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METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF UMECLIDINIUM AND VILANTEROL IN PURE FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract:

A new, simple, precise, rapid, selective and stability reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous quantification of Vilanterol and Umeclidinium in pure form and its pharmaceutical dosage form. The method is based on Phenomenex Gemini C18 (4.6×250mm) 5µ column. The separation is achieved using isocratic elution by Methanol: TEA Buffer in the ratio of 65:35% v/v, pumped at flow rate 1.0mL/min and UV detection at 265nm. The column is maintained at 40°C throughout the analysis. The total run time is about 6min. The method is validated for specificity, accuracy, precision and linearity, robustness and ruggedness, system suitability, limit of detection and limit of quantitation as per international conference of harmonization (ICH) Guidelines. The method is accurate and linear for quantification of Vilanterol, Umeclidinium between 10 - 50µg/mL and 20 - 100µg/mL respectively. Further, satisfactory results are also established in terms of mean percent- age recovery (100.37% for Vilanterol and 100.34% for Umeclidinium, intra-day and inter-day precision (<2%) and robustness. The advantages of this method are good resolution with sharper peaks and sufficient precision. The results indicate that the method is suitable for the routine quality control testing of marketed tablet formulations.

Keywords: *Vilanterol and Umeclidinium, RP-HPLC, ICH Guidelines, Accuracy, Precision.*

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INTRODUCTION:

Vilanterol is a long-acting beta2-adrenergic agonist used in combination with other bronchodilators for the management of chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema.[1] Vilanterol is a selective long-acting beta2-adrenergic agonist. 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. Vilanterol is approved for use in several combination products such as with fluticasone furoate under the tradename Breo Ellipta,[3] in combination with umeclidinium bromide as Anoro Ellipta,[5] and in combination with both fluticasone furoate and umeclidinium under the tradename Trelegy Ellipta.[4] Approved by the FDA in 2013, the use of Breo Ellipta is indicated for the long-term, once-daily maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and emphysema, as well as the once-daily maintenance treatment of asthma in patients aged 18 or older with reversible obstructive airways disease. Anoro Ellipta is indicated for the maintenance treatment of patients with COPD5. IUPAC Name is 4-[(1R)-2-[(6-{2-[(2,6-dichlorophenyl) methoxy] ethoxy} hexyl) amino]-1-hydroxyethyl]-2-(hydroxymethyl)phenol. Molecular formula is $C_{24}H_{33}Cl_2NO_5$. Molecular weight is 486.4.

Umeclidinium is a long-acting muscarinic antagonist (LAMA) used as maintenance treatment for symptoms of chronic obstructive pulmonary disease (COPD).[6] It is available as a once-daily inhalation monotherapy or as a fixed-dose combination product with the long-acting beta2-agonist vilanterol. COPD is a progressive obstructive lung disease characterized by shortness of breath, cough, sputum production, and chronically poor airflow with a forced expiratory volume in 1 second (FEV1) of less than 80%. By blocking the M3 muscarinic receptor which is highly expressed in airway smooth muscle of the lungs, umeclidinium inhibits the binding of acetylcholine and thereby opens up the airways by preventing bronchoconstriction. Its use has been shown to provide clinically significant, sustained improvements in lung function. Umeclidinium is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through the inhibition of M3 receptor at the smooth muscle leading to bronchodilation. IUPAC Name is 1-[2-(benzyloxy)ethyl]-4-(hydroxydiphenylmethyl)-1-azabicyclo[2.2.2]octan-1-ium. Molecular formula is $C_{29}H_{34}NO_2$. Molecular weight is 428.5 g/mol.

