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NEW STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF VILANTEROL AND UMECLIDINIUM BROMIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT
A simple, Accurate, precise method was developed for the simultaneous estimation of the Umeclidinium and Vilanterol in bulk and pharmaceutical dosage form. Chromatogram was run through Altima C18 150 x 4.6mm, 5.0µ. Mobile phase containing Buffer 0.01N Na2hpo4: acetonitrile taken in the ratio 60:40v/v was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 265 nm. Retention time of Umeclidinium and Vilanterol were found to be 2.280 min and 2.857 min. %Recovery was obtained as 100.06% and 99.66% for Umeclidinium and Vilanterol respectively. LOD, LOQ values obtained from regression equations of Umeclidinium and Vilanterol were 0.19, 0.31 and 0.58, 0.93 respectively. Regression equation of Umeclidinium is y = 25083x + 17380, and y = 23963x + 2725.2of Vilanterol.

KEYWORDS: Umeclidinium, Vilanterol, RP-HPLC.

INTRODUCTION
Vilanterol is a long-acting, selective beta2-adrenergic agonist (LABA) with intrinsic 24-hour action for once daily COPD and asthma diagnosis. Its pharmacological activity is due to intracellular adenylyl cyclase stimulation that catalyzes the transformation of adenosine triphosphate (ATP) into cyclic-3',5'-adenosine monophosphate (cAMP). It id chemically called as 4-[(1R)-2-[(6-{2-[(2,6-dichlorophenyl) methoxy]ethoxy} hexyl)amino]-1-hydroxyethyl]-2-(hydroxymethyl)phenol

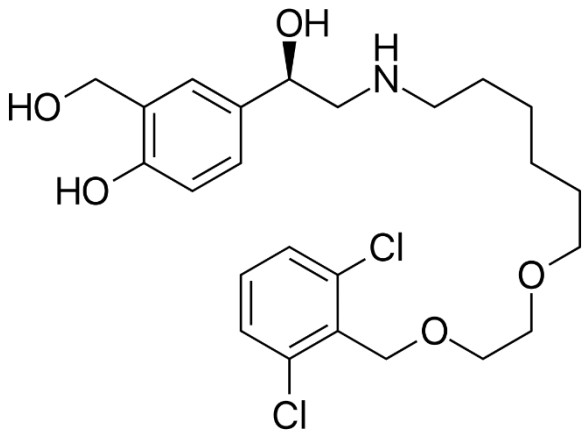

Figure 1: chemical structure of Vilanterol.

Umeclidinium is a long-acting muscarinic antagonist (LAMA), used to treat chronic obstructive pulmonary disease (COPD) symptoms as a preventive treatment. It is available as a single-daily inhalation monotherapy or a combination of a fixed-dose drug with the long-acting beta2-agonist vilanterol. COPD is a progressive

obstructive pulmonary disease characterized by shortness of breath, cough, sputum output and persistent impaired airflow. It is chemically called as 1-[2-(benzyloxy)ethyl] – 4 - (hydroxydiphenylmethyl) - 1-azabicyclo[2.2.2] octan-1-ium bromide.

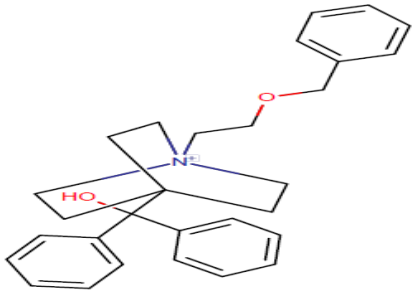

Figure 2: chemical structure of Umeclidinium.

