World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Stability indicating RP-HPLC method development and validation for the simultaneous determination of vilanterol trifinatate and umeclidinium bromide in bulk and pharmaceutical dosage forms

G. Indira Priyadarshini, N. Naga Udayasri, M. Lakshmi Sowmya and Nagaraju Pappula

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur – 522002, Andhra Pradesh, India

Received: 10-01-2010 / Revised Accepted: 31-01-2021 / Published: 01-02-2021

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Vilanterol trifinatate and Umeclidinium bromide in dosage form. Chromatogram was run through symmetry C18 (150 x 4.6 mm, 5n). Mobile phase containing Water: Acetonitrile taken in the ratio 65:35 was pumped through column at a flow rate of 1.0ml/min. Retention time of Vilanterol trifinatate and Umeclidinium bromide were found to be 2.96 min and 2.315 min. %RSD of the Vilanterol trifinatate and Umeclidinium bromide were and found to be 1.0 and 0.6 respectively. %Recovery was obtained as 101.8% and 99.78% for Vilanterol trifinatate and Umeclidinium bromide respectively. LOD, LOQ values obtained from regression equations of Vilanterol trifinatate and Umeclidinium bromide Vilanterol were 0.54, 0.18 and 0.16, 0.05 respectively. Regression equation of Vilanterol trifinatate is y = 9969.6x +1935.1 and y =9122.6x + 6120.9 of Umeclidinium bromide. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Vilanterol trifinatate, Umeclidinium bromide, RP-HPLC

INTRODUCTION

Vilanterol trifinatate is approved by the FDA in December 2013 for use in combination with umeclidinium bromide. Vilanterol is a selective long-acting beta2-adrenergic agonist (LABA) with inherent 24-hour activity for once daily treatment of COPD and asthma. The combination drug is marketed by GSK (Glaxo smith kline) under the brand Anoro Ellipta.

Umeclidinium bromide is a long-acting muscarinic antagonist (LAMA) used as maintenance treatment for symptoms of chronic obstructive pulmonary disease (COPD). It is available as a once-daily inhalation monotherapy or as a fixed-dose

Address for Correspondence: G. Indira Priyadarshini, Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur – 522002, Andhra Pradesh, India; **Email: darshinipharma@gmail.com**

How to Cite this Article: G. Indira Priyadarshini, N. Naga Udayasri, M. Lakshmi Sowmya and Nagaraju Pappula. Stability indicating RP-HPLC method development and validation for the simultaneous determination of vilanterol trifinatate and umeclidinium bromide in bulk and pharmaceutical dosage formsWorld J Pharm Sci 2021; 9(2): 137-143.

combination product with the long-acting beta2-agonist vilanterol trifinatate. Its use has been shown to provide clinically significant, sustained improvements in lung function.

The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product[4].

Literature survey revealed few analytical methods is reported for both the drugs in alone. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method with UV detection for the analysis of Vilanterol trifenatate and umeclidinium bromide in bulk samples and combined dosage formulation.

Objective: Following are the objectives of the present work:

- To develop a new stability indicating HPLC method for the simultaneous estimation of Vilanterol trifenatate and Umeclidinium bromide and to develop the validated method according to ICH guidelines.
- To apply the validated method for the simultaneous estimation of Vilanterol trifenatate and Umeclidinium bromide in pharmaceutical formulation.

EXPERIMENTAL

Chemicals and reagents: Vilanterol trifenatate and Umeclidinium bromide pure drugs (API) were gifted from Sun pharma, Ahmedabad. marketed was procured from local market. Vilanterol trifenatate and Umeclidinium bromide inhaler (Anoro Ellipta), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer and Ortho-phosphoric acid, were purchased from Rankem, Mumbai

Apparatus and chromatographic condition: The chromatographic separation was performed on a HPLC system (WATERS) Series Alliance e2695 Software EMPOWER- 2, integrated with Auto Sampler and 2998 PDA detector. Water and CAN in the ratio of 65:5 (v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1.0 mL/min and the detector wavelength was set at 260 nm. The injection volume was 10 µL. Diluent used was Acetonitrile and Water taken in the ratio of 50:50.

