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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FLUTICASONE FUROATE AND VILANTEROL TRIFENATATE BY RP-UPLC

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Abstract:

A simple, accurate, precise method was developed for the simultaneous estimation of the Fluticasone furoate (FLU) and Vilanterol trifenatate (VIL) in bulk and pharmaceutical dosage form by using UPLC. Combination of Fluticasone furoate and Vilanterol trifenatate is used for treatment of bronchospasm associated with chronic obstructive pulmonary disease. Chromatography was carried out using BEH 100 x 2.1mm, 1.8µm. column with a flow rate of 0.3 mL/min. The mobile phase consisted of 0.01N Na_2HPO_4 Buffer of pH 4.0 and Acetonitrile in the ratio of 50:50. The retention times of FLU and VIL were found to be 1.206 min and 1.582 min respectively. Temperature was maintained at 30°C. Optimized wavelength for FLU and VIL was 260nm. The method developed has been statistically validated according to ICH guidelines. %RSD of FLU and VIL were and found to be 1.2 and 0.6 respectively. %Recovery was obtained as 100.13% and 99.82% for FLU and VIL respectively. LoD, LoQ values were obtained from regression equations of Fluticasone furoate and Vilanterol trifenatate were 0.10 ppm, 0.30 ppm and 1.51 ppm, 4.56 ppm respectively. The method obeys Beer's law in the concentration range of 25-150 µg/mL (R^2 = 0.999) for FLU, and 6.25-37.5 µg/mL (R^2 = 0.999) for VIL. The method showed good reproducibility and recovery with %RSD less than 2. The stability-indicating capability of the method was established by forced degradation studies under stress conditions like acid, base, peroxide, UV, thermal, humidity. Retention times were decreased and that run time was decreased. Hence, the chromatographic method developed was simple and economical that can be adopted in regular quality control test in Industries and it is said to be rapid, specific, sensitive, robust, and reliable that can be effectively applied for routine analysis in research institutions, quality control departments in industries.

Key Words: *Fluticasone furoate, Vilanterol trifenatate, RP-UPLC.*

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INTRODUCTION:

A triphenyl acetate salt obtained by combining vilanterol with one equivalent of triphenyl acetic acid, used in combination with fluticasone furoate for treatment of bronchospasm associated with chronic obstructive pulmonary disease.[1] Indicated for the maintenance treatment of the airflow obstruction in patients with chronic obstructive pulmonary disease (COPD) including chronic bronchitis and/ or emphysema and to reduce exacerbation of COPD in patients with an exacerbation history. The combination was approved by US FDA in April 2015.[2] and by CDSCO in June 2017.[3]

Fluticasone furoate is a corticosteroid for the treatment of non-allergic and allergic rhinitis administered by a nasal spray.[4] It is also available as an inhaled corticosteroid to help prevent and control symptoms of asthma. It is derived from cortisol. Unlike fluticasone propionate, which is only approved for children four years and older, fluticasone furoate is approved in children as young as two years of age when used for allergies.[5] It is also available as an inhaled corticosteroid to help prevent and control symptoms of asthma. Fluticasone furoate works through an unknown mechanism to affect the action of various cell types and mediators of inflammation. In vitro experiments show fluticasone furoate activating glucocorticoid receptors, inhibiting nuclear factor kappa b, and inhibiting lung eosinophilia in rats.

Vilanterol trifenatate is a selective long-acting beta2-adrenergic agonist (LABA) with inherent 24-hour activity for once daily treatment of COPD and asthma. Its pharmacological effect is attributable to stimulation of intracellular adenylyl cyclase which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3',5'-adenosine monophosphate (cAMP). Increases in cyclic AMP are associated with relaxation of bronchial smooth muscle and inhibition of release of hypersensitivity mediators from mast cells in the lungs.[6]

Extensive literature survey was conducted to find out the analytical methods reported for fluticasone furoate and vilanterol trifenatate. A literature survey discloses that no UPLC method for the estimation of fluticasone furoate and vilanterol trifenatate combined dosage form was reported till now. Few stability-indicating HPLC methods, [7-15] HPTLC, [16] Spectrophotometric methods [17] have been reported for the estimation of fluticasone furoate and vilanterol trifenatate individually and or along with drug combinations in pharmaceutical preparations. The aim of this study is to develop and validate a stability-indicating method with less runtime, which would be able to separate and quantify a combination of FLU and VIL in a single run. The developed method was validated as per ICH guidelines [18-19] and can be applied lucratively to quality control purposes.