MATERIALS AND METHODS

Materials

Umeclidinium and Vilanterol pure drugs (API), Combination Umeclidinium and Vilanterol (Annora Ellipta), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instruments

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, Waters HPLC 2695 system equipped with TUV detector with Empower 2 Software, UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Umeclidinium and Vilanterol solutions.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and 0.01N KH_2PO_4 (potassium di hydrogen phosphate) taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 62.5 mg of Umeclidinium, 25 mg of Vilanterol and transferred to individual 50ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (625µg/ml of Umeclidinium and 250µg/ml of Vilanterol)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (62.5µg/ml Umeclidinium of and 25µg/ml of Vilanterol)

Preparation of Sample stock solutions: The contents of nasal spray deliveried by 50 actuations (62.5 & 25 mcg each) were collected in 100 ml volumetric flask. Then 20ml acetonitrile was added , sonicated for 25 min and made up to mark to yield 1100 & 500µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF) (625µg/ml of Umeclidinium and 250µg/ml of Vilanterol)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (62.5µg/ml of Umeclidinium and 25µg/ml of Vilanterol)

Preparation of buffer

0.1% OPA Buffer: 1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

0.01N NA_2HPO4 Buffer (Disodium hydrogen phosphate): Accurately weighed 1.42gm of Disodium hydrogen phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 4.8 with dil. Orthophosphoric acid solution.

Method Validation

System Suitability: The system suitability parameters were determined by preparing standard solutions of Umeclidinium 62.5µg/ml and Vilanterol 25 µg/ml. The solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Specificity of a method was determined by testing standard substances against potential interferences. There should not find interfering peaks in

the blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Preparation of Standard stock solutions: Accurately weighed 62.5 mg of Umeclidinium, 25 mg of Vilanterol and transferred to individual 50ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (625µg/ml of Umeclidinium and 250µg/ml of Vilanterol)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (15.625µg/ml of Umeclidinium and 6.25µg/ml of Vilanterol)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (31.25µg/ml of Umeclidinium and 12.5µg/ml of Vilanterol)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (46.875µg/ml of Umeclidinium and 18.75µg/ml of Vilanterol)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (62.5µg/ml of Umeclidinium and 25µg/ml of Vilanterol)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (78.125µg/ml of Umeclidinium and 31.25µg/ml of Vilanterol)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (93.75µg/ml of Umeclidinium and 37.5µg/ml of Vilanterol)

Accuracy

Preparation of Standard stock solutions: Accurately weighed 62.5 mg of Umeclidinium, 25 mg of Vilanterol and transferred to individual 50ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (625µg/ml of Umeclidinium and 250µg/ml of Vilanterol)

Preparation of 50% Spiked Solution: 1ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 2.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 2.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Umeclidinium, Vilanterol, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Umeclidinium, Vilanterol, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies

Oxidation

To 1 ml of stock solution of Umeclidinium and Vilanterol, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 62.5µg/ml & 25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

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

Alkali Degradation Studies

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

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 62.5µg/ml & 25µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 625µg/ml & 250µg/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 62.5µg/ml & 25µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

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 62.5µg/ml & 25µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION

Optimized method:

Mobile phase : 0.01N Na2hpo4: Aceonitril (60:40 v/v)
Flow rate : **1** ml/min
Column : Altima C18 (4.6 x 150mm, 5µm)
Detector wave length : 265.0nm
Column temperature : 30°C
Injection volume : 10.0µL
Run time : 5.0 min
Diluent : Water and Acetonitrile in the ratio 50:50 v/v

Results: Both peaks are eluted with good Peak shape and all System suitability parameters was satisfactory so, this trail was optimized.

Neutral Degradation Studies:

