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Research/Review

Method Development And Validation For The Simultaneous Estimation Of Umeclidinium And Vilanterol In Solution Using UPLC Method

J. Lavanya[*1] , K. Likhitha Lahari[2] , T. Rama Rao[3]

*[*1]Associate Professor, Department of Pharmaceutical Chemistry, CMR College of pharmacy, Hyderabad, India*
[2]M. Pharmacy student, Department of Pharmaceutical Analysis, CMR College of pharmacy, Hyderabad, India
[3]Principal and professor of CMR College of pharmacy, Medchal, Hyderabad, Telangana, India

*Author for Correspondence: J. Lavanya
Email: likhithakurapati1999@gmail.com

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Umeclidinium and Vilanterol, in its pure form as well as in powder dosage form. Chromatography was carried out on Acquity UPLC BEH C-18,50 mm × 2.1 mm and 1.7 µm µm column using a mixture of Water and Acetonitrile (60:40% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 220nm. The retention time of the Umeclidinium and Vilanterol was 3.0, 3.8±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Umeclidinium and 10- 50µg/ml of Vilanterol. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Umeclidinium, Vilanterol, UPLC and validation.

INTRODUCTION

Chromatography is a non-destructive process that uses a porous material and solvents to separate a mixture of components into their individual components. Prior to 2004, HPLC was the method of choice for decomposing a mixture of components into their constituent parts. However due to various restrictions, scientists have developed a new technology known as "Ultra Performance Liquid Chromatography (UPLC)" that is very effective and cutting-edge while also overcoming some of HPLC's limitations.[1-3]

Brief History Chromatography is a novel technology that was invented by Russian botanist Tswett in Warsaw in 1906. He was successful in separating chlorophyll, xanthophylls, and a number of other coloured compounds from vegetable extracts using a calcium carbonate column during that year. The calcium carbonate column serves as an adsorbent, and the various compounds are adsorbed to varying degrees, resulting in coloured

bands at various positions on the column. The Greek terms chroma and graphos, which imply colour and writing, respectively, inspired Tswett to use the term "chromatogram" to describe this system of coloured bands and the technique used to generate it.[4-6] Since then, significant progress has been made, and the techniques are now employed to differentiate coloured and colourless compounds. The stationary phase is the calcium carbonate column included in the Tsweet method, which remains stationary throughout. Vegetable extract solution is referred to as mobile phase because it flows or flows down the column. The separation of solutes between a stationary phase and a mobile phase occurs during the separation process of chromatography. Thin layer chromatography and ion exchange chromatography were both first introduced as a method of separation in 1930. Paper chromatography was first introduced by Martin and Synge in 1941, and gas chromatography followed in 1952. It is becoming a prospective technique for the preparation of extremely pure substances in industries like the pharmaceutical industry or in the manufacturing of pure chemicals, in addition to its usage in analysis. The chromatographic methods of biomolecule separation are totally responsible for the recent outstanding developments in the field of bioscience. Subsequently, other techniques such as HPLC were established, which have been utilised in many laboratories for a long time. More recently, a new technology known as UPLC was introduced (Ultra performance Liquid Chromatography) The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 µm. The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical formula that describes the relationship between, linear (flow rate) and plate height (HETP or column efficiency).[7-8.]

Drug profile

Table 1: Drug profile of Umeclidinium

DRUG	**Umeclidinium**
Synonym	1-[2-(benzyloxy)ethyl]-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo [2.2.2]octane
Category	Anticholinergic Agents
IUPAC	-[2-(benzyloxy)ethyl]-4- (hydroxydiphenylmethyl)-1-azabicyclo [2.2.2]octan-1-ium
Molecular formula	$C_{29}H_{34}NO_2$
Melting point	178ºC
pKa	13.04
Log p	2.88

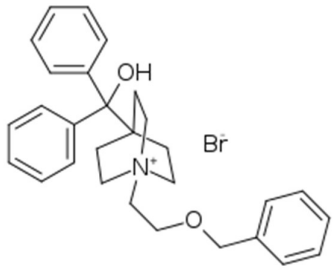

Fig 1: Structure of Umeclidinium

Table 2: Drug profile of VILANTEROL

DRUG	**Vilanterol**
Synonym	Vilanterolum
Category	Adrenergics, Inhalants
IUPAC	4-[(1R)-2-[(6-{2-[(2,6-dichlorophenyl)methoxy]ethoxy} hexyl)amino]-1-hydroxyethyl]-2- (hydroxymethyl)phenol
Molecular formula	$C_{24}H_{33}Cl_2NO_5$
Melting point	175ºC
pKa	10.12
Log p	3.39