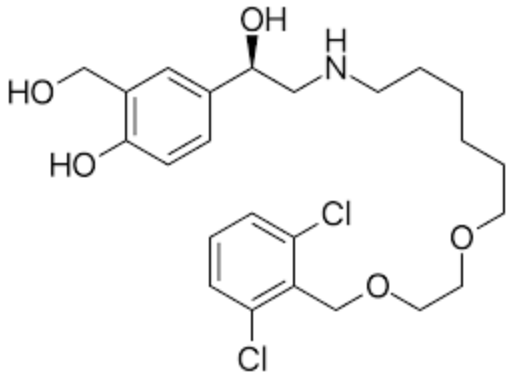

Figure 1: Structure of Vilanterol

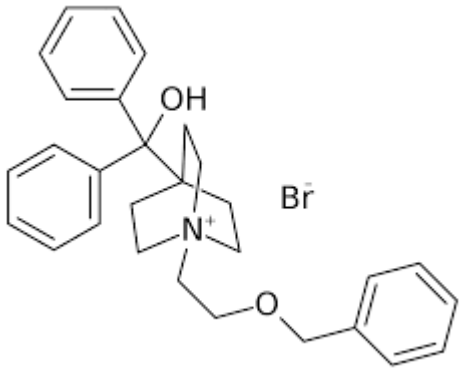

Figure 2: Structure of Umeclidinium

The literature survey revealed that There are very few methods reported in the literature for analysis of Vilanterol and Umeclidinium alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC.[7-11] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Vilanterol and Umeclidinium Simultaneous estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Vilanterol and Umeclidinium. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Vilanterol and Umeclidinium in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Vilanterol and Umeclidinium were Purchased from Hetero drugs. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 265 nm with column Phenomenex Gemini C18 (4.6×250mm) 5µ, dimensions at 40^0C temperature. The optimized mobile phase consists of Methanol: TEA Buffer (65:35 v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of Triethylamine buffer (pH-4.0):

Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.0 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase:

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Vilanterol and Umeclidinium working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.3 ml of Vilanterol and 0.6ml of Umeclidinium from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Powder &weight 10 mg equivalent weight of Vilanterol and Umeclidinium sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.3 ml of Vilanterol and 0.6ml of Umeclidinium from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

RESULTS AND DISCUSSION:

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Phenomenex Gemini C18 (4.6×250mm) 5µ, the mobile phase of composition Methanol: TEA Buffer (65:35 v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1,2.

Table 1: Results of system suitability for Vilanterol

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vilanterol	2.152	526856	78569	1.63	5856
2	Vilanterol	2.157	528794	78545	1.63	5874
3	Vilanterol	2.141	526598	78954	1.62	5869
4	Vilanterol	2.133	524875	78224	1.63	5897
5	Vilanterol	2.166	526584	78965	1.62	5829
Mean			526741.4			
Std. Dev.			1392.398			
%RSD			0.264342			

Table 2: Results of system suitability for Umeclidinium

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate	USP Tailing	Resolution
1	Umeclidinium	3.674	1645985	268542	5869	1.48	10.01
2	Umeclidinium	3.631	1648579	267854	5874	1.49	10.01
3	Umeclidinium	3.625	1645739	268598	5864	1.48	9.99
4	Umeclidinium	3.692	1645285	268745	5826	1.49	10.01
5	Umeclidinium	3.629	1648598	268598	5824	1.48	10.02
Mean			1646837				
Std. Dev.			1618.325				
%RSD			0.098269				

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Vilanterol and Umeclidinium in their pharmaceutical dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-3.