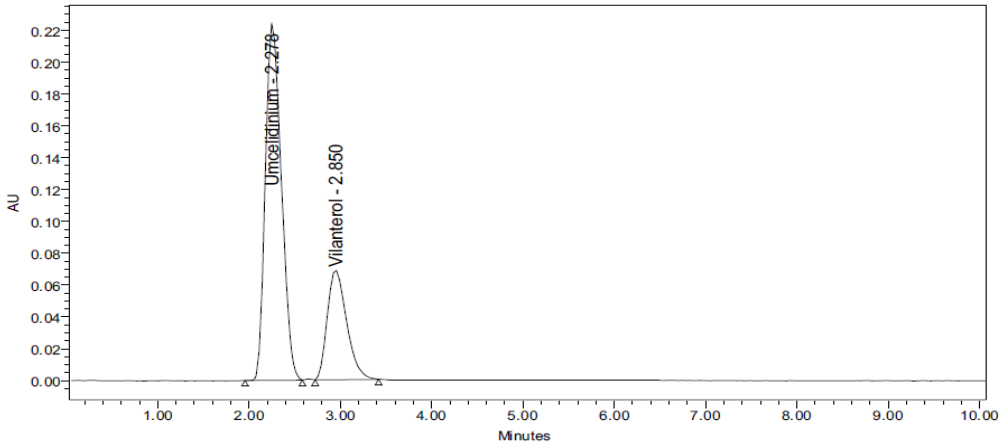

Fig 3: Optimized Chromatogram.

Observation: Umeclidinium and Vilanterol were eluted at 2.278 min and 2.850 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table: 1 System suitability parameters for Umeclidinium and Vilanterol.

S no	Umeclidinium			Vilanterol			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.280	2920	1.44	2.855	3791	1.33	3.2
2	2.280	2931	1.42	2.855	3756	1.34	3.2
3	2.280	3037	1.41	2.855	3846	1.33	3.2
4	2.280	3017	1.40	2.856	3808	1.33	3.2
5	2.280	2886	1.43	2.857	3762	1.32	3.2
6	2.281	2945	1.42	2.857	3792	1.30	3.2

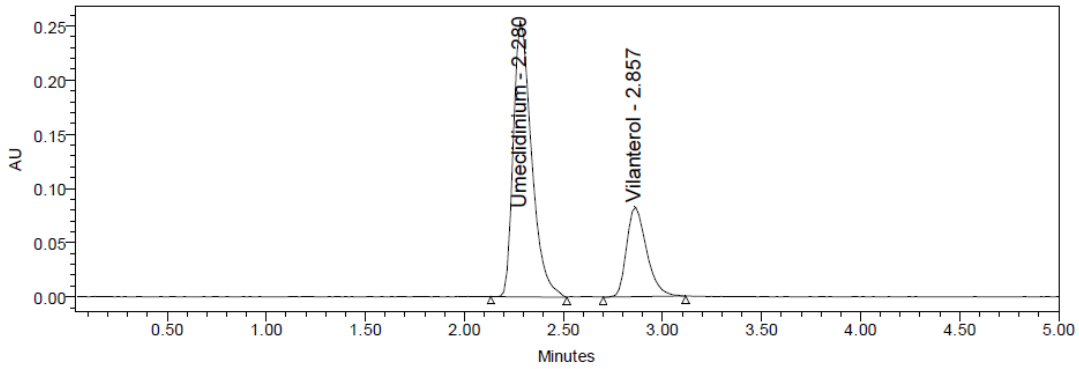

Fig 4: System suitability Chromatogram.

Discussion

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2.

All the system suitable parameters were passed and were within the limits.

Method Validation

Specificity

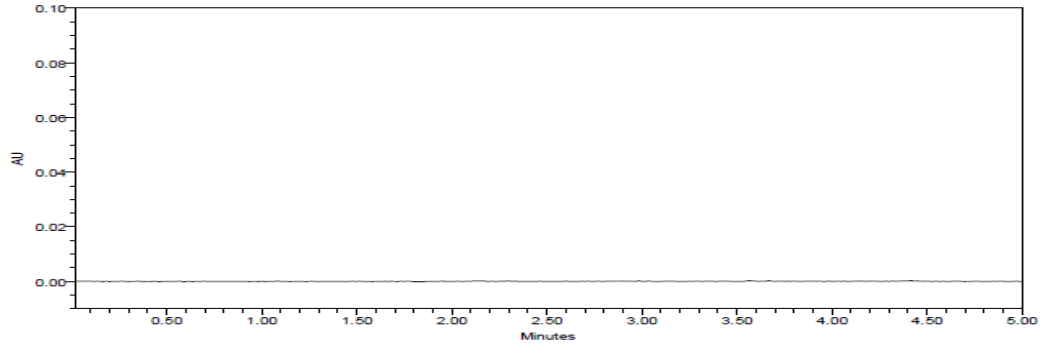

Figure No. 5: Chromatogram of blank.