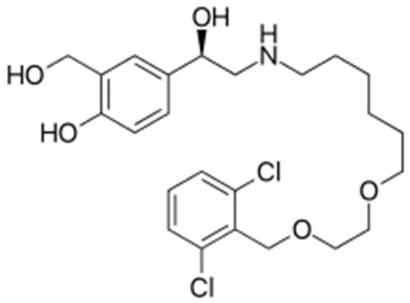

Fig 2: Structure of Vilanterol

UPLC

UPLC is also called Ultra-Performance Liquid Chromatography (UPLC) is a technique that uses high pressure and small particles to separate sample components. It's a development of High Performance Liquid Chromatography (HPLC) and offers several advantages over traditional HPLC. UPLC is a variation of High Performance Liquid Chromatography (HPLC) that uses smaller particles in the stationary phase and shorter columns. This requires higher pressures to force the mobile phase through, but it also leads to better resolution and sensitivity.[9-11]

Applications

UPLC is used in a variety of fields, including pharmaceuticals, environmental analysis, and food and beverage testing. It's especially useful for identifying and quantifying components in complex mixtures.[12]

Advantages

UPLC offers better resolution and sensitivity than HPLC, and it uses less solvent and has shorter run times.[13]

METHODS AND MATERIALS

Table 3: Instruments used

S.No	Instruments and Glass wares	Name of the manufacturer
1	UPLC	WATERS Alliance 2695 separation module, Software: Empower 2, Acquity Tunable Ultra Violet (TUV) detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 4: Chemicals used

S.No	Chemical	Brand names
1	Umeclidinium	Sura labs
2	Vilanterol	Sura labs
3	Water and Methanol for UPLC	LICHROSOLV (MERCK)
4	Acetonitrile for UPLC	Merck
5	Triethylamine	Merck

Uplc method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Umeclidinium and Vilanterol 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 0.15ml of the Umeclidinium and 0.3ml of the Vilanterol 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 and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 40:60 v/v respectively.

Optimization of Column

The method was performed with various columns like Symmetry, Hypersil and Sunfire C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 600ml (60%) of Water, 400ml of Acetonitrile (40%) were mixed and degassed in 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.

Validation parameters

System suitability

Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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 0.15ml of the Umeclidinium and 0.3ml of the Vilanterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity study of drug

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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 0.15ml of the Umeclidinium and 0.3ml of the Vilanterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution

Take weight 10 mg equivalent weight of Umeclidinium and Vilanterol 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. Further pipette 0.3ml of the Sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of drug solutions for linearity

Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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).

Preparation of Level – I (5ppm of Umeclidinium & 10ppm of Vilanterol)

Pipette out 0.05ml of Umeclidinium and 0.1ml of Vilanterol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10ppm of Umeclidinium & 20ppm of Vilanterol)

Pipette out 0.1ml of Umeclidinium and 0.2ml of Vilanterol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15ppm of Umeclidinium & 30ppm of Vilanterol)
Pipette out 0.15ml of Umeclidinium and 0.3ml of Vilanterol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20ppm of Umeclidinium & 40ppm of Vilanterol)
Pipette out 0.2ml of Umeclidinium and 0.4ml of Vilanterol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25ppm of Umeclidinium & 50ppm of Vilanterol)
Pipette out 0.25ml of Umeclidinium and 0.5ml of Vilanterol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure
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.

Precision
Preparation of Umeclidinium and Vilanterol Product Solution for Precision
Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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 0.15ml of the Umeclidinium and 0.3ml of the Vilanterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. The standard solution was injected for five times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure
DAY 1: The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
DAY 2: The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy
For preparation of 50% Standard stock solution
Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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 0.075ml of the Umeclidinium and 0.15ml of the Vilanterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution
Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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 0.15ml of the Umeclidinium and 0.3ml of the Vilanterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution
Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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 0.225ml of the Umeclidinium and 0.45ml of the Vilanterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Umeclidinium and Vilanterol and calculate the individual recovery and mean recovery values.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Umeclidinium and 10mg of Vilanterol 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 0.15ml of the Umeclidinium and 0.45ml of the Vilanterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions

The sample was analyzed at 0.8ml/min and 1.0ml/min instead of 0.9ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile and Water was taken in the ratio and 35:65, 45:55 instead (40:60), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

SYSTEM SUTABILITY

Chromatographic conditions

The method was performed with various C18 columns. Acquity UPLC BEH C-18,50 mm × 2.1 mm and 1.7 µm was found to be ideal as it gave good peak shape and resolution at 0.9 ml/min flow, equilibrated with Acetonitrile: Water (65:45v/v) as mobile phase. Run time was 6 minutes and here the peaks were seperated and showed better resolutions. Conditions of optimized chromatography are shown in table No.5.

