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HPLC Method Development and Validation for the Estimation of Umeclidinium and Vilanterol in Bulk Dosage Forms

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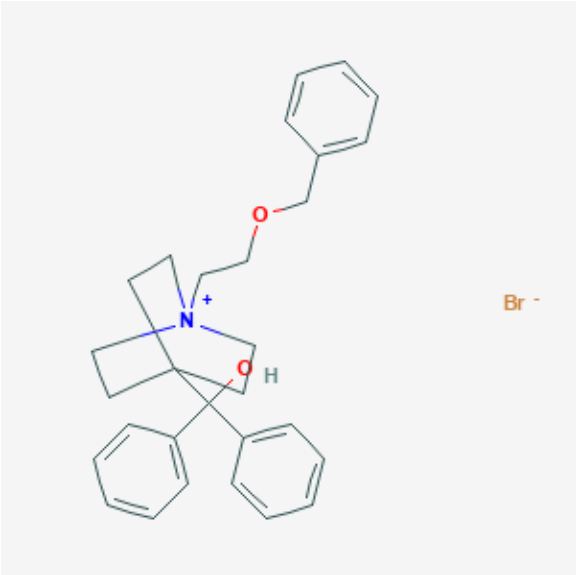
ABSTRACT

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.

INTRODUCTION



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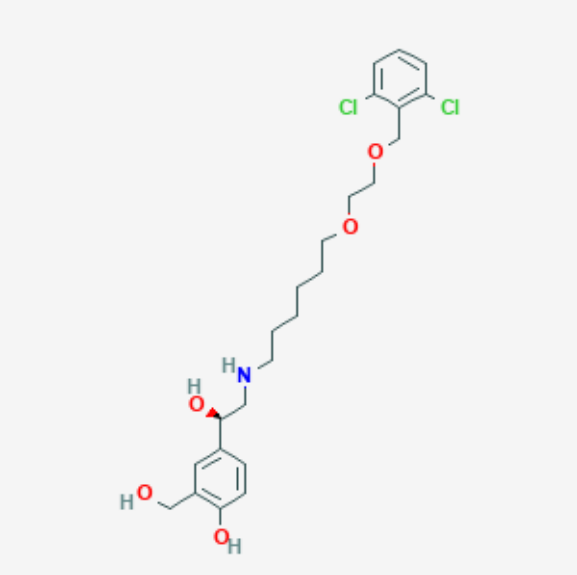

Fig: 1 Structure of Umeclidinium **Fig: 2 Structure of Vilanterol**

Umeclidinium bromide (diphenyl-[1-(2-phenylmethoxyethyl)-1-azoniabicyclo[2.2.2]octan-4-yl]methanol) bromide is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. The solubility of umeclidinium (bromide) in these solvents is approximately 0.14, 15, and 10 mg/ml, respectively. Umeclidinium (bromide) is sparingly soluble in aqueous buffers. In vitro data showed that umeclidinium is primarily metabolized by the enzyme cytochrome P450 2D6 (CYP2D6) and is a substrate for the P-glycoprotein (P-gp) transporter. The primary metabolic routes for umeclidinium are oxidative (hydroxylation, Odealkylation) followed by conjugation (e.g., glucuronidation), resulting in a range of metabolites with either reduced pharmacological activity or for which the pharmacological activity has not been established. Systemic exposure to the metabolites is low. 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.

Vilanterol 4-[(1*R*)-2-[6-[2-[(2,6-dichlorophenyl)methoxy]ethoxy]hexylamino]-1-hydroxyethyl]-2-(hydroxymethyl)phenol 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. 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.

Table: 1 Marketed Formulation

S.No	Drug name	Label Claim	Brand name	Company
1	umeclidinium-vilanterol	62.5mcg/25mcg)/actuation	Anoro Ellipta	GlaxoSmithKline/Innovia

Materials

Chemical-Brand names, Vilanterol-Sura labs, Umeclidinium-Sura labs, Water and Methanol for HPLC LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

HPLC Method Development:

Trails

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 Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 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 Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Water, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to TEA buffer (pH 4.0), Methanol in proportion 65:35 v/v respectively.

