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METHOD DEVELOPMENT AND VALIDATION OF UMECLIDINIUM AND VILANTEROL IN 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 Agilent Zorbax XDB C18(150mm×4.6mm,5µ). The separation is achieved using isocratic elution by Potassium dihydrogen phosphate: Methanol (40:60) v/v, pumped at flow rate 1.0mL/min and UV detection at 235nm. The column is maintained at 25°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 50 - 150µg/mL and 50 - 150µg/mL respectively. Further, satisfactory results are also established in terms of mean percent- age recovery 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.

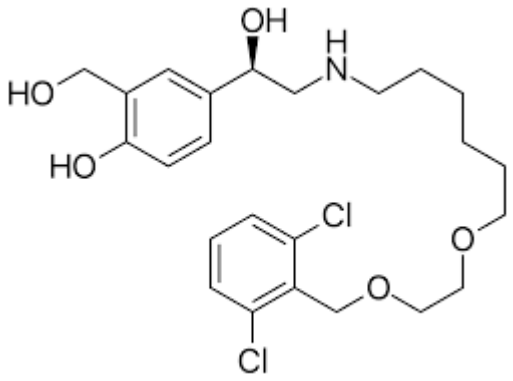

Figure 1: Structure of Vilanterol

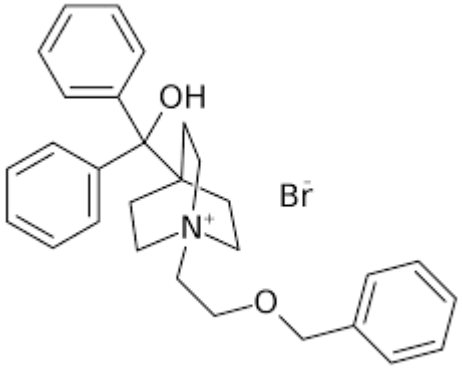

Figure 2: Structure of Umeclidinium

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 235 nm with column Agilent Zorbax XDB C18(150mm×4.6mm,5µ), dimensions at 25^0C temperature. The optimized mobile phase consists of Potassium dihydrogen phosphate: Methanol (40:60). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

PREPARATION OF MOBILE PHASE:

Transfer 1.36086gof Potassium dihydrogen phosphate into 1000ml of beaker and adjust pH 4.80 with orthophosphoric acid (OPA).

Transfer the above solution 400ml and 600ml of methanol is used as mobile phase. They are mixed and sonicated for 20min.

PREPARATION OF THE VILANTEROL AND UMECLIDINIUM STANDARD AND SAMPLE SOLUTION:

PREPARATION OF STANDARD SOLUTION:

Accurately weigh and transfer 10 mg of Vilanterol and 10 mg of Umeclidinium into 50 ml of volumetric flask and add 10ml of water and sonicate 10min (or) shake 5min and make with water.

Transfers 5ml of the above solution into 25ml volumetric flask, make up the volume with water**.**

PREPARATION OF SAMPLE STOCK SOLUTION:

Commercially available six tablets ware weighed and powdered the powdered equivalent to the 10 mg of Vilanterol and Umeclidinium of active ingredients were transfer into a 50 ml of volumetric flask and add 10ml of methanol and sonicate for 20min (or) shake 10min and makeup with water.

Transfers above solution 5ml into 25ml of the volumetric flask dilute the volume with water. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

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 AgilentZorbax XDB C18(150mm×4.6mm,5µ), the mobile phase of composition Potassium dihydrogen phosphate: Methanol (40:60) 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.

Table 1: System suitability parameters

parameter	Vilanterol	Simethicone	Acceptance criteria
Retention time	1.694	3.234	_
Theoretical plates	4508	5387	>2500
Tailing factor	1.69	1.55	<2.00
% RSD	0.02	0.03	<2.00

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-2.