Preparation of standard and sample solutions

Standard solution: Accurately weighed 2.5mg of Vilanterol trifinatate, 6.25mg of Umeclidinium bromide and transferred to 10ml volumetric flask and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (250µg/ml of Vilanterol and 625µg/ml of Umeclidinium)

Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (25µg/ml of Vilanterol and 62.5µg/ml of Umeclidinium)

Sample Solution: The contents of nasal spray delivered by 50 actuations (25&62.5 mcg each) were collected in 50 ml volumetric flask. Then 20ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 1250&3125µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF). 2ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (25µg/ml of Vilanterol and 62.5µg/ml of Umeclidinium)

Procedure: Inject 10µL of the standard and sample solution separately into the chromatographic system and measure the peak areas forvilanterol trifenatate and umeclidinium bromide and calculate the % assay value.

RESULTS AND DISCUSSIONS

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines[6]. Obtained validation parameters are presented in Table 1.

Linearity: The calibration curve was constructed by plotting response factor against respective concentration of vilanterol and umeclidium. The plots of peak area Vs respective concentration of vilanterol trifinatate and umeclidium bromide were found to be linear in the range of 6.25-37.5 µg/mL and 15.625-93.75 µg/mL with coefficient of correlation (r^2) 0.999 for two drugs. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for vilanterol trifinatate and umeclidium bromide were given in Fig. 1 and Fig. 2.

Recovery: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy

and mean %Recovery was obtained as 101.8% and 99.78% for Vilanterol trifinatate and Umeclidinium bromide respectively. The obtained results are presented in Table 2.

Sensitivity: The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to 0.05µg/ml and 0.18µg/ml for vilanterol trifinatate and 0.16µg/ml and 0.54µg/ml for umeclidinium bromide. The LOD and LOQ showed that the method is sensitive for vilanterol trifinatate and umeclidinium bromide.

System suitability test: The specificity of this method was determined by complete separation of Vilanterol trifinatate and Umeclidinium bromide as shown in Fig. 3 with parameters like retention time, resolution and tailing factor. The tailing factor for peaks of Vilanterol trifinatate and Umeclidinium bromide was less than 2% and resolution was satisfactory. The average retention time for Vilanterol trifinatate and Umeclidinium bromide were 2.956 min and 2.316 min respectively for five replicates. The peaks obtained for Vilanterol and Umeclidinium bromide were sharp and have clear baseline separation. Analysis was also performed for active Vilanterol trifinatate and Umeclidinium bromide, placebo sample (All the ingredients except active Vilanterol trifinatate and Umeclidinium bromide) both at stressed and unstressed condition. After analysis it was found that there is no interference of peak in the placebo & active sample. Hence the developed method was specific for the analysis of this product**.**

Precision: From a single volumetric flask of working standard solution six injections were given. A study was carried out for intermediate precision with the same analyst on the different day for six sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, temperature and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The assay results of tablet dosage formulation by the proposed method are presented in Table 3.

Stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of vilanterol trifinatate and umeclidinium bromide remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table 4.

Control sample: The contents of nasal spray delivered by 50 actuations (25&62.5 mcg each) were collected in 50 ml volumetric flask. Then 20ml acetonitrile was added , sonicated for 25 min and made up to mark to yield 1250&3125µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF). 2ml from sample stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (25µg/ml of Vilanterol and 62.5µg/ml of Umeclidinium)

Acid degradation sample: To 1 ml of stock s solution Vilanterol trifinatate and Umeclidinium bromide, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.The resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Fig. 4.

Base degradation sample: To 1 ml of stock solution Vilanterol trifinatate and Umeclidinium bromide, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Fig. 5.

Peroxide degradation sample: To 1 ml of stock solution of Vilanterol trifinatate and Umeclidinium bromide, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of oxidative degradation was given in Fig. 6.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For

HPLC study, the resultant solution was diluted to 25µg/ml & 62.5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The typical chromatogram of oxidative degradation was given in Fig. 7.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 250µg/ml & 625µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 25µg/ml & 62.5µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of thermal degradation was given in Fig. 8.

CONCLUSION

A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc. In the present method a SymmetryC18 (4.6 x 150mm, 5µm) column has been used for vilanterol trifinatate and umeclidinium bromide respectively. Mobile phase used was Water: Acetonitrile(65:35) for vilanterol and umeclidinium respectively, Retention of vilanterol trifinatate and umeclidinium bromide has more dependence on the mobile phase. The separation of the two peaks was also dependent on the buffer and the percentage of mobile phases. vilanterol trifinatate and umeclidinium bromide were eluted at acceptable retention times and got good resolution. Several assay methods has been developed for the determination of vilanterol trifinatate and umeclidinium bromide in pharmaceutical dosage forms and in biological fluids but this method is most economic and accurate so this method is very useful for the determination of vilanterol and umeclidinium bromide in bulk and pharmaceutical dosageforms. This method was validated as per ICH-Q2 (R1) guidelines and met the regulatory requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of vilanterol and umeclidinium bromide.