Optimization of Column:

The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized Chromatographic Conditions:

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	40ºC
Column	:	Phenomenex Gemini C18 (4.6×250mm) 5µ
Mobile phase	:	Methanol: TEA Buffer (65:35 v/v)
Flow rate	:	1ml/min
Wavelength	:	265nm
Injection volume	:	10µl
Run time	:	6minutes

Validation

Preparation Of Buffer And Mobile Phase:

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.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: TEA Buffer (65:35 v/v)

Column : Phenomenex Gemini C18 (4.6×250mm) 5µ

Column temperature : 40ºC

Wavelength : 265nm

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 6minutes

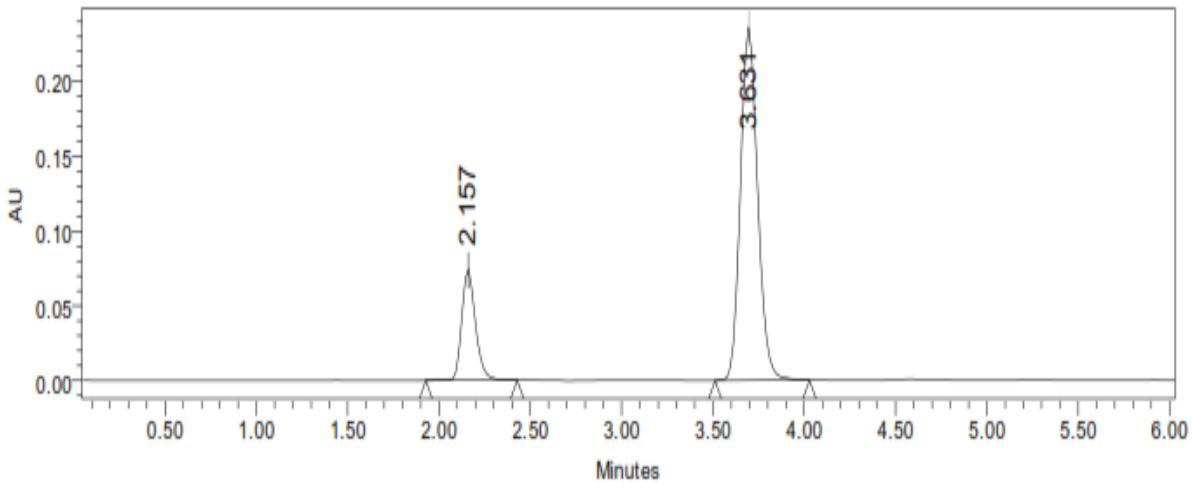

Figure:3 Optimized Chromatogram (Standard)

Table: 2 Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Vilanterol	2.157	526541	78564	1.62	5859	
2	Umeclidinium	3.631	1645875	265842	1.48	7965	9.9

Observation: From the above chromatogram it was observed that the Vilanterol and Umeclidinium peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So, it's optimized trial.

System Suitability: System suitability of the method was assessed by five replicate injections. Parameters like USP Plate count, USP Tailing were recorded and tabulated**.**

Table:3 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:4 Results of system suitability for Umeclidinium

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	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				

The method has passed the system suitability as the number of theoretical plates, USP tailing and resolution were within the limits.

Assay (Sample): Table 5: Peak results for Assay sample of Vilanterol

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Vilanterol	2.152	536598	79856	1.64	5969	1
2	Vilanterol	2.150	536589	79265	1.65	5997	2
3	Vilanterol	2.187	534658	79898	1.65	5986	3

Table:6 Peak results for Assay sample of Umeclidinium

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Umeclidinium	3.646	1658952	278598	1.49	8016	1
2	Umeclidinium	3.651	1658954	276984	1.48	8041	2
3	Umeclidinium	3.601	1653659	275849	1.49	8079	3

%ASSAY

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Vilanterol and Umeclidinium in pharmaceutical dosage form was found to be 99.63%.