Table 3: Assay results for Vilanterol and Umeclidinium

	Label Claim (mg)	% Assay
Vilanterol	10	99.63
Umeclidinium	10	99.63

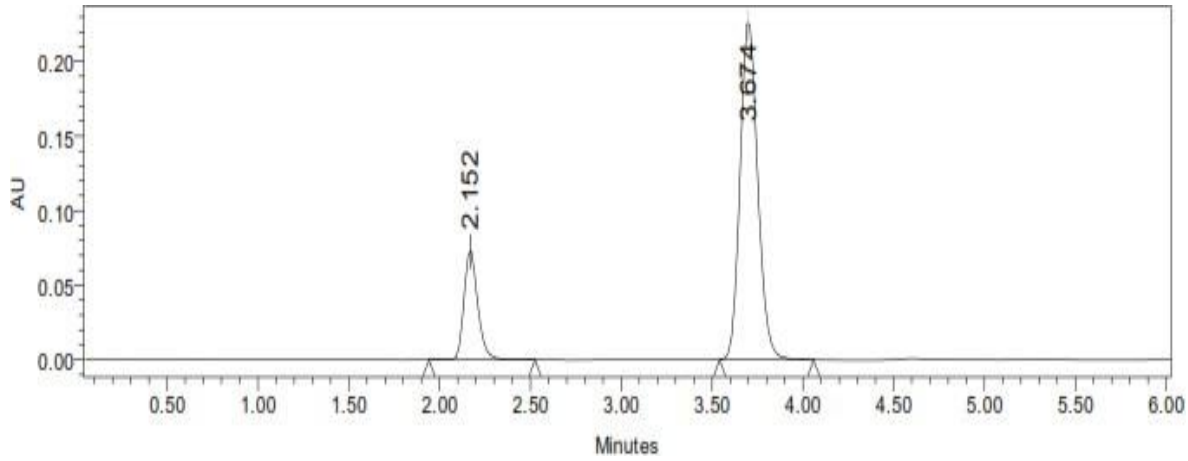

Figure 3: Standard chromatogram

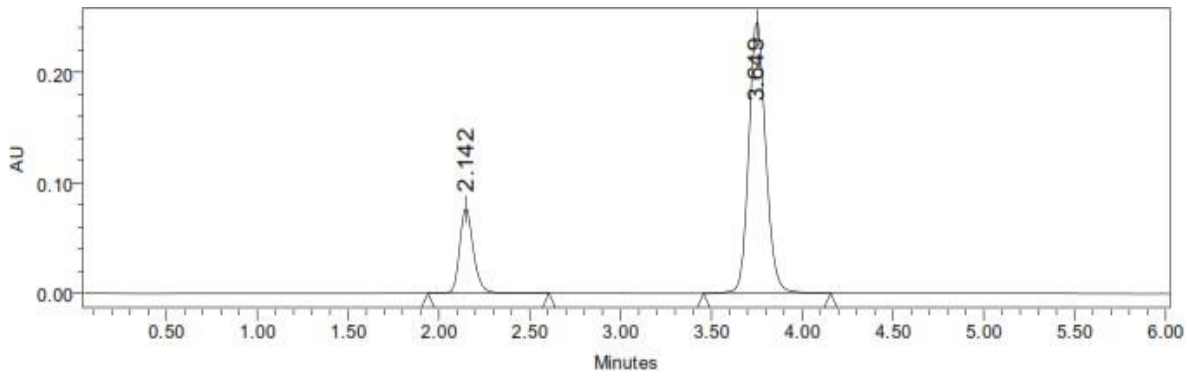

Figure 4: Sample chromatogram

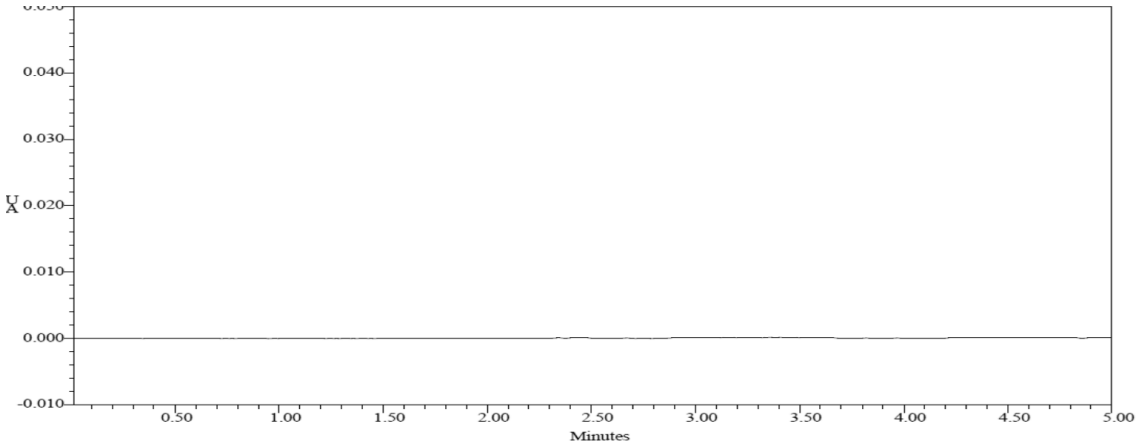

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 10 ppm to 50 ppm and 20 ppm to 100 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 4,5.

