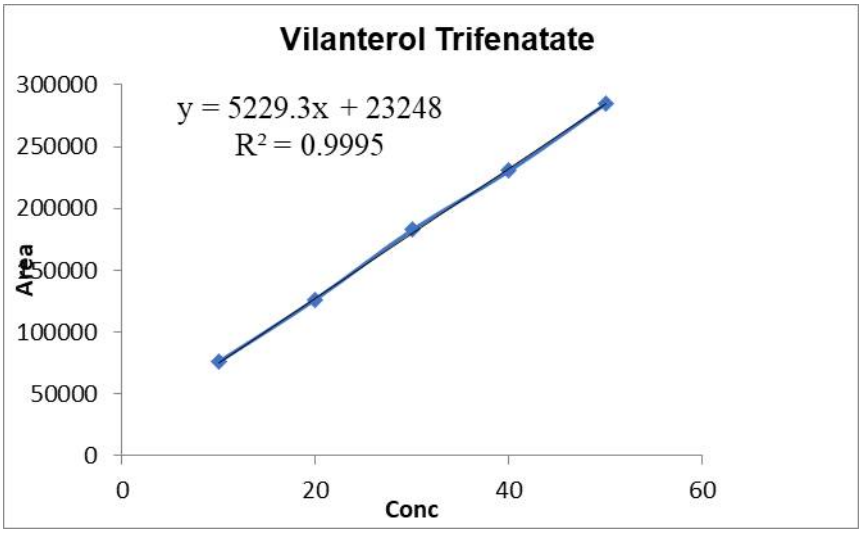

Figure 6: -Plot of linearity and range study for VIL

System suitability:

The system suitability parameters for the developed RP-HPLC method were evaluated and are presented in Table No. 5. The results showed consistent peak areas, retention times, asymmetry factors, and theoretical plate counts for both FLU and VIL. The low %RSD values indicate good repeatability and reliability of the chromatographic system. These findings confirm that the system is suitable for the intended analysis.

Table 5: Result of System Suitability Study

Sr. No	Peak area		Retention Time		Asymmetry		T Plate	
	FLU	VIL	FLU	VIL	FLU	VIL	FLU	VIL
1	810228	285145	6.373	4.573	1.12	1.30	8221	5779
2	810245	285136	6.379	4.581	1.11	1.39	8224	5773
3	810214	285133	6.377	4.582	1.16	1.36	8225	5788
4	810221	285141	6.373	4.588	1.12	1.30	8225	5779
5	810239	285149	6.374	4.573	1.14	1.31	8232	5782
mean	810229.4	285140.8	6.375	4.579	1.13	1.332	8225.4	5780.2
± S.D	12.7004	6.4962	0.00268	0.00642	0.02	0.0408	4.0373258	5.4497706
C.V	0.00002	0.00002	0.00042	0.00140	0.01769	0.03067	0.0004908	0.0009428

Specificity and sensitivity:

The specificity of RP-HPLC method was determined by complete separation of FLU and VIL. The tailing factor for peaks of FLU and VIL was less than 2% and resolution was satisfactory. The average retention time for FLU and VIL were found to be 6.339 and 4.509 respectively, for five replicates. The peaks obtained for FLU and VIL were sharp and have clear baseline separation. Analysis was also performed for active FLU and VIL, as well as blank/mobile phase sample at different conditions. The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix also after analysis it was found that there is no interference of peak in the FLU and VIL region for the blank solution & active sample. Hence the developed method was specific for the analysis of this product.

Figure 7: Typical Chromatogram showing blank (mobile phase)

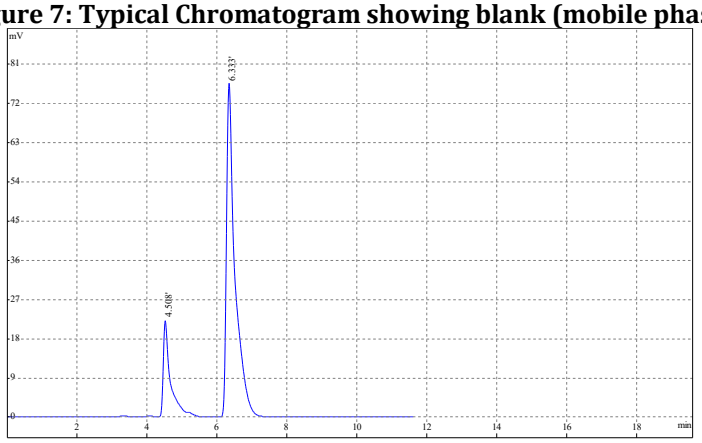

Figure 8: Typical Chromatogram obtained for standard solution (FLU & VIL)