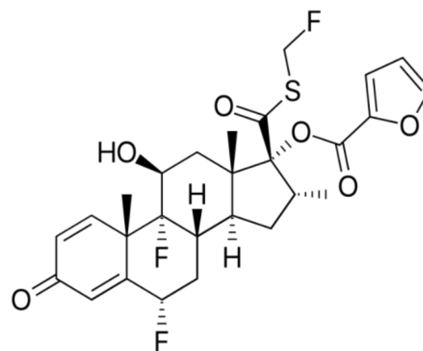

Fig. 1: Structure of fluticasone furoate

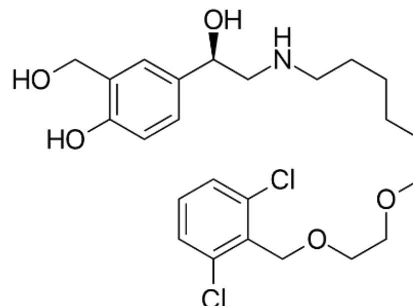

Fig. 2: Structure of Vilanterol trifenatate

MATERIALS AND METHODS:

Instrumentation:

Waters UPLC 2695 System equipped with quaternary pumps, photodiode array detector and auto sampler integrated with Empower 2 Software was used in the current investigation. Ultrasonic bath (Labman Scientific Instruments Pvt. Ltd. Chennai, India) for sonication and digital pH meter (Metsar Technologies Pvt. Ltd. Hyderabad, India) for measuring pH were used in the study. Electronic balance ELB 300 was used for weighing the materials. For degradation studies, hot air oven and a UV cross inker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used.

Materials:

Fluticasone furoate and vilanterol trifenatate reference standards were procured from Spectrum Pharma Limited, Hyderabad. HPLC grade acetonitrile,

analytical grade orthophosphoric acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide were from Ramkem (Haryana, India). Sodium hydrogen phosphate were purchased from E. Merck Limited, Mumbai, India. Milli-Q-water was used throughout the process.

Chromatographic conditions:
UPLC analysis was carried out on Waters UPLC System equipped with 2695-separation module connected to 2996-photo diode array detector and the data was acquired by Empower® version 2. Separation was achieved using BEH C18 100 x 2.1mm,1.8µ.as a column with mobile phase 0.01N Na_2HPO_4 Buffer of pH 4.0 and Acetonitrile in the ratio of 50:50. The samples were analyzed using 1.0 µL injection volume, Flow rate was maintained at 0.3 mL/min with runtime of 2.5 min and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs were achieved using PDA detector at 260 nm wavelength.

Preparation of working standard solutions:
Accurately Weighed and transferred 25mg of FLU and 6.25mg of VIL working Standards into a 25mL clean dry volumetric flask, added 25mL of diluent and sonicated for 30 minutes to dissolve completely. The final volume was made with the diluent to obtain a concentration of 1000µg/mL of FLU and 250µg/mL VIL. From the above stock solution 1 mL was pipetted out in to a 10mL volumetric flask and then made up to the final volume with diluent. The final concentration was found to be 100µg/mL of FLU and 25µg/mL of VIL.

Preparation of sample solution:
The contents of nasal spray delivered by 50 actuations were collected in 10 mL volumetric flask. Now 8mL of acetonitrile was added and sonicated for 25 min. The final volume was made using diluent which gives the concentration of 5000 µg/mL and 1250 µg/mL. Then it was centrifuged for 20 min and the supernatant liquid was collected and filtered using 0.45 µm filters (Millipore, Milford, PVDF). From the above stock solution 2 mL was pipetted out and taken into a 10mL volumetric flask and made up with diluent to obtain a final concentration of 100µg/mL of FLU and 25µg/mL of VIL.

METHOD VALIDATION:
The developed and optimized RP-UPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) in order to determine the system suitability, linearity, limit of detection (LoD), limit of quantification (LoQ), precision, accuracy, ruggedness and robustness.