LINEARITY: The linearity of the method was established by injecting 10-50µg/ml concentrations for vilanterol and 20-100 µg/ml for umeclidinium in replicates. Peak areas at each injection were recorded and a plot was also constructed between concentrations and peak areas.

Table: 7 Linearity data of Vialanterol and Umeclidinium

Vilanterol		Umeclidinium	
Concentration µg/ml	Average Peak Area	Concentration µg/ml	Average Peak Area
10	185689	20	665985
20	349852	40	1298698
30	521541	60	1927852
40	685986	80	2548545
50	848265	100	3162468

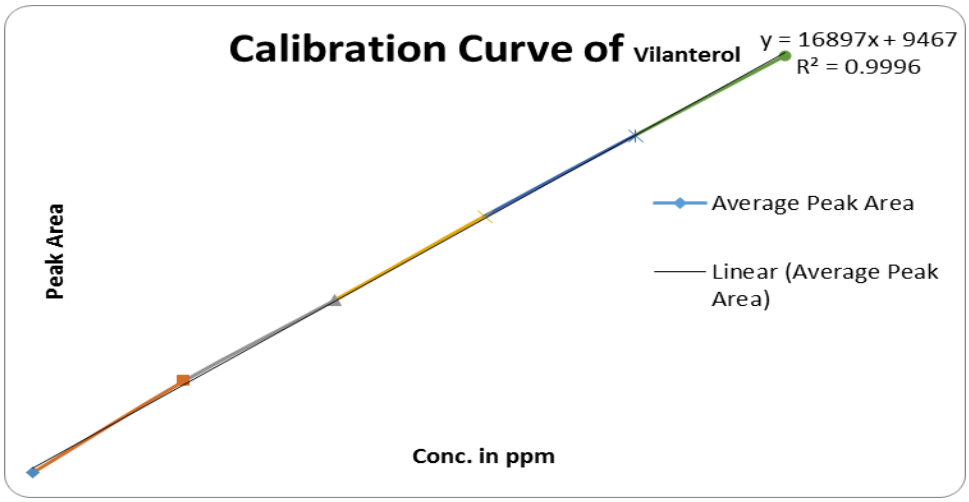

Fig:4 Calibration Curve of Vilanterol

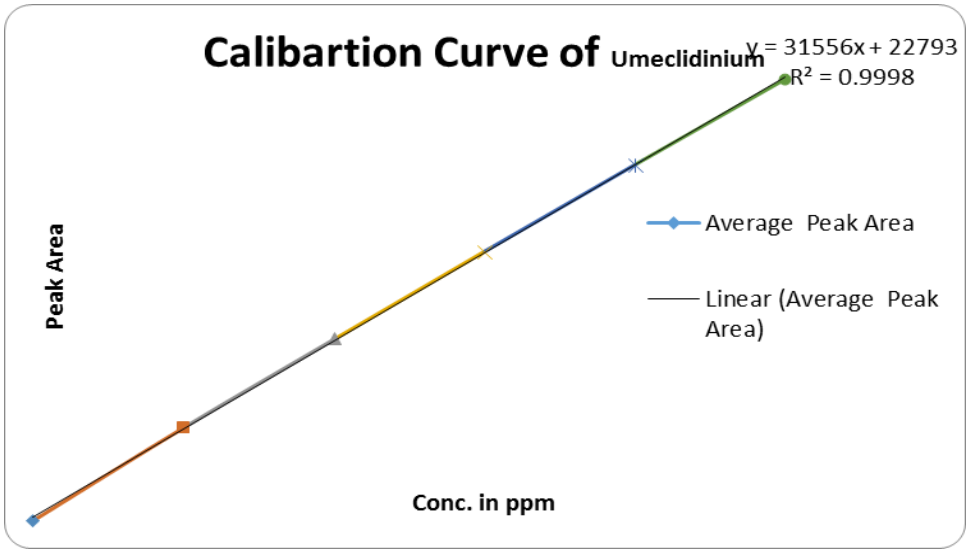

Fig:5 Calibration Curve of Umeclidinium

The method was found to be linear as the correlation coeffecient is 0.9999.