API	RT	Area	Resolution	T. Plate Num	Asymmetry
VIL	4.508	182764	4.31	7779	1.23
FLU	6.333	631034	0.00	7900	1.14

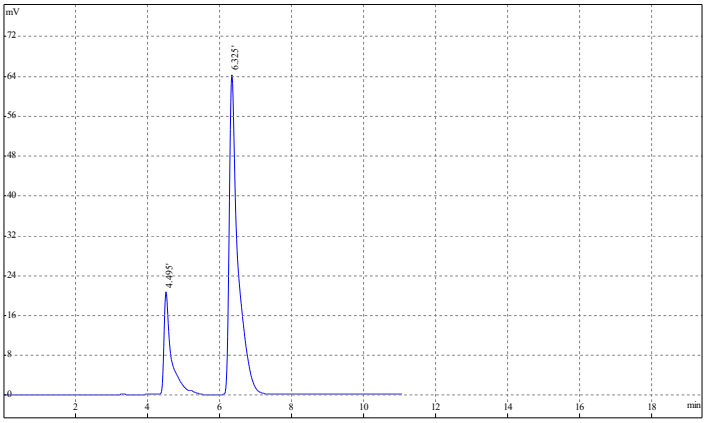

Figure 9: Typical Chromatogram obtained for Sample solution (FLU & VIL)

API	RT	Area	Resolution	T. Plate Num	Asymmetry
VIL	4.495	182033	4.36	7741	1.26
FLU	6.325	634820	0.00	7930	1.15

Sensitivity:
LOD and LOQ were found to be 0.41µg/mL and 0.1.25µg/mL for FLP and 0.3245µg/mL and 0.98µg/mL for VIL. The LOD and LOQ showed that the method is sensitive for FLP and VIL.

Precision

The precision of the developed RP-HPLC method was evaluated by analyzing replicate samples, and the results are presented in Table No. 7. The % label claim values for both FLU and VIL were found to be close to 100%, indicating good agreement with the labeled amount. The low standard deviation and %RSD values demonstrate excellent repeatability of the method. These results confirm that the method is precise for the simultaneous estimation of FLU and VIL.

Table No.7: Results and statistical data of Precision Study -Brand Name: Vilor-F 100 Powder for Inhalation

Sr. No	Weight of std.(mg)		Wt. of sample (g)	Peak area of std.		Peak area of sample		% Label claim	
	FLU	VIL		FLU	VIL	FLU	VIL	FLU	VIL
1.	10	25	2.51	631034	182764	626617	182947	99.30	100.10
2.			2.50			634820	182033	100.60	99.60
3.			2.50			636082	183312	100.80	100.30
							Mean	100.23	100.00
							±S.D.	0.814	0.361
							C.V.	0.008	0.004

Ruggedness:

The studies of ruggedness were carried out under two different conditions like different days and by different analysts

i) Interday (Different days):

The interday precision of the developed RP-HPLC method was evaluated by performing the analysis on different days, and the results are presented in Table No. 8. The % label claim values for both FLU and VIL were found to be close to 100%, indicating consistency in the method over time. The low standard deviation and %RSD values demonstrate minimal variability between days. These results confirm that the method is reproducible and reliable under interday conditions.

Table No. 8: Results and statistical data of Interday Study: Brand Name: Vilor-F 100

Sr. No.	Weight of std.(mg)		Wt. of sample (g)	Peak area of std.		Peak area of sample		% Label claim	
	FLU	VIL		FLU	VIL	FLU	VIL	FLU	VIL
1.	10	25	2.50	631034	182764	634189	182398	100.5	99.8
2.			2.50			636713	183130	100.9	100.2
3.			2.52			635451	182581	100.7	99.9
							Mean	100.70	99.97
							±S.D.	0.200	0.208
							C.V.	0.002	0.002

ii) Intraday:

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The percent label claim was calculated using formula & Result and statistical data are shown in Table No. 9

Table No.9: Results and statistical data of Intraday Study: Brand Name: Vilor-F 100