System suitability:
System suitability parameters were evaluated to verify system performance. 1 µL of standard solution was injected five times into the system, the chromatograms were recorded. Parameters such as number of theoretical plates and peak tailing were determined and all the parameters were within the limits.

Specificity:
The specificity of the analytical method was established by injecting the solutions of diluent (blank), placebo, working standards and sample solution individually to investigate interference from the representative peaks.

Precision:
Repeatability/method precision was performed by injecting six replicates of FLU and VIL and calculated % assay and %RSD for each compound. Reproducibility/Ruggedness/Intermediate precision was performed using different analysts and a different instrument in the same laboratory.

Accuracy:
Accuracy of the proposed method was determined using recovery studies by spiking method. The recovery studies were carried out by adding known amounts (50%, 100% and 150%) of the working standard solution to the pre-analysed sample. The solutions were prepared in triplicates to determine the accuracy.

Linearity:
Linearity was evaluated by analyzing different concentrations of the standard solutions of FLU and VIL. Six working standard solutions ranging between 25-150 µg/mL for FLU and 3.25-37.5 µg/mL for VIL were prepared and injected. The response was a linear function of concentration over peak area and were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient.

Limit of detection and limit of quantification:
Limit of detection (LoD) and limit of quantification (LoQ) of FLU and VIL were determined by calibration curve method. Solutions of FLU and VIL were prepared in linearity range and injected (n = 3). Average peak areas were plotted against concentration.

Robustness:

To examine the robustness of the developed method, experimental conditions were deliberately changed, and the resolution, tailing factor, and theoretical plates of FLU and VIL peaks were evaluated. To study the outcome of the flow rate on the developed method, it was changed ± 0.1 mL/min. The effect of column temperature on the developed method was studied at ± 5°C. and the mobile phase composition was changed ±5 % from the initial composition of the organic phase. In all the above varied conditions, the aqueous component of the mobile phase was held constant.

Forced Degradation Studies:

Stress studies were performed by considering the working standard solutions of concentrations 100µg/mL of FLU and 25µg/mL of VIL to provide the stability-indicating property of the proposed method. Intended degradation was attempted by the stress conditions of exposure to photolytic stress (1.2 million lux hours followed by 200 Watt hours), heat (exposed at 105°C for 6 hours), acid (2 N HCl for 2 hours at 60°C), base (2 N NaOH for 2 hours at 60°C), oxidation (20% peroxide for 30 minutes at 60°C), and water (refluxed for 12 hours at 60°C). The solutions were injected into the system; chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION:

System Suitability:

The column efficiency for FLU and VIL peaks was identified from the theoretical plate count is more than 3000 and tailing factor was between 0.80 to 2.0. %RSD for peak areas from six replicate injections was found to be less than 2.0%. The results of other system suitability parameters, such as, resolution, peak tailing, and theoretical plates are presented in Table 1. All system-suitable parameters were found to be satisfactory.

Table 1: System suitability data

Parameter	FLU	VIL	Acceptance criteria
USP Plate count*	3208	3645	NLT 3000
%RSD	1.2	0.6	NMT 2.0
Peak Tailing*	1.35	1.07	NMT 2.0
Resolution*	--	2.5	>1.5

* = Average of 6 replicate injections

Specificity:

From the obtained chromatograms in figures 3 to 6 it can be inferred that there were no co-eluting peaks at the retention time of FLU and VIL, which shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analyte of interest. Figures 3 and 4 show that there is no interference of blank and placebo at the retention time of FLU and VIL from the other excipients.

