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Research Article

Stability indicating RP-HPLC method development and validation for the simultaneous estimation of vilanterol and umeclidinium bromide in bulk and pharmaceutical dosage forms

Diviti Sai Sindhu [1], S. Shoba Rani[2]

[1]M. Pharmacy Scholar, Department of Pharmaceutical Analysis, Institute of Science and Technology, JNTUH, Hyderabad
[2]Associate Professor, NSS Co-Ordinator & PIU at Centre for Pharmaceutical Sciences, Institute of Science and Technology, JNTUH, Kukatpally, Hyderabad, Telangana

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Vilanterol and Umeclidinium bromide in dosage form. Chromatogram was run through BDS C18 (150 x 4.6 mm, 5.0µ). Mobile phase containing Buffer 0.1% Formic acid: Acetonitrile taken in the ratio 65:35 was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 265 nm. Retention time of Umeclidinium and Vilanterol were found to be 2.363 min and 3.101 min. %RSD of the Vilanterol and Umeclidinium bromide were and found to be 0.4and0.7respectively. %Recovery was obtained as 99.49%and 101.14% for Vilanterol and Umeclidinium bromide respectively. LOD, LOQ values obtained from regression equations of Vilanterol and Umeclidinium bromide were 0.19, 0.56 and 0.59, 1.8 respectively. Regression equation of Vilanterol is y = 69945x + 7045 and y = 89939x + 60718 of Umeclidinium bromide. Retention times were decreased and that runtime was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Vilanterol, Umeclidinium bromide, RP-HPLC

INTRODUCTION

Vilanterol is approved by the FDA in December 2013 for use in combination with umeclidinium bromide. Vilanterol is a selective long-acting beta2-adrenergic agonist (LABA) with in herent24-hour activity for once daily treatment of COPD and asthma. The combination drug is marketed by GSK (Glaxo smith kline) under the brand Anoro Ellipta.

Umeclidinium bromide is a long-acting muscarinic antagonist (LAMA) used as maintenance treatment for symptoms of chronic obstructive pulmonary disease (COPD). It is available as a once-daily inhalation mono therapy or as a fixed-dose.

Combination product with the long-acting beta2-agonist vilanterol Its use has been shown to provide

Address for Correspondence: Diviti Sai Sindhu, M. Pharmacy Scholar, Department of Pharmaceutical Analysis, Institute of Science and Technology, JNTUH, Hyderabad.

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clinically significant, sustained improvements in lung function.

The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product[4].

Literature survey revealed few analytical methods is reported for both the drugs in alone. The aim ofthe present study was to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method with UV detection for the analysis of Vilanterol and umeclidinium bromide in bulk samples and combined dosage formulation.

Objective: Following are the objectives of the present work:

- To develop a new stability indicating HPLC method for the simultaneous estimation of Vilanterol and Umeclidinium bromide and to develop the validated method according to ICH guidelines.
- To apply the validated method for the simultaneous estimation of Vilanterol and Umeclidinium bromide in pharmaceutical formulation.

EXPERIMENTAL

Chemicals and reagents: Vilanterol and Umeclidinium bromide pure drugs (API) were from Rankem and marked formulation Vilanterol and Umeclidinium bromide inhaler (AnoroEllipta), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer and Ortho-phosphoric acid, were purchased from Rankem, Mumbai

Apparatus and chromatographic condition: 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 2mm and 10mm and matched quartz cells integrated with UV-win 6 The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1.0mL/min and the detector wavelength was set at 260nm injection volume was 10µL. Diluent used was Acetonitrile and Water taken in the ratio of 50:50.

Preparation of standard and sample solutions
Standard solution: Accurately weighed 2.5mg of Vilanterol, 6.25 mg of Umeclidinium bromide and transferred to 10ml volumetric flask and 3/4th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (250µg/ml of Vilanterol and 625µg/ml of Umeclidinium)

Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (25µg/ml of Vilanterol and 62.5µg/ml of Umeclidinium)

Sample Solution: The contents of nasal spray delivered by 50actuations (25 & 62.5mcg each) were collected in 100ml volumetric flask. Then 20ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 1110 & 500µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45µm filters using (Millipore, Milford, PVDF). 2ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (25µg/ml of Vilanterol and 62.5µg/ml of Umeclidinium)

Procedure: Inject 10µL of the standard and sample solution separately into the chromatographic system and measure the peak areas for vilanterol and umeclidinium bromide and calculate the %assay value.

RESULTS AND DISCUSSIONS

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines. Obtained validation parameters are presented in Table 1.

