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Research

Method development & validation for the simultaneous estimation of umeclidinium & vilanterol in solution using rp-hplc method

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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 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.

INTRODUCTION

Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research

are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- Qualitative analysis is the identification of elements, species and/or compounds present in sample.
- Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital.

Quality control (QC) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Chromatography [2]

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

"] *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*". The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient. "In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process". "Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

MATERIALS AND METHODS

Vilanterol, Umeclidinium-Sura labs, Water and Methanol for HPLC- LICHROSOLV (MERCK), Acetonitrile for HPLC- Merck, Potassium Dihydrogen Phosphate-Finar Chemicals.

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

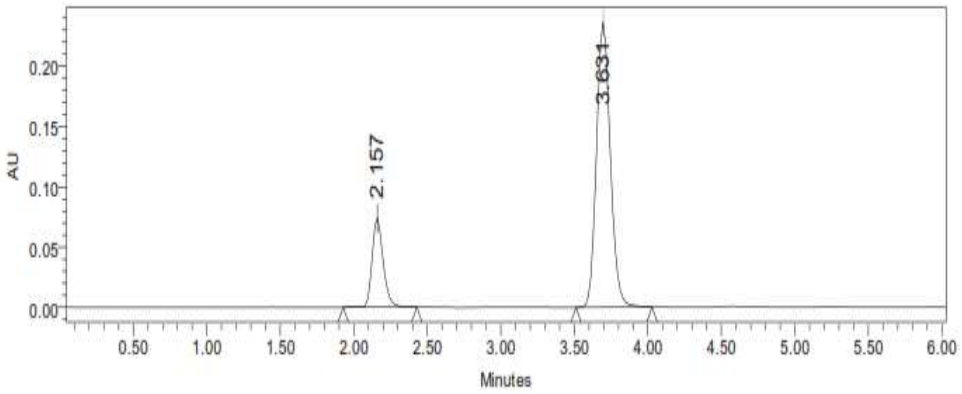

Fig 1: Optimized Chromatogram (Standard)

Table 1: 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

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

Optimized Chromatogram (Sample)

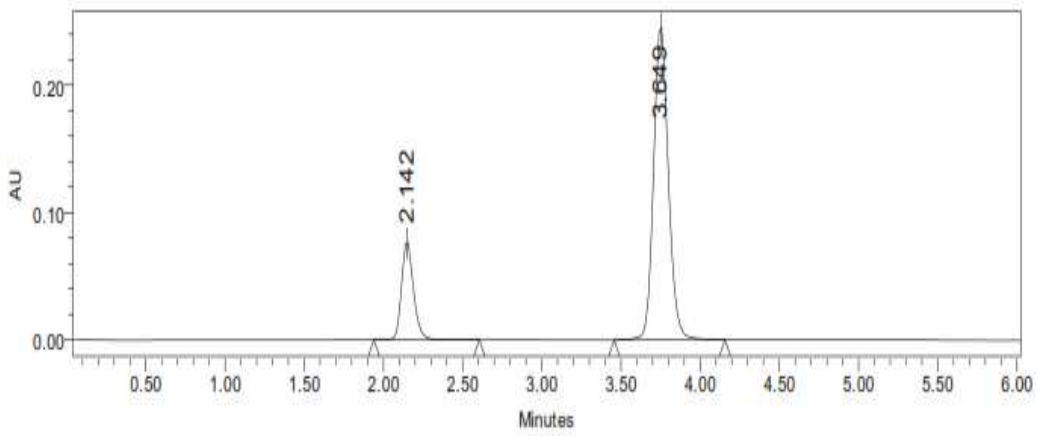

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Vilanterol	2.142	538954	79658	1.63	5986	
2	Umeclidinium	3.649	1658745	275854	1.49	8056	10.1

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System suitability

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			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

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				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Standard)