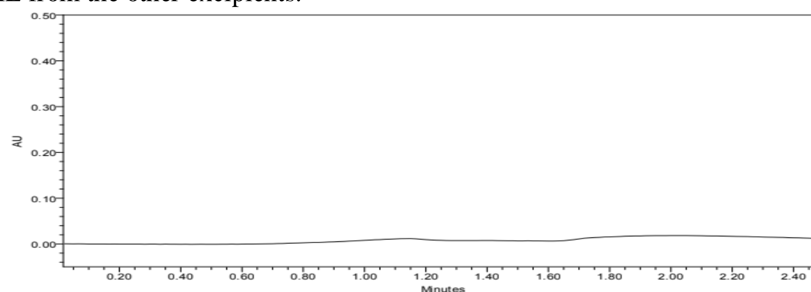

Fig. 3: Chromatogram of blank

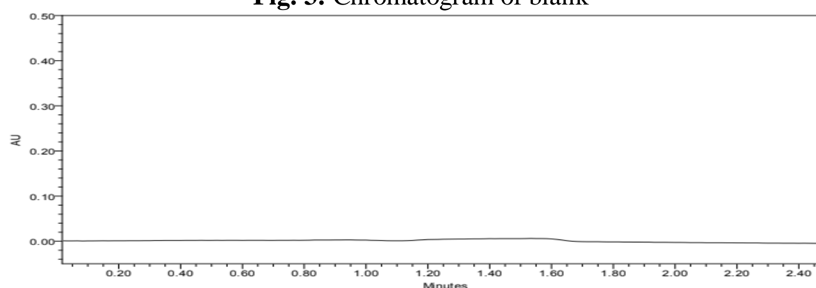

Fig. 4: Chromatogram of placebo

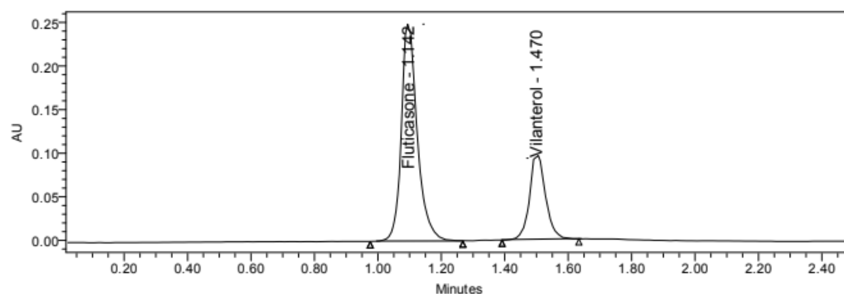

Fig. 5: Chromatogram of Fluticasone furoate and Vilanterol trifenatate standards

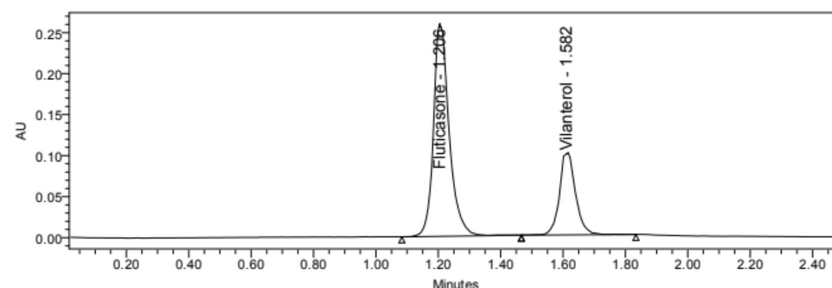

Fig. 6: Chromatogram of Fluticasone furoate and Vilanterol trifenatate sample

Precision:

% Assay for FLU and VIL was found to be in the range of 98 – 102%, and the % RSD for FLU and VIL were found to be within 2%. Hence the method is precise, reproducible and rugged for 48 hours' study and the results are summarized in table 2.

Table 2: Precision data

S.NO	Peak Areas	% Assay	Peak Areas	% Assay
	FLU		VIL	
1	1343425	98.66	504925	99.93
2	1369939	100.61	499617	98.88
3	1364280	100.42	497804	98.52
4	1357725	99.71	495308	98.02
5	1366930	100.39	509206	100.77
6	1352520	99.33	504586	99.86
Mean	1359637	99.85	501908	99.93
SD	10342.8	0.76	5199.7	1.0290
% RSD	0.8	0.76	1.0	1.0

Accuracy:

The % recovery for FLU and VIL were within the range of 98 –102%. The % RSD for FLU and VIL were found to be within 2%. Hence the proposed method was accurate and the results are summarized in table 3.