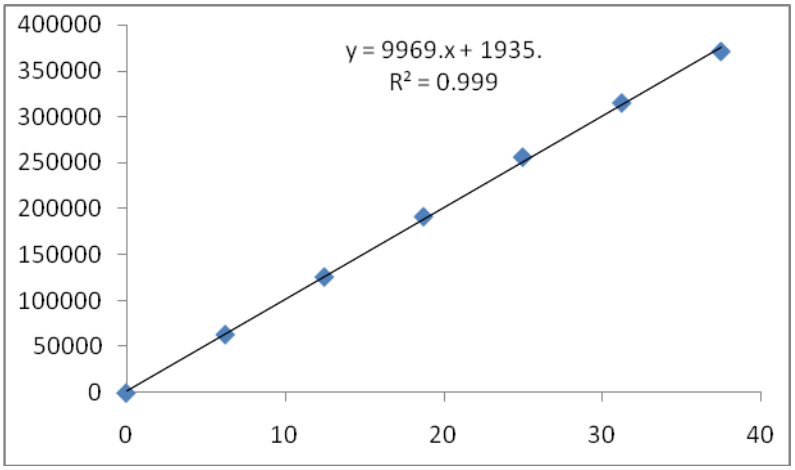

Fig. 1: Calibration curve of Vilanterol

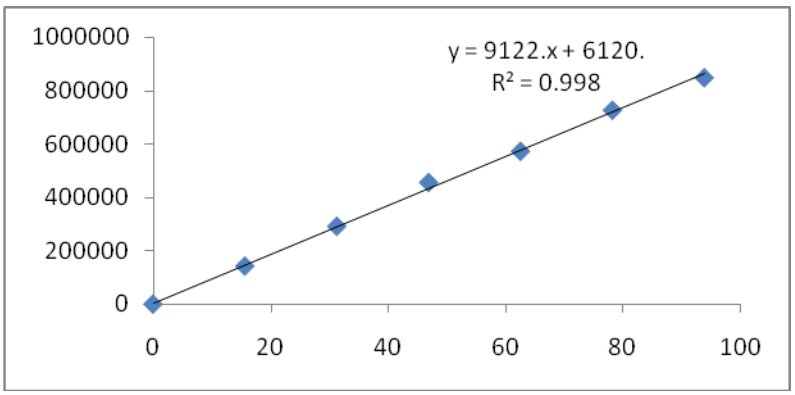

Fig. 2: Calibration curve for umeclidinium

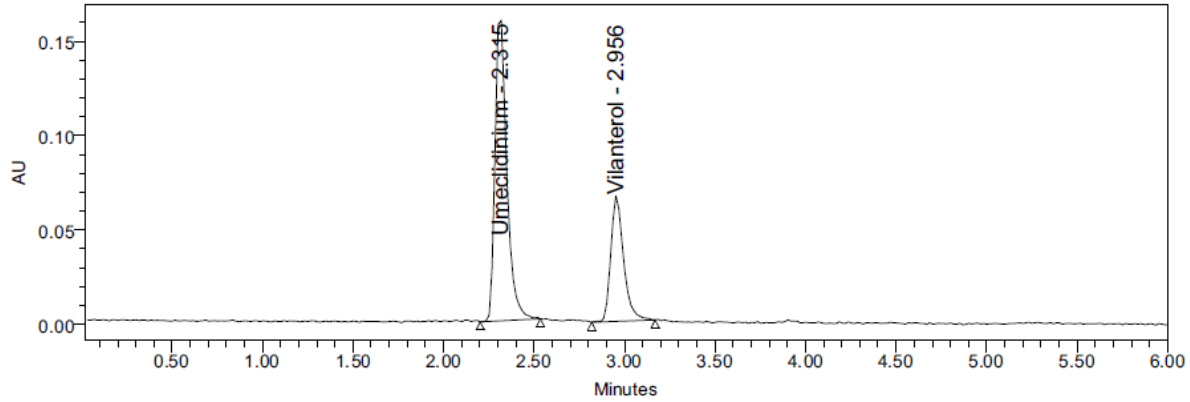

Fig. 3: Typical chromatogram of vilanterol and umeclidinium

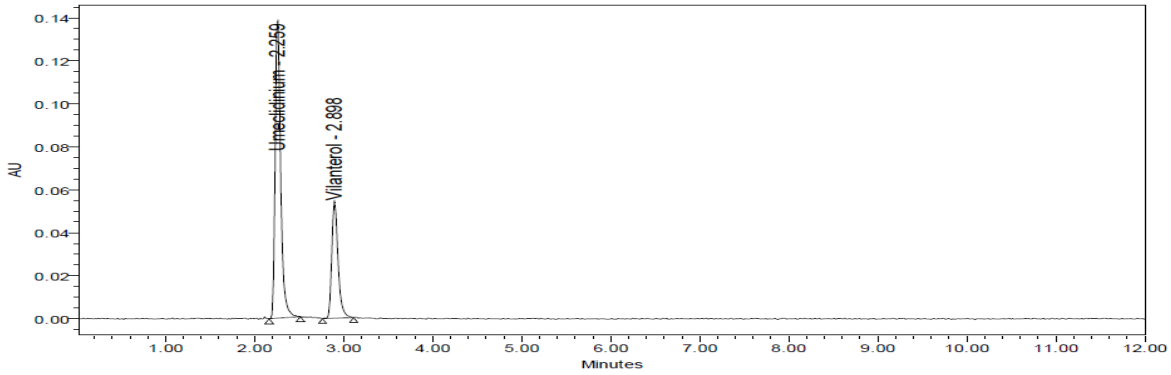

Fig. 4: Acid degradation chromatogram of vilanterol and umeclidinium

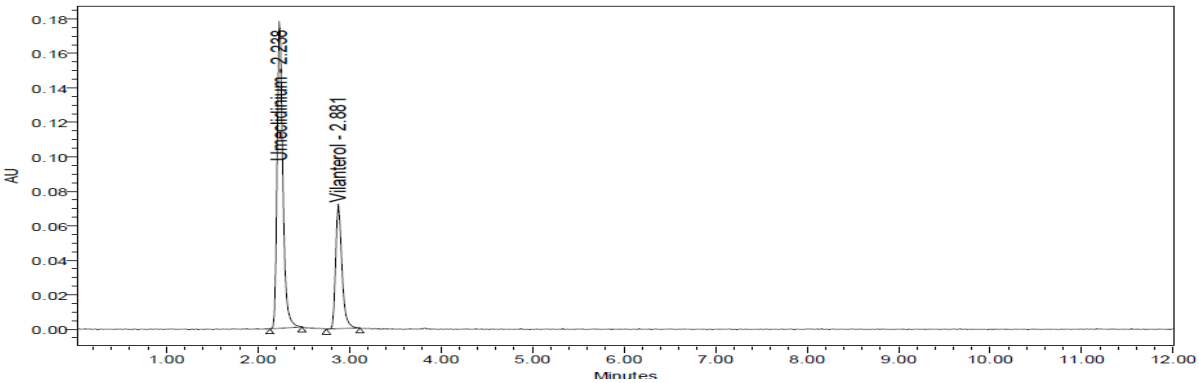

Fig. 5: Base degradation chromatogram of vilanterol and umeclidinium

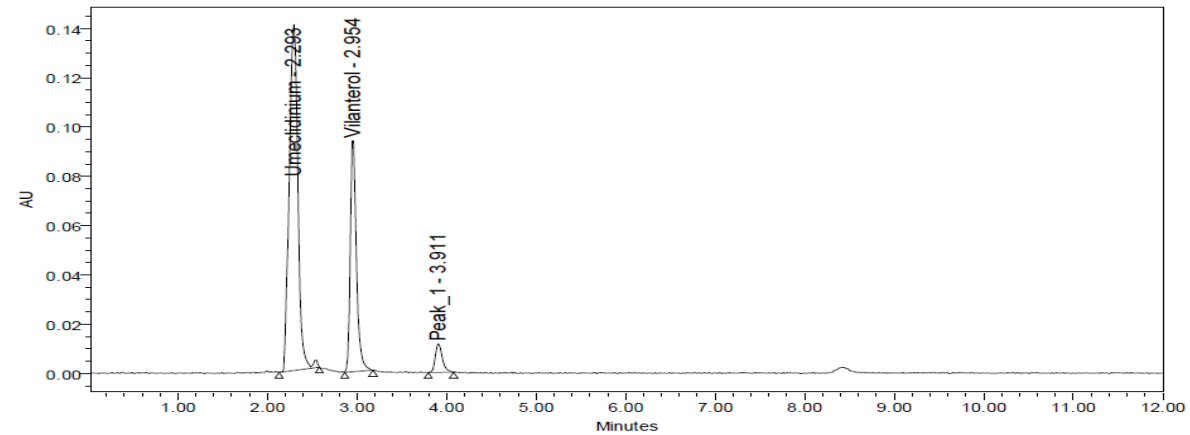

Fig. 6: Peroxide degradation chromatogram of vilanterol and umeclidinium