Table 2: Assay results for Vilanterol and Umeclidinium

	Label Claim (mg)	% Assay
Vilanterol	10	100.25
Umeclidinium	10	98.99

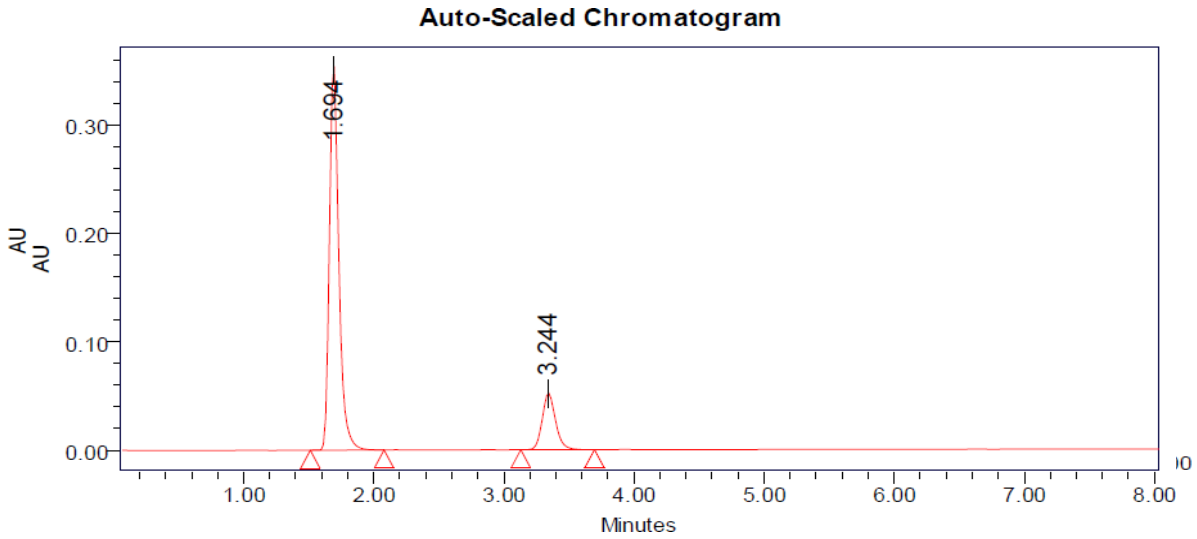

Figure 3: Standard chromatogram

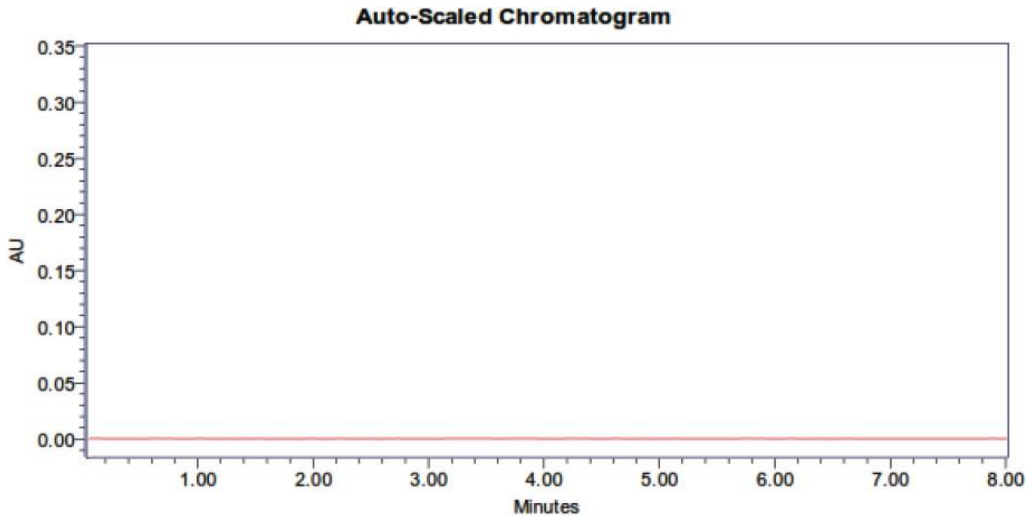

Figure 4: Sample chromatogram

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 50 ppm to 150 ppm and 50 ppm to 150 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 3,4.

Table 3: Linearity results of Vilanterol

S. no	Concentration (µg/ml)	Rt	Area
1.	50	1.689	1254871
2.	75	1.691	1895454
3.	100	1.692	2653415
4.	125	1.689	3258474
5.	150	1.688	3986547

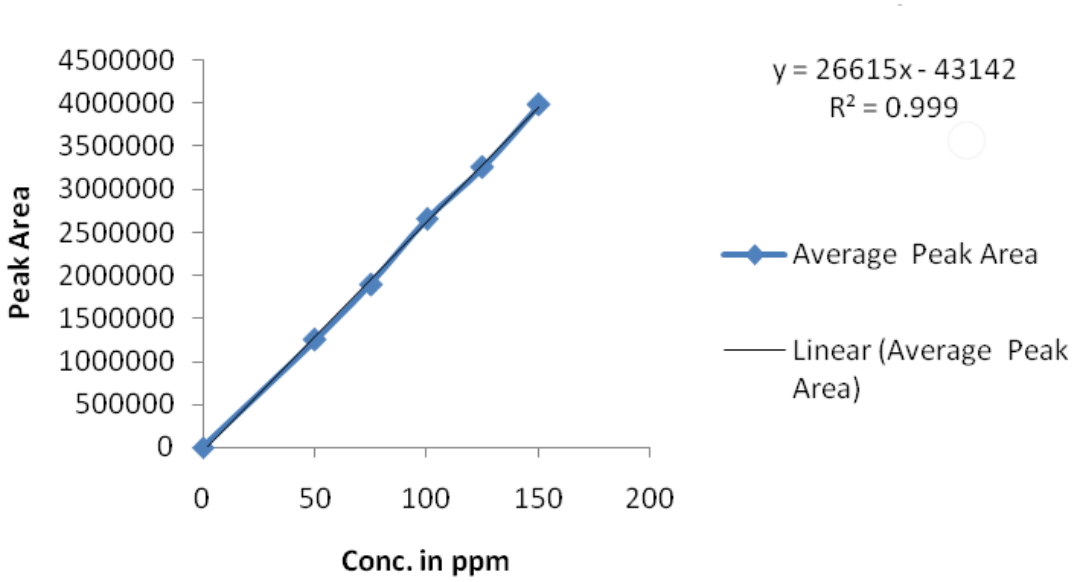

Figure 6: Linearity graph for Vilanterol

Table 4: Linearity results of Umeclidinium

S. no	Concentration (µg/ml)	Rt	Area
1.	50	3.203	269658
2.	75	3.299	418753
3.	100	3.294	559858
4.	125	3.290	695847
5.	150	3.288	828654