Table 5: Optimized Chromatographic Conditions

Mobile phase ratio	Acetonitrile: Water (65:45v/v)
Column	Acquity UPLC BEH C-18,50 mm × 2.1 mm and 1.7 µm
Column temperature	40ºC
Wavelength	235nm
Flow rate	0.9ml/min
Injection volume	10µl
Run time	6 minutes

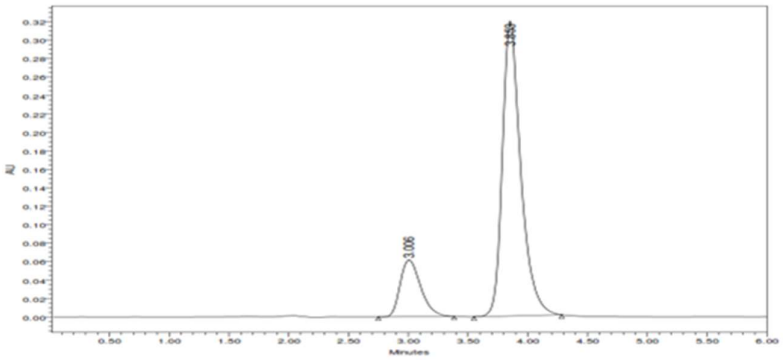

Fig 3: Optimized chromatogram of Umeclidinium (RT = 3.006) min) & Vilanterol (RT = 3.853 min)

Specificity

There were no other components were present at the elution time for Umeclidinium and Vilanterol. As seen in the figure 3, the blank chromatogram is present.

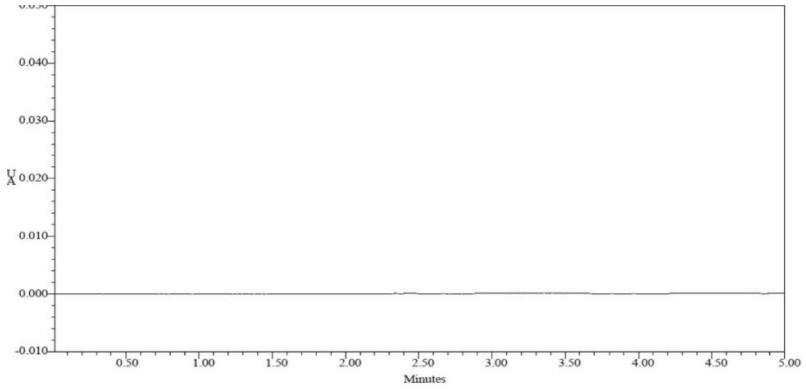

Fig 4: Chromatogram of blank

Linearity

The linearity range was found to be 5-10 µg/ml of Umeclidinium and 25-50 µg/ml of Vilanterol and chromatograms are shown in table no-6.

Table 6: Linearity data of Umeclidinium and Vilanterol

S.No	Umeclidinium		Vilanterol	
	Working conc (µg/ml)	Peak Area	Working conc (µg/ml)	Peak Area
1	5	230247	10	1215225
2	10	462332	20	2135937
3	15	659905	30	3020839
4	20	892989	40	4078841
5	25	1101075	50	5058145
Correlation Coefficient (r)		0.999		0.999
Slope (m)		43950		9933
Intercept (c)		8388		10151