Table 4: Linearity results of Vilanterol

Concentration µg/ml	Average Peak Area
10	185689
20	349852
30	521541
40	685986
50	848265

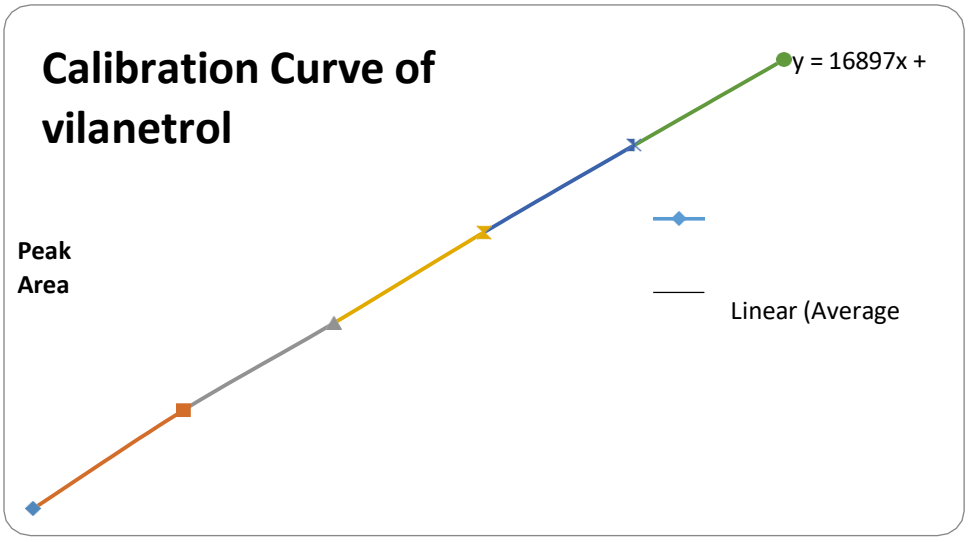

Figure 6: Linearity graph for Vilanterol

Table 5: Linearity results of Umeclidinium

Concentration µg/ml	Average Peak Area
20	665985
40	1298698
60	1927852
80	2548545
100	3162468

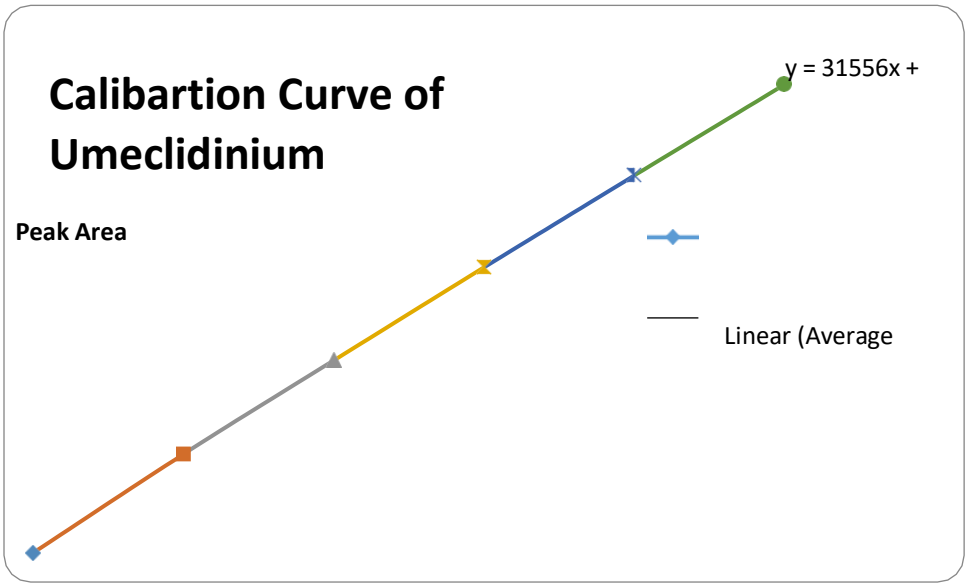

Figure 6: Linearity graph for Umeclidinium

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Vilanterol and Umeclidinium and calculate the individual recovery and mean recovery values. The results are shown in table 6,7.