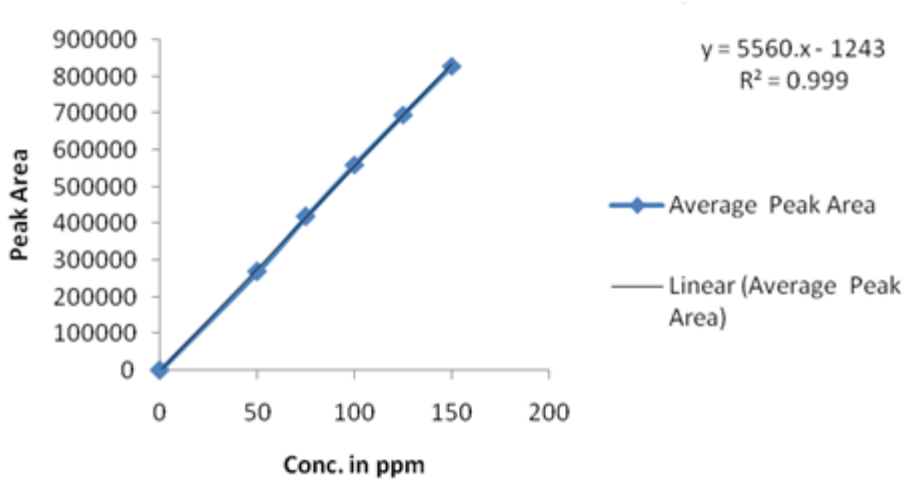

Figure 6: Linearity graph for Umeclidinium

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 5,6.

Table 5: Showing accuracy results for Vilanterol

S. no	Accuracy Level	Sample name	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	50	49.761	99.522	99.99
		2	50	50.053	100.106	
		3	50	50.171	100.342	
2	100%	1	100	99.581	99.581	100.073
		2	100	100.446	100.446	
		3	100	100.194	100.194	
3	150%	1	150	149.885	99.923	99.956
		2	150	149.757	99.838	
		3	150	150.164	100.109	

Table 6: Showing accuracy results for Umeclidinium

S. no	Accuracy Level	Sample name	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	50	50.358	100.716	100.66
		2	50	50.518	101.036	
		3	50	50.114	100.228	
2	100%	1	100	100.454	100.454	100.25
		2	100	100.822	100.822	
		3	100	99.475	99.475	
3	150%	1	150	150.379	100.252	100.03
		2	150	149.462	99.641	
		3	150	150.297	100.198	

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 7.

Table 7: Precision results for Vilanterol and Umeclidinium

S. no	Intraday precision for Vilanterol			Intraday precision for Umeclidinium		
	Peak area	Mean peak area	%RSD	Peak area	Mean peak area	%RSD
1	2653415	2667028	0.556	567898	568727	0.137
2	2654514			568887		
3	2685475			569275		
4	2658426			569858		
5	2664858			568586		
6	2685479			567858		

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 0.8 ml/min to 1.2 ml/min. The results are shown in table 8,9.

Table 8: Robustness results for Vilanterol

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	1.868	4052	1.60
Increased flow rate (1.2ml/min)	1.544	4941	1.49
Decreased temperature (20^0c)	1.731	4475	1.61
Increased temperature (30^0c)	1.675	4581	1.61

Table 9: Robustness results for Umeclidinium

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	3.621	5230	1.45
Increased flow rate(1.2ml/min)	2.998	5828	1.41
Decreased temperature (20^0c)	6.242	5484	1.50
Increased temperature (30^0c)	2.302	5494	1.50

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 10.

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 10: LOD, LOQ of Vilanterol and Umeclidinium

Drug	LOD	LOQ
Vilanterol	1.36	1.0
Umeclidinium	4.12	3.0

CONCLUSION:

The validated HPLC method developed for the quantitative quality control determination of Vilanterol and Umeclidinium in combination was evaluated for system suitability, specificity, sensitivity, linearity, range, accuracy (recovery), precision (repeatability and intermediate precision), and robustness. All the validation results were within the allowed specifications of ICH guidelines. The developed method has proven to be rapid, accurate, and stability-indicating for the simultaneous determination of combined Vilanterol and Umeclidinium in pharmaceutical dosage form in the presence of excipients and the degradation products. As a result, the proposed HPLC method could be adopted for the quantitative quality control and routine analysis of the pharmaceutical dosage form.

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