Precision: The reproducibility of the method was assessed by precision studies which were evaluated by repeatability and intermediate precision. Sample was injected in replicates and peak areas were recorded.

REPEATABILITY

Table: 8 Results of Repeatability for Vilanterol:

S. No.	Peak name	Retention 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 Results of repeatability 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:

Table:10 Results of Intermediate precision Day-1 for Vilanterol

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
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 Day-1 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				

Table: 12 Results of Intermediate precision Day 2 for Vilanterol

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Vilanterol	2.198	519689	77859	5749	1.61
2	Vilanterol	2.196	518957	77985	5792	1.60
3	Vilanterol	2.178	519856	77854	5746	1.60
4	Vilanterol	2.142	519857	77869	5749	1.61
5	Vilanterol	2.177	519869	77935	5718	1.61
6	Vilanterol	2.177	519687	77954	5795	1.60
Mean			519652.5			
Std.Dev.			351.0976			
%RSD			0.067564			

Table: 13 Results of Intermediate precision Day 2 for Umeclidinium

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Umeclidinium	3.611	1638598	256985	7968	1.47	9.90
2	Umeclidinium	3.623	1637849	257589	7952	1.46	9.91
3	Umeclidinium	3.684	1635982	256985	7934	1.46	9.90
4	Umeclidinium	3.697	1636598	254613	7986	1.47	9.90
5	Umeclidinium	3.684	1635874	258487	7924	1.46	9.91
6	Umeclidinium	3.684	1635984	259861	7915	1.47	9.91
Mean			1636814				
Std.Dev.			1145.885				
%RSD			0.070007				

The method was precise since the %RSD of peak areas was less than 2**.**

ACCURACY: The accuracy of the method was evaluated by recovery studies. Three target concentrations were selected and injected in triplicates. Percentage recovery at each level was recorded from which mean recovery was calculated.

Table:14 The 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: 15 The 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%	

The mean recoveries were found to be 100.37 and 100.34 for vilanterol and umeclidinium respectively indicate the accuracy of the method.

Robustness: It was assessed by deliberate changes in variables like flow rate and organic phase composition from the normal with respect to optimized conditions.

Table: 16 Results For Robustness 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: 17 Results For Robustness Vilanterol 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

The method was found to be robust as there is no considerable deviation was observed in parameters like retention time, theoretical plates and tailing factor with respect to optimized conditions.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Vilanterol and Umeclidinium in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Vilanterol was found to

be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide; it is very slightly soluble in water, slightly soluble in Acetonitrile and ethanol, sparingly soluble in methanol, practically insoluble in toluene. Umeclidinium was found to be very slightly soluble in water (0.9 mg/mL). Umeclidinium is soluble in methanol (ca. 60 mg/mL), sparingly soluble in ethanol (ca. 10 mg/mL), very slightly soluble in isopropanol (<1 mg/mL), and very slightly soluble in acetone. After a series of trials the method was optimized at a flow rate 1ml/min, column Phenomenex Gemini C18 (4.6×250mm) 5µ and mobile phase Methanol: TEA Buffer (65:35 v/v). The detection wavelength 265nm was used during the entire study. The method was duly validated as per ICH guidelines. The method was found to be linear in the range 10-50 µg/l for vilanterol and 20-100 µg/l for umeclidinium. The proposed method was precise as the % RSD values of peak areas were found to be below 2. The percentage recovery values were 100.37 and 100.34 for umeclidinium and vilanterol respectively which signifies the method was accurate. The LOD and LOQ values indicate the sensitivity of the method. The robustness was established on deliberate changes on organic composition and flow rate. The parameters were checked and there is no much deviation was observed.

The % RSD values were within 2 and the method was found to be precise.

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