Linearity: The calibration curve was constructed by plotting response factor against respective concentration of vilanterol and umeclidinium. The plots of peak area Vs respective concentration of vilanterol and umeclidinium bromide were found to be linear in the range of 6.25-37.5 µg/mL and 15.625-93.75µg/mL with coefficient of correlation (r^2) 0.999 for two drugs. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for vilanterol and umeclidinium bromide were given in Fig.5 and Fig.6.

Accuracy: 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 101.14%

and 99.49% for Vilanterol and Umeclidinium bromide respectively. The obtained results are presented in Table 2.

Sensitivity: The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to 0.19µg/ml and 0.56µg/ml for vilanterol and 0.59µg/ml and 1.80µg/ml for umeclidinium bromide. The LOD and LOQ showed that the method is sensitive for vilanterol and umeclidinium bromide in table 4.

System suitability test: The specificity of this method was determined by complete separation of Vilanterol and Umeclidinium bromide as shown in Fig. 3 with parameters like retention time, resolution and tailing factor. The tailing factor for peaks of Vilanterol and Umeclidinium bromide was less than 2% and resolution was satisfactory. The average retention time for Vilanterol and Umeclidinium bromide were 2.358min and 3.099min respectively for five replicates. The peaks obtained for Vilanterol and Umeclidinium bromide were sharp and have clear baseline separation. Analysis was also performed for active Vilanterol and Umeclidinium bromide, placebo sample (All the ingredients except active Vilanterol and Umeclidinium bromide) both at stressed and unstressed condition. After analysis it was found that there is no interference of peak in the placebo& active sample. Hence the developed method was specific for the analysis of this product**.**

Precision: From a single volumetric flask of working standard solution six injections were given. A study was carried out for intermediate precision with the same analyst on the different day for six sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, temperature and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The assay results of tablet dosage formulation by the proposed method are presented in Table 3.

Stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of vilanterol and umeclidinium bromide remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table 7.

Assay sample: The contents of nasal spray delivered by 50actuations (25 & 62.5mcg each) were collected in 50ml volumetric flask. Then 20ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 1110 & 500µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45µm filters using (Millipore, Milford, PVDF). 2ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (25µg/ml of Vilanterol and 62.5µg/ml of Umeclidinium)

Acid degradation sample: To 1ml of stock solution Vilanterol and Umeclidinium bromide, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solution and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Fig.7.

Base degradation sample: To 1ml of stock solution Vilanterol and Umeclidinium bromide, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Fig. 8.

Peroxide degradation sample: To 1 ml of stock solution of Vilanterol and Umeclidinium bromide, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of oxidative degradation was giveninFig.9.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 25µg/ml & 62.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

The typical chromatogram of oxidative degradation was given in Fig.10.

Photo Stability studies: The photo chemical stability of the drug was also studied by exposing the 250µg/ml & 625µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200-Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of thermal degradation was given in Fig. 11.

CONCLUSION

A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc. In the present method a BDS C18 (4.6x150mm, 5µm) column has been used For vilanterol and umeclidinium bromide respectively. Mobile phase used was Buffer 0.1% Formic acid: Acetonitrile (65:35) for vilanterol and umeclidinium respectively, Retention of vilanterol and umeclidinium bromide has more dependence on the mobile phase. The separation of the two peaks was also dependent on the buffer and the percentage of mobile phases. Vilanterol and umeclidinium bromide were eluted at acceptable retention times and got good resolution. Several assay methods has been developed for the determination of vilanterol and umeclidinium bromide in pharmaceutical dosage forms and in biological fluids but this method is most economic and accurate so this method is very useful for the determination of vilanterol and umeclidinium bromide in bulk and pharmaceutical dosage forms. This method was validated as per ICH-Q2(R1) guidelines and met the regulatory requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of vilanterol and umeclidinium bromide.