Table 5: Peak results for assay standard of Vilanterol

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Vilanterol	2.152	526595	78569	1.63	5896	1
2	Vilanterol	2.198	524658	78496	1.63	5879	2
3	Vilanterol	2.179	528476	78459	1.62	5895	3

Table 6: Peak results for assay standard of Umeclidinium

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Umeclidinium	3.646	1648546	265845	1.48	8012	1
2	Umeclidinium	3.604	1648598	265418	1.49	7955	2
3	Umeclidinium	3.610	1648574	265365	1.48	7989	3

Assay (Sample)

Table 7: 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 8: 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

Chromatographic data for linearity study of vilanterol

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

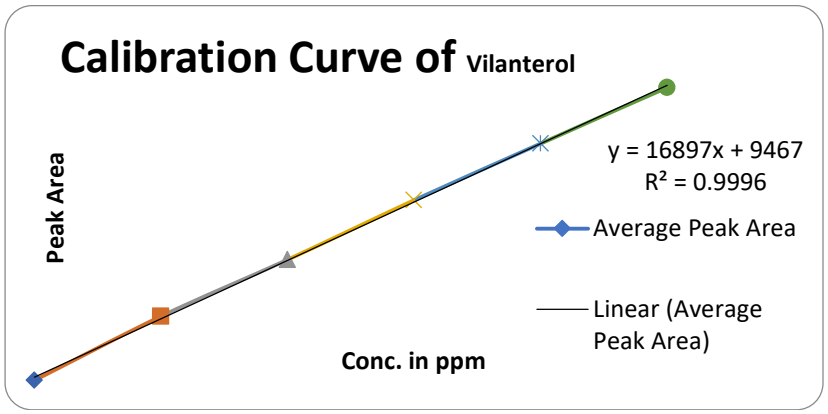

Fig 3: Calibration Curve of Vilanterol

Chromatographic data for linearity study of umeclidinium

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

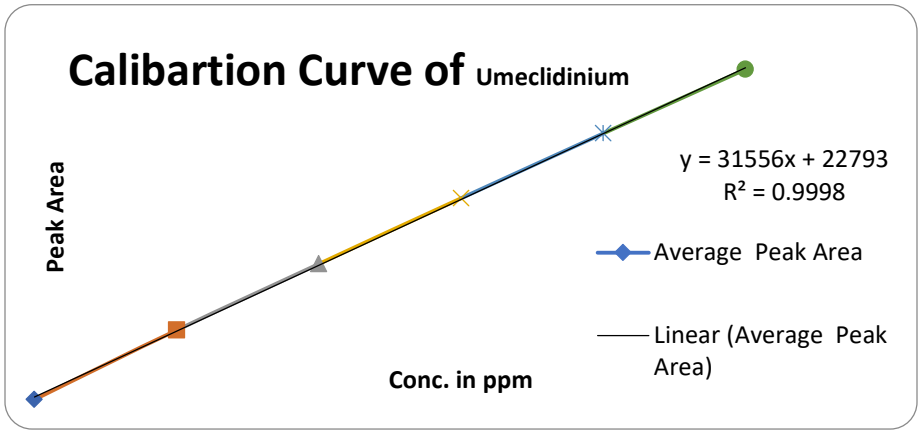

Fig 4: Calibration Curve of Umeclidinium

Precision
Repeatability

Table 9: 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			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 10: 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
Day 1

Table 11: Results of Intermediate precision 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			

- *%RSD of five different sample solutions should not more than 2.*

Table 12: 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				

- *%RSD of five different sample solutions should not more than 2.*

Table 13: 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			

- *%RSD of five different sample solutions should not more than 2.*

Day 2

Table 14: 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				

- *%RSD of five different sample solutions should not more than 2.*

Accuracy

Table 15: 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%	

- The percentage recovery was found to be within the limit (98-102%).

Table 16: 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 results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

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

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

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 tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

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. Methanol: TEA Buffer (65:35 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Vilanterol and Umeclidinium in bulk drug and in Pharmaceutical dosage forms.

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