Sr. No.	Weight of std.(mg)		Wt. of sample (g)	Peak area of std.		Peak area of sample		% Label claim	
	FLU	VIL		FLU	VIL	FLU	VIL	FLU	VIL
1.	10	25	2.50	631034	182764	633558	183495	100.4	100.4
2.			2.50			634189	181667	100.5	99.4
3.			2.52			637344	182398	101	99.8
							Mean	100.63	99.87
							±S.D.	0.321	0.503
							C.V.	0.003	0.005

iii) Different analyst:

The ruggedness of the developed RP-HPLC method was evaluated by performing the analysis using two different analysts, and the results are presented in Table No. 10. The % label claim values for both FLU and VIL were found to be consistent and close to 100% for both analysts. The low standard deviation and %RSD values indicate minimal variability between analysts. These findings confirm that the method is rugged and reproducible under normal operating conditions.

Table No.10: Result and statistical data of Different analyst study

Sr. No.	% Label claim			
	ANALYST I		ANALYST II	
	FLU	VIL	FLU	VIL
1	100.4	100.8	99.9	101
2	100.5	100.9	100.3	101.1
3	101	101.1	100.4	99.3
4	101.1	101.5	100.5	100.6
5	99.3	100.2	101	100.8
Mean	100.46	100.9	100.42	100.56
± S.D	0.7162	0.4743	0.3962	0.7300
C.V	0.0071	0.0047	0.0039	0.0072

Robustness and ruggedness:

The robustness of the developed RP-HPLC method was evaluated by introducing small deliberate variations in analytical parameters such as wavelength and pH, and the results are presented in Table No. 11 and Table No. 12. The peak areas of both FLU and VIL remained consistent under these varied conditions. The low %RSD values indicate that minor changes in method parameters do not significantly affect the results. These findings confirm that the method is robust and reliable for routine analysis.

Table No.11: Result of Robustness study of FLU

Change in Wavelength	Concentration µg/ml	Area	Mean	SD	%RSD
223	30	633555	633549	6.5574	0.001035
225	30	633542			
227	30	633550			
Change in pH	**Concentration µg/ml**	**Area**	**Mean**	**SD**	**%RSD**
2.8	30	633547	633547	4.0000	0.000631
3.0	30	633543			
3.2	30	633551			

Table No.12: Result of Robustness study of VIL

Change in Wavelength	Concentration µg/ml	Area	Mean	SD	%RSD
223	30	182766	182760	6.0277	0.003298
225	30	182754			
227	30	182761			
Change in pH	**Concentration µg/ml**	**Area**	**Mean**	**SD**	**%RSD**
2.8	30	182760	182758	5.6862	0.003111
3.0	30	182752			
3.2	30	182763			

Tables No. 11 and 12 present the robustness study results for FLU and VIL under small deliberate variations in wavelength and pH. For both drugs, the peak areas remained nearly constant despite changes in wavelength (223–227 nm) and pH (2.8–3.2), indicating that the method performance was not significantly affected. The very low standard deviation and %RSD values further confirm minimal variability under these altered conditions.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Table No. 13 presents the LOD and LOQ for FLU and VIL, indicating the sensitivity of the developed RP-HPLC method. FLU shows an LOD of 0.4155 µg/mL and LOQ of 1.2593 µg/mL, whereas VIL exhibits lower values (LOD: 0.3245 µg/mL, LOQ: 0.9833 µg/mL), suggesting that VIL can be detected and quantified at slightly lower concentrations compared to FLU. The low values for both drugs confirm that the method is sufficiently sensitive for detecting and quantifying trace levels.

Table 13: LOD & LOQ of FLU & VIL

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
FLU	0.4155	1.2593
VIL	0.3245	0.9833

DISCUSSION

The present study successfully developed and validated a robust RP-HPLC method for the simultaneous estimation of FLU and VIL in bulk and pharmaceutical dosage forms. The method was evaluated as per ICH guidelines, and the obtained results confirm its suitability for routine analytical applications. The accuracy of the method was confirmed through recovery studies at three concentration levels for both drugs [15]. For FLU, the mean peak areas were found to be 461859.00, 631832.33, and 814754.66 at concentrations of 10, 30, and 50 µg/mL, respectively, with a %RSD of 0.1535. Similarly, for VIL, the mean peak areas were 75440.66, 181609.66, and 285671.00 at concentrations of 2.5, 7.5, and 12.5 µg/mL, respectively, with a %RSD of 0.1544. The closeness of observed values with expected concentrations and low %RSD values indicate high accuracy and minimal variability.