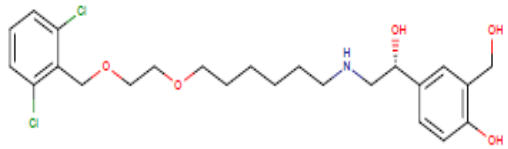

Figure-1: Chemical Structure of Telmisartan

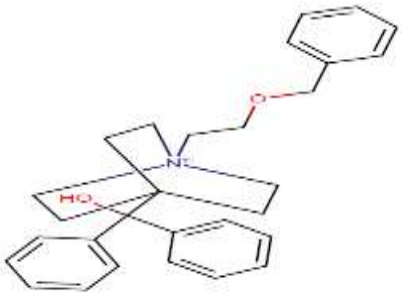

Figure-2: Chemical Structure of umeclidinium bromide

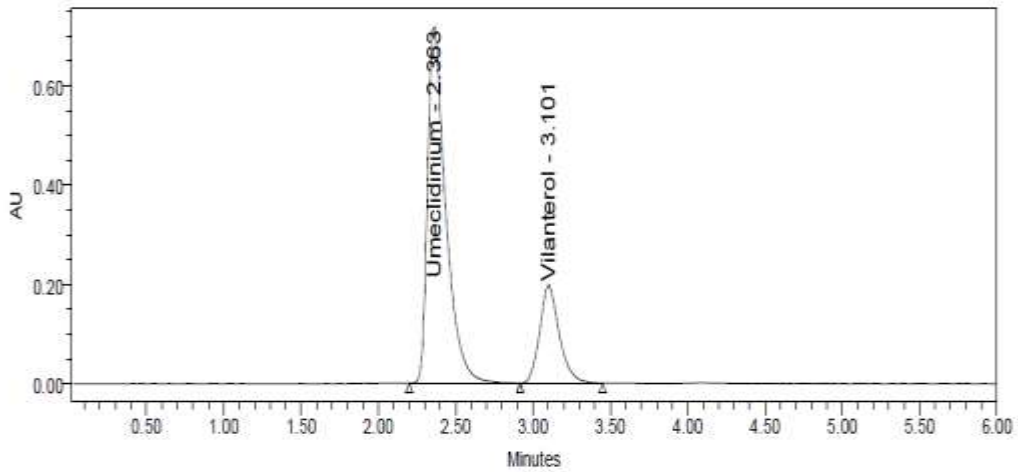

Fig -3 Optimized Chromatogram of vilanterol and umeclidinium

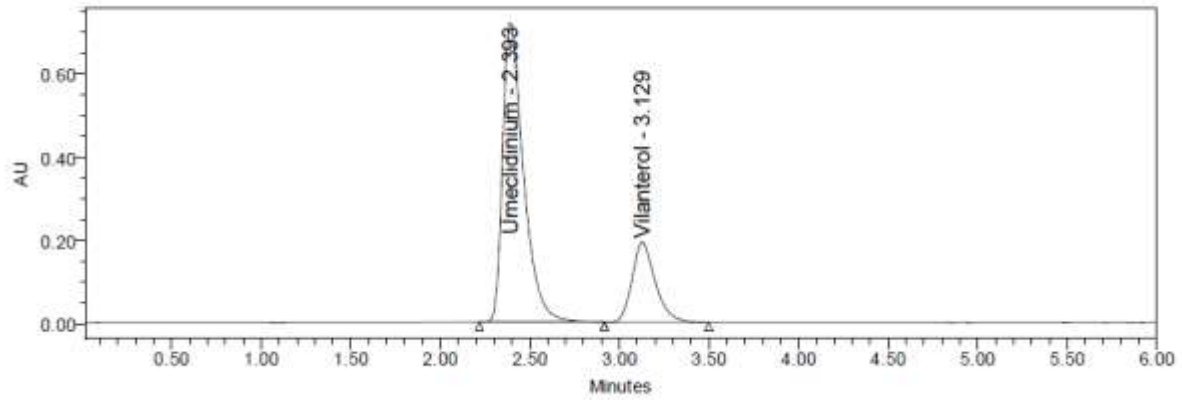

Fig No. 4 Chromatogram of working sample solution

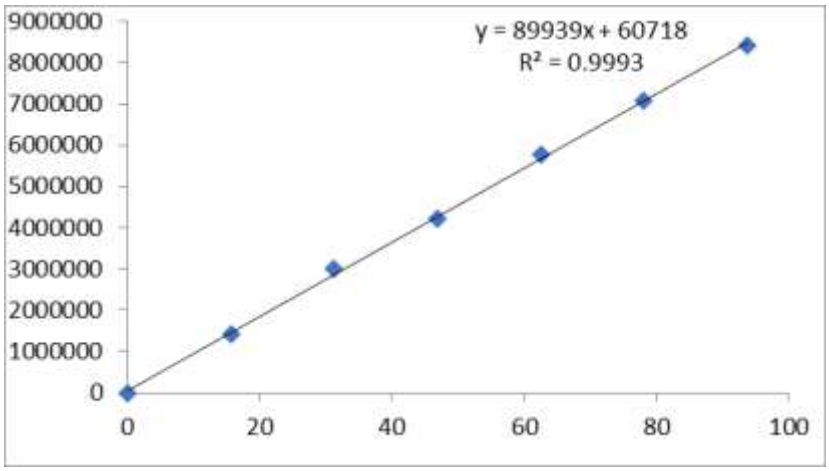

Fig.5: Calibration curve of umeclidinium

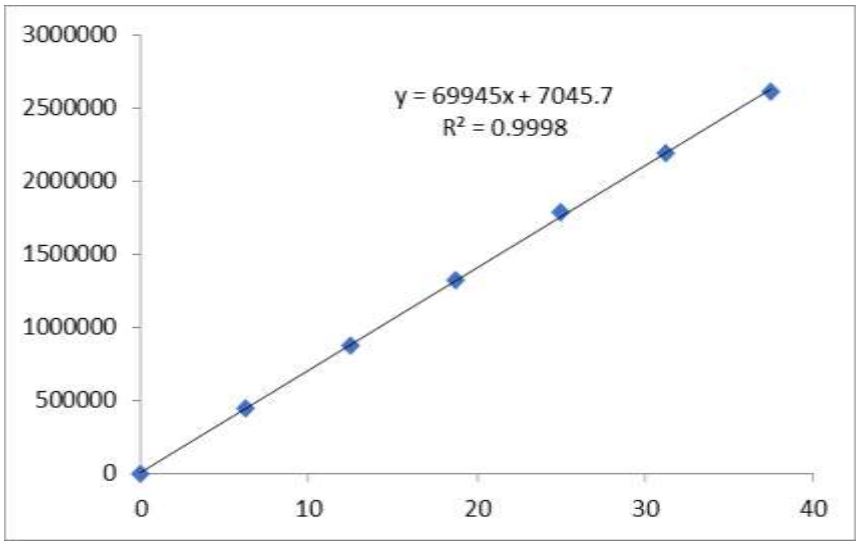

Fig.6: Calibration curve for vilanterol

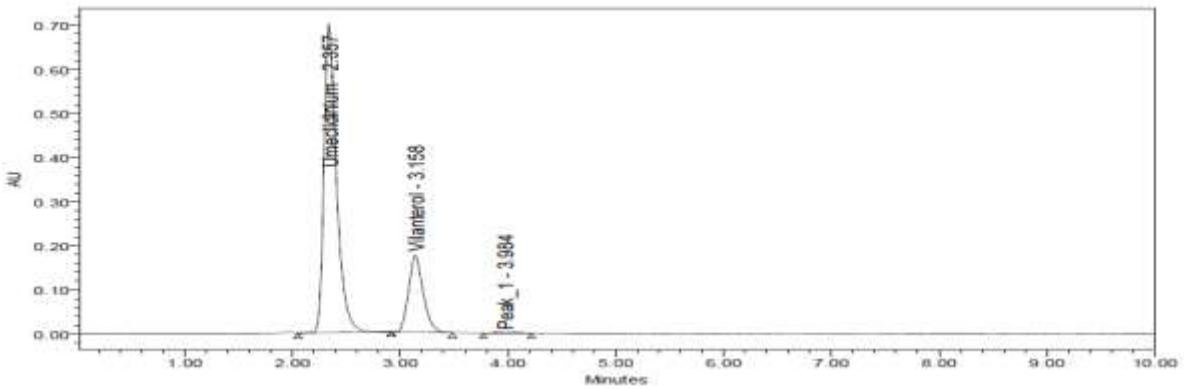

Fig.7: Acid degradation chromatogram of vilanterol and umeclidinium

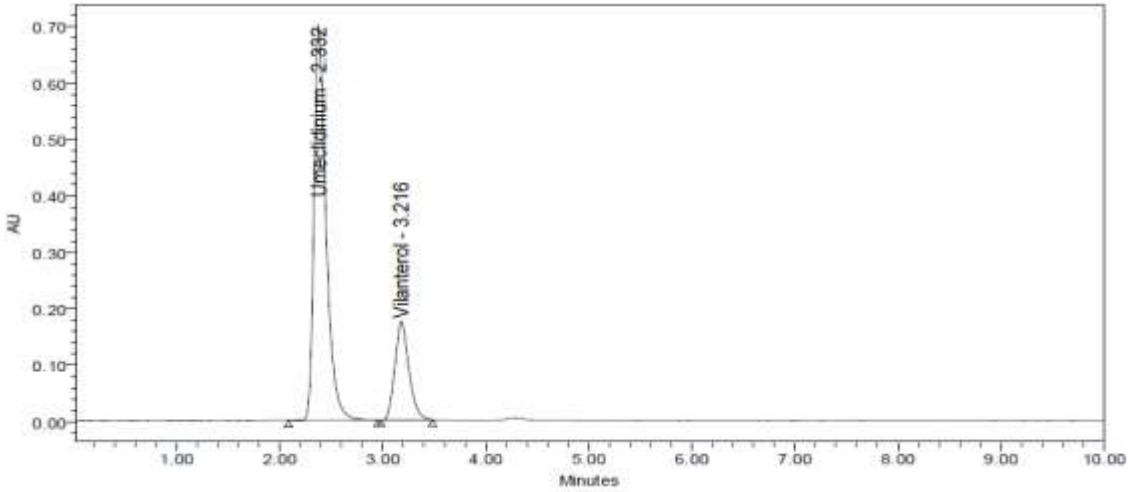

Fig.8: Base degradation chromatogram of vilanterol and umeclidinium

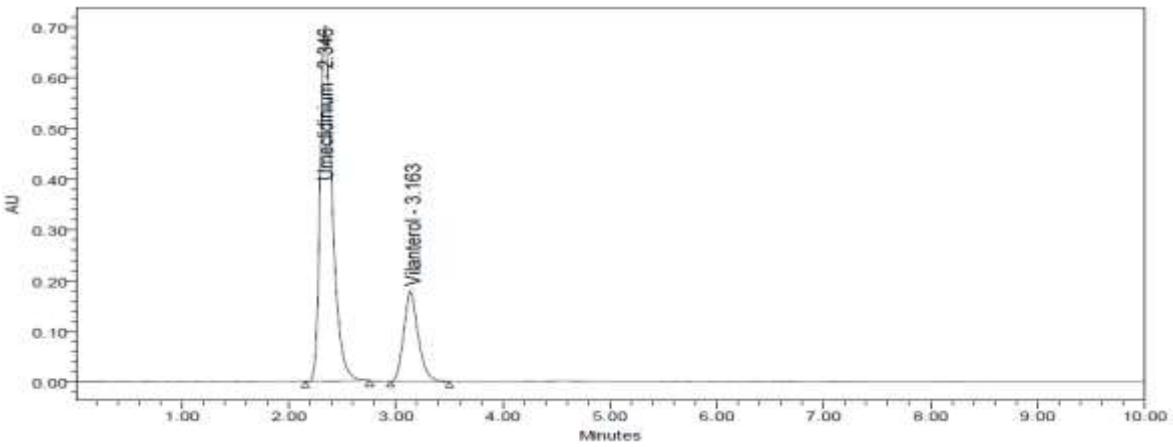