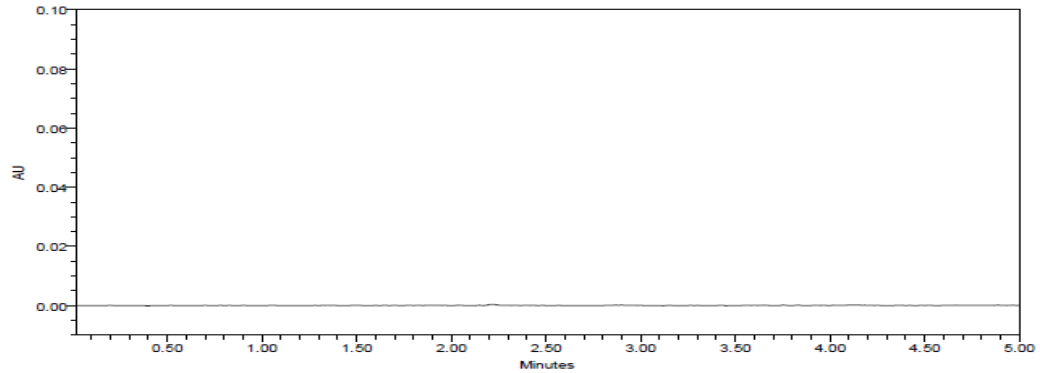

Figure No. 6: Chromatogram of placebo.

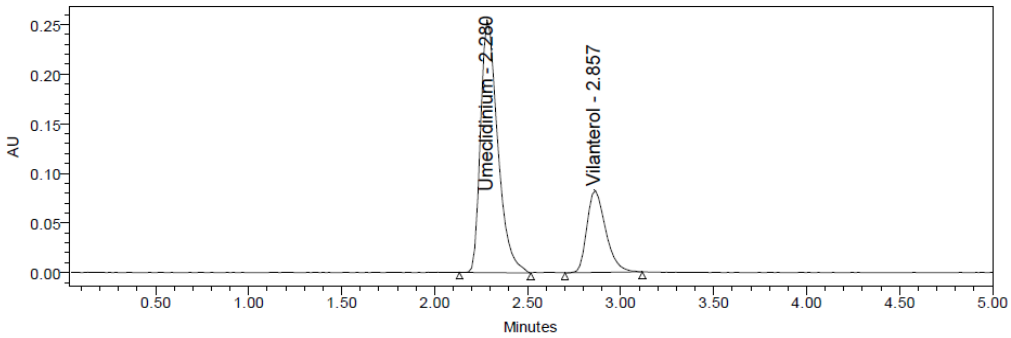

Figure No. 7: Optimized chromatogram.

Discussion

Retention times of Umeclidinium and Vilanterol were 2.280 min and 2.857 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Table 2: Linearity table for Umeclidinium and Vilanterol.

Umeclidinium		Vilanterol	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
15.625	404999	6.25	150754
31.25	819090	12.5	304712
46.875	1226473	18.75	454343
62.5	1583535	25	603269
78.125	1934332	31.25	757052
93.75	2383550	37.5	894150