Table 3: Accuracy data

Drug name	Conc. (%)	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% recovery	Statistical parameters
FLU	50	50	49.84	99.71	Mean %: 99.64
	100	100	99.91	99.91	SD: 0.96
	150	150	149.89	99.32	%RSD: 0.9
VIL	50	12.5	12.46	99.69	Mean %: 99.81
	100	25	25.08	100.31	SD: 0.55
	150	37.5	37.29	99.45	%RSD: 0.5

Linearity:
Linearity was evaluated by analysing different concentrations. The correlation coefficient obtained was greater than 0.999 for all the components. The slope and y-intercept values were also provided in Table 4, which confirmed good linearity between peak areas and concentration. The linearity graphs of FLU and VIL were shown in Figures 7a and b respectively.

Table 4: Linearity data

S.NO	FLU		VIL	
	Concentration (µg/mL)	**Peak area***	**Concentration (µg/mL)**	**Peak area***
1	25	346062	6.25	128062
2	50	687264	12.5	261164
3	75	1056513	18.75	378313
4	100	1382531	25	502516
5	125	1701130	31.25	634177
6	150	2059146	37.5	763640
	Regression equation $y = 13690x + 6502.3$ $R^2 = 0.9997$		Regression equation $y = 20254x + 1356.5$ $R^2 = 0.9998$	

* = Average peak area of 3 replicate injections for each concentration

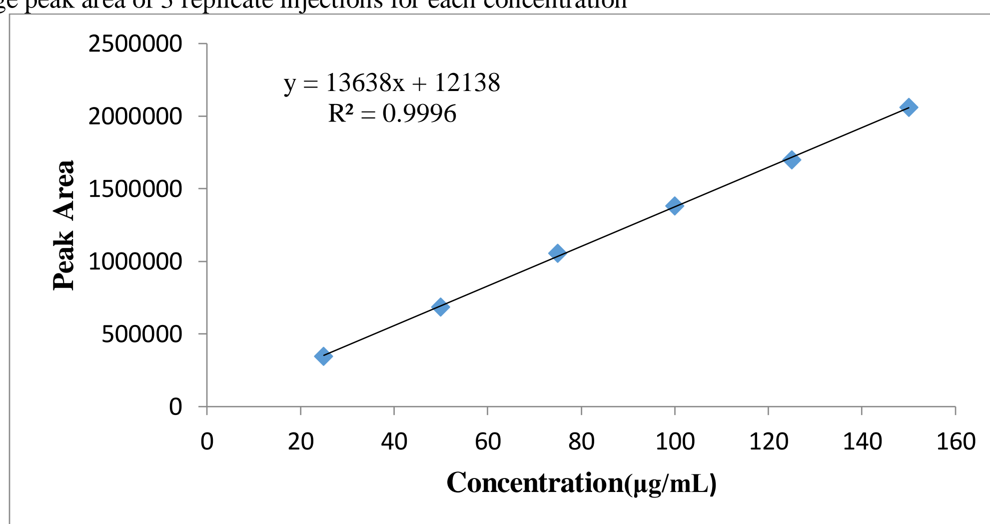

Fig. 7a: Standard curve of Fluticasone furoate

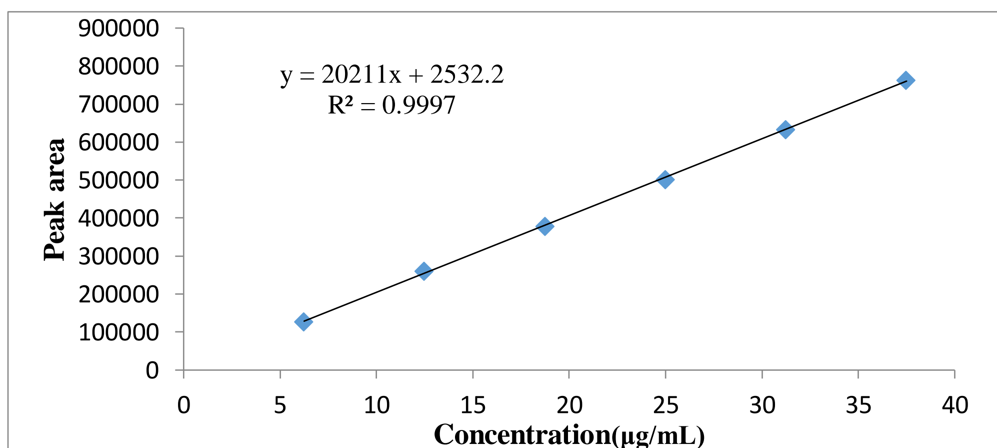

Fig. 7b: Standard curve of Vilanterol trifenatate

LoD and LoQ:
The Limit of Detection and Limit of Quantification of FLU and VIL were calculated by using following equations (ICH, Q2 (R1)). The LoD and LoQ values are reported in table 5.
These $LoD = 3.3 \times \sigma/S$ and $LoQ = 10 \times \sigma/S$
Where σ = the standard deviation of the response and S = slope of the calibration curve.