Linearity studies demonstrated a direct relationship between concentration and peak area over the range of 80–120% of the label claim. For FLU, the peak area increased from 460608 to 810228, while for VIL it increased from 75746 to 285145 across the studied range. This consistent increase confirms excellent linearity and adherence to Beer's law, validating the method for quantitative estimation. System suitability parameters showed consistent and reproducible results. The mean peak areas were 810229.4 for FLU and 285140.8 for VIL [16]. The average retention times were 6.375 min for FLU and 4.579 min for VIL. The asymmetry factors were 1.13 (FLU) and 1.332 (VIL), while the theoretical plate counts were 8225.4 and 5780.2, respectively. The %CV values were extremely low (0.00002 for peak area), indicating excellent system performance and repeatability.

The specificity of the method was confirmed by clear baseline separation of FLU and VIL with retention times around 6.33 min and 4.50 min, respectively. No interference was observed from blank or excipients, and the resolution between peaks was satisfactory (approximately 4.31–4.36), indicating high selectivity. Sensitivity of the method was demonstrated by low LOD and LOQ values. For FLU, LOD and LOQ were 0.4155 µg/mL and 1.2593 µg/mL, respectively, whereas for VIL they were 0.3245 µg/mL and 0.9833 µg/mL. These results indicate that the method is capable of detecting and quantifying very low concentrations, with slightly higher sensitivity for VIL. Precision studies showed % label claim values close to 100%. The mean % label claim was 100.23% for FLU and 100.00% for VIL, with %RSD values of 0.008 and 0.004, respectively, indicating excellent repeatability [17].

Ruggedness studies further confirmed method reliability. In interday analysis, mean % label claims were 100.70% (FLU) and 99.97% (VIL) with %RSD of 0.002. Intraday results showed mean values of 100.63% (FLU) and 99.87% (VIL) with %RSD of 0.003 and 0.005, respectively. Analysis by different analysts also produced consistent results, with mean values of 100.46% and 100.42% for FLU and 100.9% and 100.56% for VIL, confirming reproducibility under varied conditions. Robustness studies indicated that small variations in wavelength (223–227 nm) and pH (2.8–3.2) did not significantly affect the results. For FLU, the mean peak area was 633549 with %RSD of 0.001035 (wavelength variation) and 0.000631 (pH variation). For VIL, the mean peak area was 182760 with %RSD of 0.003298 (wavelength variation) and 0.003111 (pH variation). These very low %RSD values confirm that the method remains stable under minor changes in analytical conditions [18-19].

CONCLUSION

A simple, rapid, and reliable RP-HPLC method was successfully developed and validated for the simultaneous estimation of FLU and VIL in bulk and pharmaceutical dosage forms. The optimized chromatographic conditions provided well-resolved peaks with retention times of approximately 6.3 min for FLU and 4.5 min for VIL, indicating efficient separation within a short run time. The method demonstrated excellent linearity over the concentration range of 10–50 µg/mL for FLU and 2.5–12.5 µg/mL for VIL, with consistent increases in peak area. Accuracy studies showed reliable recovery with %RSD values below 0.2%, confirming minimal variability. Precision results indicated % label claim values close to 100% with very low %RSD, demonstrating high repeatability. The system suitability parameters were within acceptable limits, ensuring consistent chromatographic performance. The method was found to be highly sensitive, with LOD and LOQ values of 0.4155 µg/mL and 1.2593 µg/mL for FLU, and 0.3245 µg/mL and 0.9833 µg/mL for VIL. Ruggedness and robustness studies confirmed that the method

remains unaffected by small variations in analytical conditions and is reproducible under different environments.