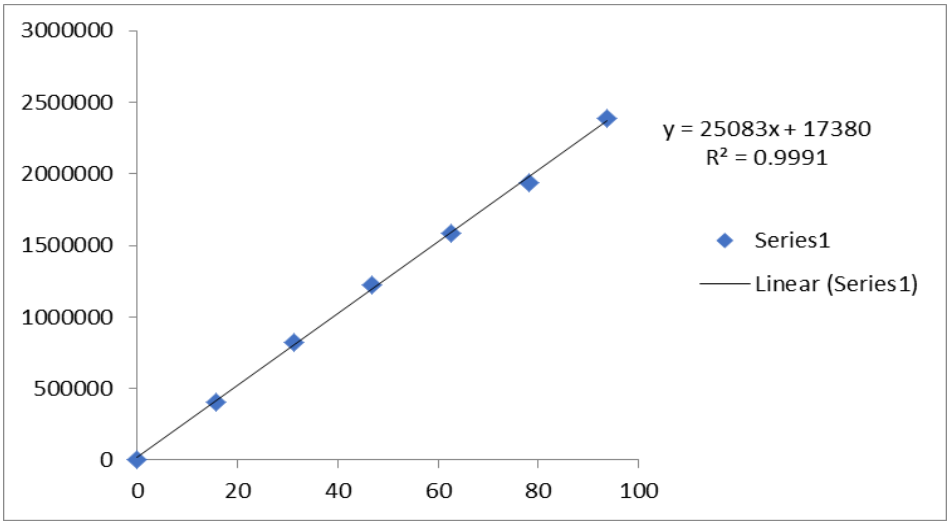

Fig No. 8: Calibration curve of Umeclidinium.

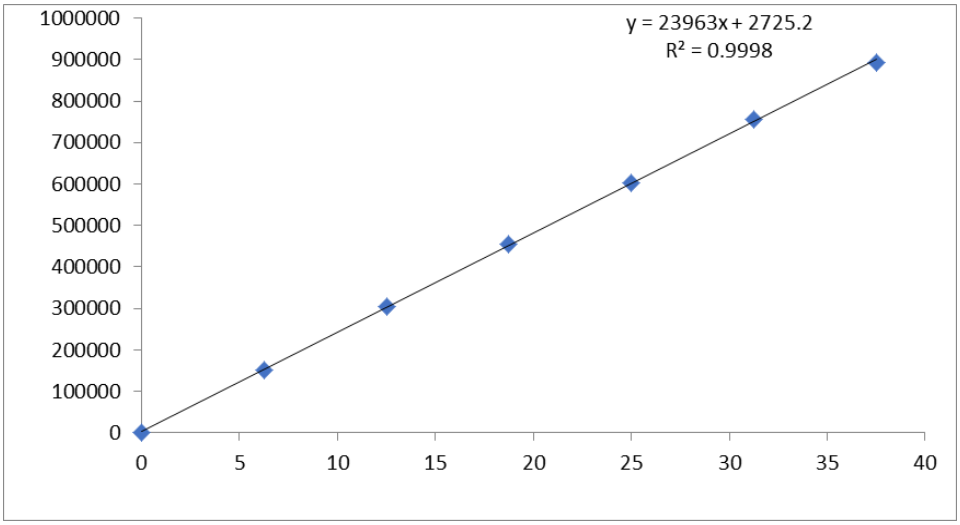

Fig No. 9: Calibration curve of Vilanterol.

Discussion

Six linear concentrations of Umeclidinium (15.625-93.75µg/ml) and Vilanterol (6.25-37.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Umeclidiniumwas y = 25083x + 17380 and of Vilanterolwas y = 23963x + 2725.2.Correlation coefficient obtained was 0.999 for the two drugs.

Precision

System Precision

Table 3 System precision table of Umeclidinium and Vilanterol.

S. No	Area of Umeclidinium	Area of Vilanterol
1.	1529150	606413
2.	1554755	605365
3.	1542763	606231
4.	1521695	607785
5.	1516671	602667
6.	1522754	605600
Mean	1531298	605677
S.D	14597.7	1700.3
%RSD	1.0	0.3

Discussion

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 1.0% and 0.3% respectively for Umeclidinium and Vilanterol. As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability

Table 4 Repeatability table of Umeclidinium and Vilanterol.