Table 5: LoD and LoQ data

Drug name	LoD (µg/mL)	LoQ (µg/mL)
FLU	1.51	4.56
VIL	0.10	0.30

Robustness:
The system suitability parameters such as resolution, RSD, tailing factor, or the theoretical plates of FLU, and VIL remained unaffected by deliberate changes. The results were presented in Table 6, along with the system suitability parameters of normal conditions. Thus, the method was found to be robust with respect to variability in applied conditions.

Table 6: Robustness data

Parameter		System Suitability Parameters				
		RT (min)	Plate count	Peak tailing	Resolution	% RSD
Optimized method	FLU	1.206	3208	1.35	-	1.2
	VIL	1.582	3645	1.07	2.5	0.6
Flow rate (0.4 mL/min)	FLU	1.092	3069	1.23	-	0.9
	VIL	1.286	3298	1.14	2.51	0.7
Flow rate (0.2 mL/min)	FLU	1.309	3195	1.24	-	0.6
	VIL	1.675	3386	1.16	2.53	0.9
Organic Phase (55:45)	FLU	1.132	3246	1.21	-	0.4
	VIL	1.361	3510	1.13	2.86	0.5
Organic Phase (45:55)	FLU	1.311	3498	1.17	-	0.9
	VIL	1.735	3385	1.12	2.5	0.4
Temperature (35°C)	FLU	1.207	3168	1.22	-	0.3
	VIL	1.564	3291	1.04	2.56	0.3
Temperature (25°C)	FLU	1.204	3195	1.17	-	0.3
	VIL	1.575	3324	1.12	2.71	0.2

Forced Degradation Studies:
Blank, placebo, and degradation samples were analyzed with the above mentioned UPLC conditions using a PDA detector to monitor the homogeneity and purity of the FLU and VIL. Degradation was not observed in photolytic stress, humidity, acid, base, water hydrolysis, and thermal stress studies. It was interesting to note that all the peaks due to degradation were well resolved from the peaks of FLU and VIL. Further, the peak purity of FLU and VIL was found to be homogeneous based on the evaluation parameters such as purity angle and purity threshold. The verification of peak purity indicates that there is no interference from degradants, facilitating error-free quantification of FLU and VIL. Hence, the method is considered to be "stability-indicating." The obtained results were shown in Table 7.

Table 7: Forced degradation studies at different stress conditions

Type of degradation	Fluticasone furoate			Vilanterol trifenatate		
	Peak area	Recovered (%)	Degraded (%)	Peak area	Recovered (%)	Degraded (%)
Acid	1269961	93.27	6.73	484860	95.95	4.05
Base	1286862	94.51	5.49	483866	95.76	4.24
Neutral	1350495	99.18	0.82	502579	99.46	0.54
Peroxide	1256307	92.27	7.73	486263	96.23	3.77
Thermal	1318732	96.85	3.15	495221	98.01	1.99
UV light	1344150	98.72	1.28	498571	98.67	1.33

CONCLUSION:

A simple and rugged RP-UPLC method has been developed for the simultaneous determination of fluticasone furoate and vilanterol trifenatate in active pharmaceutical ingredients and sample. The proposed method was validated in accordance with ICH guidelines by testing its parameters which include system suitability, specificity, precision, linearity, LoD, LoQ, accuracy and robustness. The method was very specific to separate the peaks of active pharmaceutical ingredients from the degradation products which were obtained with good resolution after forced degradation studies.

Thus, stress induced studies prove the effectiveness of the proposed stability indicating RP-UPLC method which can be adopted in routine analysis in pharmaceutical industries.

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