REFERENCES

1. Malerba, M., Radaeli, A., Montuschi, P. & Morjaria, J.B. (2016). Vilanterol trifenatate for the treatment of COPD. Expert Rev. Respir. Med., 10(7): 719–731.
2. Gray, E.L., Chang, V. & Thomas, P.S. (2016). Fluticasone furoate and vilanterol trifenatate combination therapy for the treatment of asthma. Expert Rev. Respir. Med., 10(8): 839–847.
3. Guo, Y., Yang, Y., Peng, J., Zhu, X. & Ji, Z. et al. (2019). Enantiomeric separation of vilanterol trifenatate by chiral liquid chromatography. Chirality, 31(10): 813–817.
4. Yadav, S., Jain, V., Magar, H., Kumar, M.V. & Warde, S. et al. (2023). A novel RP-HPLC method for simultaneous estimation of vilanterol trifenatate, umeclidinium bromide and fluticasone furoate in inhalation dry powder formulation. J. Chromatogr. Sci., 61(10): 075.
5. Valizadeh, M., Braki, Z.A., Smiley, E., Arghand, A. & Dastafkan, P. et al. (2023). Simultaneous quantitative analysis of salmeterol and fluticasone in inhalation spray using HPLC and a fast spectrophotometric technique combined with a time series neural network and multivariate calibration methods. J. AOAC Int., 106(4): 1109–1117.
6. Masimukku, S.K. & Chintala, R. (2018). Development and validation of stability indicating RP-HPLC method for fluticasone furoate and vilanterol in pharmaceutical formulations. Indian Drugs, 55(3): 27–31.
7. Valizadeh, M., Sohrabi, M.R. & Motiee, F. (2018). Simple spectrophotometric method for simultaneous determination of salmeterol and fluticasone as anti-asthma drugs in inhalation spray based on artificial neural network and support vector regression. Optik, 240: 166879.
8. Patel, B. & Patel, S. (2023). Simultaneous quantification of fluticasone propionate and olopatadine hydrochloride by high-performance thin-layer chromatography. Chromatography, 6(9): 2300070.
9. Babu, G.R., Sowjanya, M., Ramayyappa, M., Karishma, M.D.A. & Bharathi, V. et al. (2023). Rapid RP-HPLC method for simultaneous estimation of umeclidinium and vilanterol in human plasma. Res. J. Pharm. Technol., 16(3): 1017–1022.
10. Kabra, RP, Kadam, SC, Mane, VB, Kadam, SS, Mamde, CG. (2014).Simple novel UV-spectroscopic method for estimation of ezetimibe in tablet dosage form. Am. J Pharm Health Res.2(9).
11. Jadhav PA, Raut CS, Bidada JP, Buwa BB, Dhabale PN, Dhawale SC. Development and Statistical Validation of UV Spectrophotometric Method for Estimation of Griseofulvin in Tablet Dosage Form. Asian Journal of Research in Chemistry. 2010;3(2):404-6.
12. Ahmad S, Khairnar M, Bakhshi AR, Tare M, Baheti D, Tare H. QBD approach to develop stability indicating RP-HPLC method development for Levosulpiride and Ilaprazole. International Journal of Health Sciences. 2022;6(S5):7413-29.
13. Pund S, Nalawade S, Rajurkar V, Jayatpal S, Deshmukh N, Tare H. A brief review on recent advances in reverse phase HPLC. Multidiscip Rev. 2024 Jan 18;7(4):2024072.
14. Mane V, Killedar S, More H, Salunkhe S, Tare H. Development and validation of a novel bioanalytical method for estimating Epigallocatechin 3 gallate in Wistar rat plasma by RP-HPLC employing gradient elution techniques. Journal of Research in Pharmacy. 2023 May 1;27(3):1039-55.
15. Patil K, Narkhede S, Nemade M, Rane S, Chaudhari R, Dhobale G, Tare H. A Validated Sensitive Stability Indicating HPLC Method for the Determination of Etoricoxib in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):352-7.
16. Mane VA, Killedar SU, More HA, Gaikwad AS, Tare HA. A novel RP-HPLC gradient elution technique for bioanalytical method development and validation for estimating gallic acid in Wistar rat plasma. Int J App Pharm. 2023 Mar 7;15(2):153-60.
17. Kharate V, Kuchekar M, Harde M, Pimple B, Patole V, Salunkhe M, Wadgave P, Bhise M, Gaikwad A, Tare H. Development of validated stability indicating HPTLC method for estimation of febuxostat in bulk and tablet dosage form by using QBD approach. International Journal of Drug Delivery Technology. 2023;13(2):542-50.
18. ICH Harmonised Guideline Q2(R2). (2022). Validation of analytical procedures: text and methodology. International Council for Harmonisation.
19. ICH Q2(R1). (2005). Validation of analytical procedures: text and methodology. International Council for Harmonisation.