S. No	Area of Umeclidinium	Area of Vilanterol
1.	1528899	608153
2.	1517491	598489
3.	1552532	605841
4.	1516621	601414
5.	1542446	603340
6.	1539144	604783
Mean	1532856	603670
S.D	14380.3	3408.5
%RSD	0.9	0.6

Discussion

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.9% and 0.6% respectively for Umeclidinium and Vilanterol. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_ Day Precision)

Table: 5 Intermediate precision table of Umeclidinium and Vilanterol.

S. No	Area of Umeclidinium	Area of Vilanterol
1.	1503513	590338
2.	1517082	598754
3.	1530168	599310
4.	1520839	599024
5.	1527635	592436
6.	1517765	598372
Mean	1519500	801125
S.D	9446.9	3930.4
%RSD	0.6	0.5

DISCUSSION

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and

obtained as 0.6% and 0.5% respectively for Umeclidinium and Vilanterol. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Table: 6 Accuracy table of Umeclidinium.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	31.25	31.47	100.69	100.06%
	31.25	31.35	100.31	
	31.25	31.33	100.25	
100%	62.5	62.25	99.60	
	62.5	62.69	100.31	
	62.5	62.94	100.70	
150%	93.75	92.44	98.61	
	93.75	94.18	100.46	
	93.75	93.41	99.64	

Table: 7 Accuracy table of Vilanterol.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.45	99.57	99.66%
	12.5	12.41	99.29	
	12.5	12.30	98.40	
100%	25	24.75	98.98	
	25	24.95	99.80	
	25	25.21	100.83	
150%	37.5	37.80	100.81	
	37.5	37.24	99.31	
	37.5	37.48	99.94	

Discussion

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.06% and 99.66% for Umeclidinium and Vilanterol respectively.

Sensitivity

Table 8: Sensitivity table of Umeclidinium and Vilanterol.

Molecule	LOD	LOQ
Umeclidinium	0.31	0.93
Vilanterol	0.19	0.58

Robustness

Table 9: Robustness data for Umeclidinium and Vilanterol.

S.no	Condition	%RSD of Umeclidinium	%RSD of Vilanterol
1	Flow rate (-) 0.9ml/min	0.3	1.3
2	Flow rate (+) 1.1ml/min	0.5	0.3
3	Mobile phase (-) 65B:35A	1.3	0.6
4	Mobile phase (+) 55B:45A	1.0	1.6
5	Temperature (-) 25°C	0.9	0.8
6	Temperature (+) 35°C	0.7	1.0

DISCUSSION

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay: Annora Ellipta bearing the label claim Umeclidinium 62.5mcg, Vilanterol 25mcg. Assay was performed with the above formulation. Average % Assay for Umeclidinium and Vilanterol obtained was 99.70% and 99.27% respectively

Table 10: Assay Data of Umeclidinium.

S.no	Standard Area	Sample area	% Assay
1	1529150	1528899	99.44
2	1554755	1517491	98.70
3	1542763	1552532	100.98
4	1521695	1516621	98.65
5	1516671	1542446	100.33
6	1522754	1539144	100.11
Avg	1531298	1532856	99.70
Stdev	14597.7	14380.3	0.94
%RSD	1.0	0.9	0.9

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Table 11: Assay Data of Vilanterol.

S. no	Standard Area	Sample area	% Assay
1	606413	608153	100.01
2	605365	598489	98.42
3	606231	605841	99.63
4	607785	601414	98.90
5	602667	603340	99.22
6	605600	604783	99.45
Avg	605677	603670	99.27
Stdev	1700.3	3408.5	0.56
%RSD	0.3	0.6	0.6