Fig.9: Peroxide degradation chromatogram of vilanterol and umeclidinium

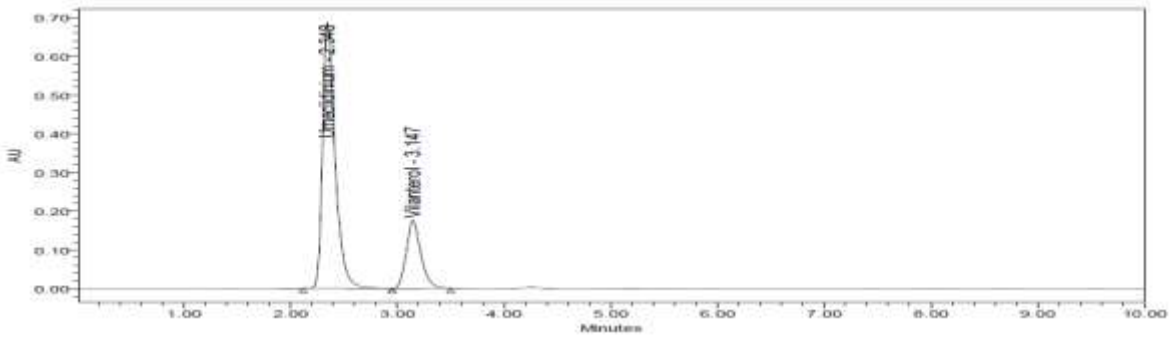

Fig.10: Water degradation chromatogram of vilanterol and umeclidinium

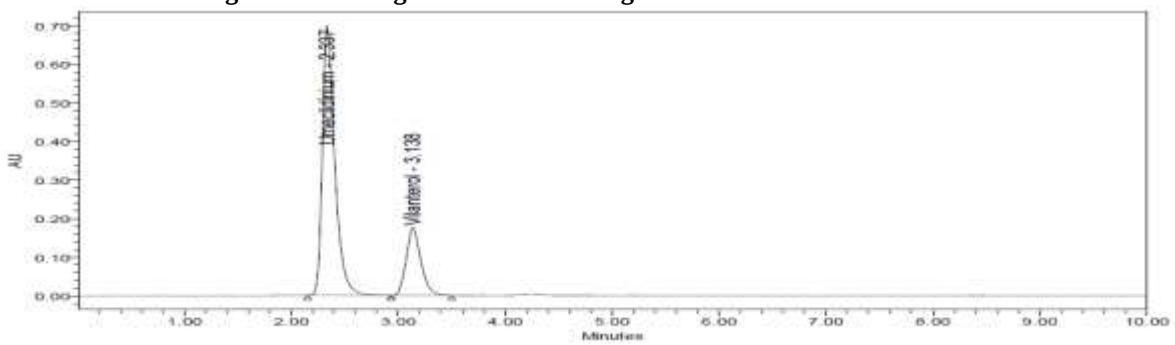

Fig. 11: Thermal degradation chromatogram of vilanterol and umeclidinium

Table1: Analytical validation parameters (System suitability and Linearity)

Parameter	**Vilanterol**	**Umeclidinium bromide**
Linearity	6.25-37.5 µg/ml	15.625-93.75µg/ml
Slope	69945	89939
Intercept	7045	60718
Regression equation (Y=mx+c)	y = 69945x + 7045	y = 89939x + 60718
Linearity Range (µg/ml)	6.25-37.5 µg/ml	15.625-93.75µg/ml
System precision %RSD	0.7	0.4
Method precision %RSD	0.8	0.6
LOD	0.19	0.59
LOQ	0.56	1.80
Theoretical Plates	2913	2109
Tailing Factor	1.22	1.53
Retention Time(min)	3.101	2.363

Table2: Accuracy table of vilanterol and umeclidinium