Table 6: Showing accuracy results for Vilanterol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	263572	15	15.038	100.253%	100.37%
100%	518870.3	30	30.147	100.490%	
150%	772572.3	45	45.162	100.360%	

Table 7: Showing accuracy results for Umeclidinium

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972935.7	30	30.109	100.363%	100.34%
100%	1919319	60	60.100	100.166%	
150%	2877020	90	90.449	100.498%	

Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 8,9.

Table 8: Precision results for Vilanterol

S. No.	Peak name	Retentio n time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vilanterol	2.157	526854	78569	5869	1.62
2	Vilanterol	2.159	523659	78469	5874	1.63
3	Vilanterol	2.186	523856	78525	5896	1.63
4	Vilanterol	2.160	523485	78548	5818	1.62
5	Vilanterol	2.170	523485	78594	5879	1.63
Mean			524267.8			
Std .dev			1453.805			
%RSD			0.277302			

Table 9: Precision results for Umeclidinium

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Umeclidinium	3.603	1645879	265845	7985	5869
2	Umeclidinium	3.608	1648578	265487	7964	5849
3	Umeclidinium	3.600	1645985	265982	7915	5879
4	Umeclidinium	3.696	1648759	265478	7928	5874
5	Umeclidinium	3.629	1648572	265422	7964	5829
Mean			1647555			
Std. dev			1483.603			
%RSD			0.090049			

Intermediate precision: Intermediate precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 10,11.

Table 10: Results of Intermediate precision for Vilanterol

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USPTailing
1	Vilanterol	2.198	536598	79584	5963	1.64
2	Vilanterol	2.196	536985	79685	5978	1.65
3	Vilanterol	2.160	534587	79654	5947	1.64
4	Vilanterol	2.160	536985	79845	5982	1.65
5	Vilanterol	2.160	536985	79864	5971	1.65
6	Vilanterol	2.186	538568	79685	5968	1.64
Mean			536784.7			
Std.Dev.			1277.909			
%RSD			0.238067			

Table 11: Results of Intermediate precision for Umeclidinium

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Umeclidinium	3.623	1658254	266598	8036	1.50	10.06
2	Umeclidinium	3.611	1659872	266473	8045	1.51	10.04
3	Umeclidinium	3.696	1653589	266958	8075	1.50	10.05
4	Umeclidinium	3.696	1658458	266451	8049	1.50	10.06
5	Umeclidinium	3.696	1653652	266352	8069	1.50	10.05
6	Umeclidinium	3.642	1652395	266954	8024	1.51	10.06
Mean			1656037				
Std. Dev.			3175.804				
%RSD			0.191771				

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1 ml/min. The results are shown in table 12,13.

Table 12: Robustness results for Vilanterol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526541	2.157	5859	1.62
Less Flow rate of 0.9 mL/min	589564	2.210	5635	1.61
More Flow rate of 1.1 mL/min	515246	2.184	5569	1.64
Less organic phase	502659	2.200	5154	1.63
More Organic phase	526485	2.172	5365	1.62

Table 13: Robustness results for Umeclidinium

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1645875	3.643	7965	1.48
Less Flow rate of 0.9 mL/min	1635985	4.498	7856	1.46
More Flow rate of 1.1 mL/min	1624587	3.505	7425	1.43
Less organic phase	1652834	4.504	7621	1.45
More organic phase	1625548	3.512	7582	1.42

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 14.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 14: LOD, LOQ of Vilanterol and Umeclidinium

Drug	LOD	LOQ
Vilanterol	0.9	2.7
Umeclidinium	1.2	3.6

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Vilanterol and Umeclidinium in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Vilanterol and Umeclidinium in pure and its pharmaceutical dosage forms.

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