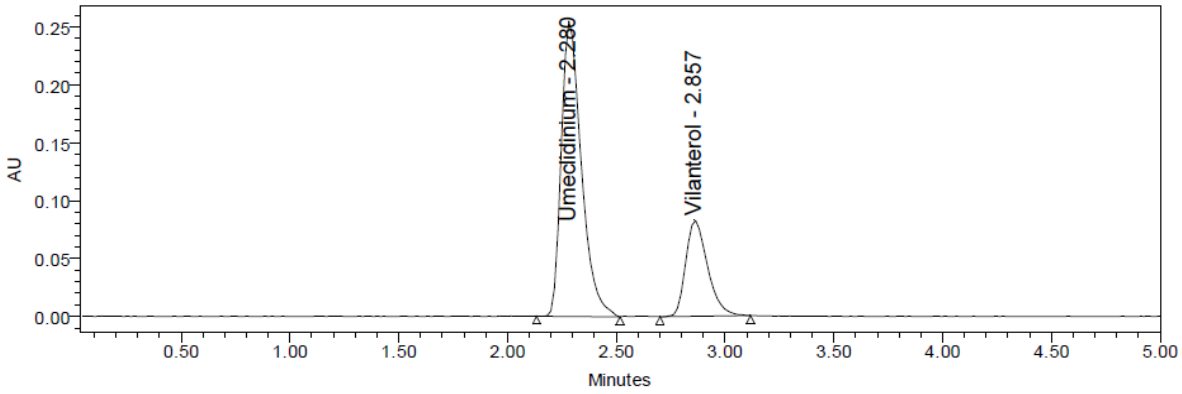

Fig 10: Chromatogram of working standard solution.

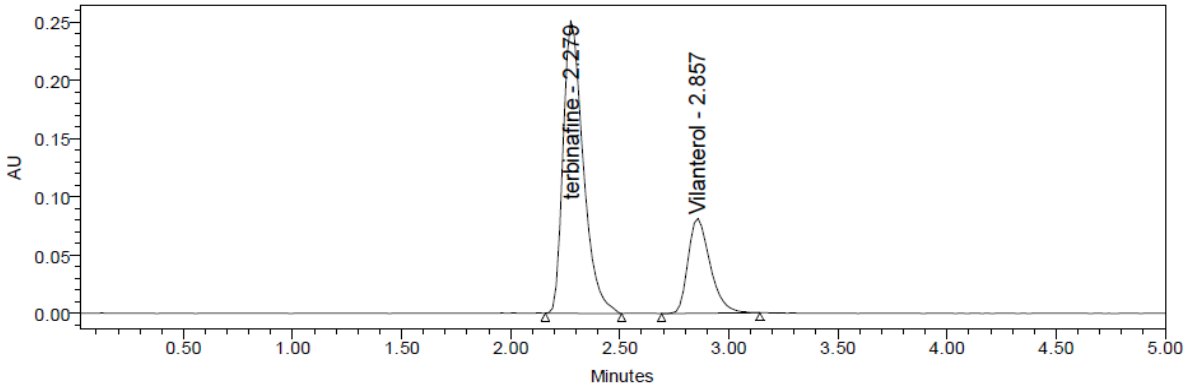

Fig No. 11 Chromatogram of working sample solution.

Degradation data

Table 12: Degradation data of Umeclidinium and Vilanterol.

Type of degradation	Umeclidinium			Vilanterol		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	1446570	94.09	5.91	576970	94.88	5.12
Base	1457527	94.80	5.20	584715	96.15	3.85
Peroxide	1486503	96.69	3.31	583578	95.97	4.03
Thermal	1491961	97.04	2.96	592001	97.35	2.65
Uv	1499150	97.51	2.49	596231	98.05	1.95
Water	1526671	99.30	0.70	602667	99.11	0.89

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Umeclidinium and Vilanterol in bulk and pharmaceutical dosage form. Chromatogram was run through Altima C18 150 x 4.6mm, 5.0µ. Mobile phase containing Buffer 0.01N Na2hpo4: acetonitrile taken in the ratio 60:40v/v was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 265 nm. Retention time of Umeclidinium and Vilanterol were found to be 2.280 min and 2.857 min. %Recovery was obtained as 100.06% and 99.66% for Umeclidinium and Vilanterol respectively. LOD, LOQ values obtained from regression equations of Umeclidinium and Vilanterol were 0.19, 0.31 and 0.58, 0.93 respectively. Regression equation of Umeclidinium is y = 25083x + 17380, and y = 23963x + 2725.2of Vilanterol.

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