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery	% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.44	98.48	101.13%	50%	31.25	31.04	99.34	100.11%
	12.5	12.49	99.94			31.25	31.21	99.88	
	12.5	12.65	101.18			31.25	31.29	100.14	
100%	25	25.93	99.70		100%	62.5	61.73	100.55	
	25	25.07	100.30			62.5	62.98	100.77	
	25	24.93	99.92			62.5	63.02	100.82	
150%	37.5	37.43	99.82		150%	93.75	93.22	99.44	
	37.5	37.65	100.41			93.75	93.51	99.74	
	37.5	37.65	101.40			93.75	94.08	100.36	

Table3: Precision of vilanterol and umeclidinium

Drug	Sample Weight(mg)	Inter-day precision		System precision		Repeatability	
		SD	%RSD	SD	%RSD	SD	%RSD
Vilanterol	2.5	7470.9	0.4	12161.6	0.7	13076.0	0.8
Umeclidinium	6.25	12243.7	0.2	24323.9	0.4	36309.0	0.6

Table 4 Sensitivity table of Umeclidinium and Vilanterol

Molecule	LOD	LOQ
Umeclidinium	0.59	1.80
Vilanterol	0.19	0.56

Table 5: Robustness data for Umeclidinium and Vilanterol.

S.no	Condition	%RSD of Umeclidinium	%RSD of Vilanterol
1	Flow rate (-) 0.9ml/min	0.3	0.5
2	Flow rate (+) 1.1ml/min	0.8	1.4
3	Mobile phase (-) 70B:30A	0.5	0.8
4	Mobile phase (+) 60B:40A	0.6	1.2
5	Temperature (-) 25°C	0.7	1.1
6	Temperature (+) 35°C	0.3	1.5

Table6: Assay result of pharmaceutical dosage formulation

Drug	Label strength(mcg)	%Assay
Vilanterol	25	99.49%
Umeclidinium	62.5	100.14%

Table7: Forced degradation studies of vilanterol and umeclidinium

Type of degradation	Umeclidinium		Vilanterol	
	%RECOVERED	% DEGRADED	%RECOVERED	% DEGRADED
Acid	94.56	5.44	94.57	5.43
Base	95.35	4.65	95.52	4.48
Peroxide	95.89	4.11	95.74	4.26
Thermal	97.40	2.60	96.91	3.09
Uv	97.94	2.06	97.79	2.21
Water	99.15	0.85	99.48	0.52

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