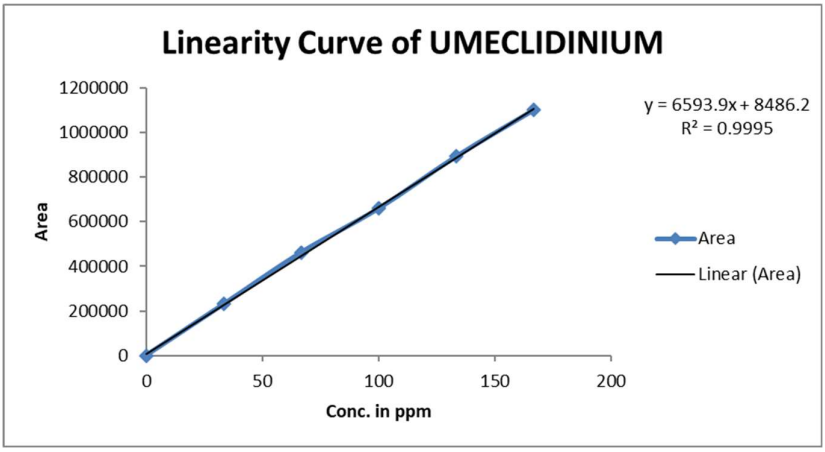

Fig 5: Calibration plot of Umeclidinium

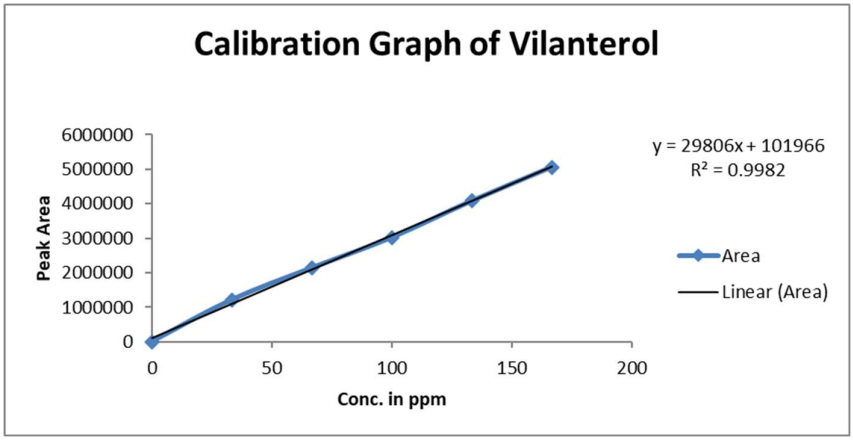

Fig 6: Calibration plot of Vilanterol

Precision

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatogram and results are shown in table 7.

Table 7: Results of method Precision

S.No	Umeclidinium		Vilanterol	
	Retention time (min)	Peak Area	Retention time (min)	Peak Area
1	3.007	658911	3.851	3021731
2	3.005	650383	3.848	3019183
3	3.005	658813	3.848	3029847
4	3.005	651138	3.850	3028471
5	3.005	659937	3.849	3088641
6	3.010	653715	3.860	3056633
Mean		655482.8		3040751
Std.Dev.		4258.945		26990.09
%RSD		0.649742		0.887613

Table 8: Results of intermediate Precision

S.No	Umeclidinium		Vilanterol	
	Retention time (min)	Peak Area	Retention time (min)	Peak Area
1	3.006	3.006	3.853	3075833
2	3.008	3.008	3.857	3029583
3	3.008	3.008	3.854	3021991
4	3.007	3.007	3.855	3022485
5	3.007	3.007	3.854	3085833
6	3.005	3.005	3.853	3019482
Mean		3.006		3042535
Std.Dev.		3.008		30022.42
%RSD		3.008		0.986757

Accuracy

Sample solutions at different concentrations (50%, 100%, 150%) were prepared and the % recovery was calculated.

Table 9: The Accuracy results of Umeclidinium & Vilanterol

Accuracy level	Umeclidinium			Vilanterol		
	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery

50%	7.5	7.3	99.88	7.5	7.49	99.7%
100%	15	14.7	98.89	15	14.9	99%
150%	22.5	22.2	101	22.5	22.48	99%
Mean% Recovery		100.166			99%	

Limit of detection and Limit of quantification (LOD & LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

Table 10: LOD & LOQ data for Umeclidinium and Vilanterol

Drug	LOD (µg/ml)	LOQ (µg/ml)
Umeclidinium	0.7µg/ml	2.1µg/ml
Vilanterol	1.8µg/ml	5.5µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.0ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Umeclidinium and Vilanterol. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

Table 11: Robustness data for Umeclidinium and Vilanterol

Parameter used for sample analysis	Umeclidinium		Vilanterol	
	Retention Time	Tailing factor	Retention Time	Tailing factor
Actual Flow rate of 0.9mL/min	3.006	1.2	3.853	1.59
Less Flow rate of 0.8mL/min	3.441	1.3	4.426	1.58
More Flow rate of 1.0mL/min	2.663	1.2	3.415	1.54
Less organic phase	3.185	1.1	4.291	1.61
More organic phase	2.867	1.3	3.583	1.50

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate UPLC method was developed for the quantitative estimation of Umeclidinium and Vilanterol 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. Umeclidinium and Vilanterol was freely soluble in ethanol, methanol and sparingly soluble in water. Water and Acetonitrile (60:40% 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 UPLC method was promising. The UPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Umeclidinium and Vilanterol in bulk drug and in pharmaceutical dosage forms.

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