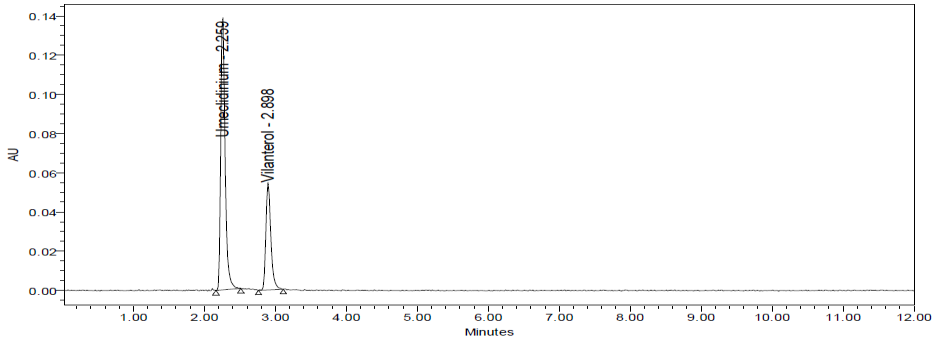

Fig. 7: Water degradation chromatogram of vilanterol and umeclidinium

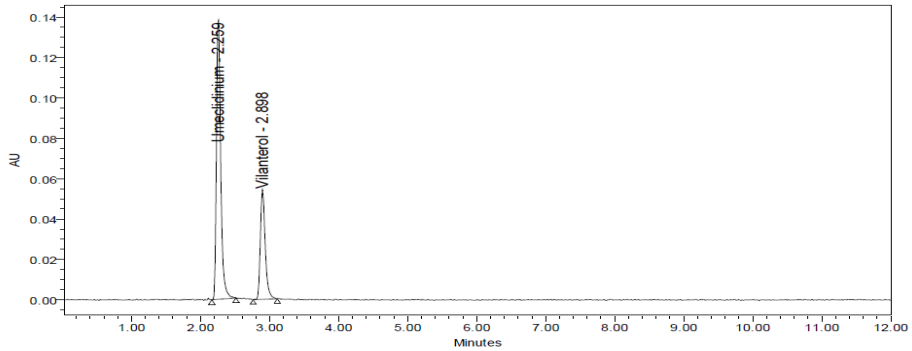

Fig. 8: Thermal degradation chromatogram of vilanterol and umeclidinium

Table 1: Analytical validation parameters (System suitability and Linearity)

Parameter	**Vilanterol trifinatate**	**Umeclidinium bromide**
Linearity	15.625-93.75 µg/mL	6.25-37.5 µg/Ml
Slope	9122.6	9969.6
Intercept	6120.9	1935.1
Regression equation (Y=mx+c)	y = 9122.6x + 6120.9	y = 9969.9x + 1935.1
Linearity Range(µg/ml)	15.625-93.75µg/ml	6.25-37.5 µg/ml
System precision %RSD	0.6	1.0
Method precision %RSD	1.3	1.3
LOD	0.18	0.05
LOQ	0.54	0.16
Theoretical Plates	6959	8848
Tailing Factor	1.3	1.37
Retention Time (min)	2.316	2.956

Table 2: Recovery studies of vilanterol and umeclidinium

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery	% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.35	98.84	101.8%	50%	31.25	31.18	99.76	99.78%
	12.5	12.47	99.72			31.25	31.52	100.86	
	12.5	12.34	98.69			31.25	31.21	99.88	
100%	25	25.32	101.30		100%	62.5	62.31	99.70	
	25	24.61	98.44			62.5	61.63	98.61	
	25	24.79	99.17			62.5	62.41	99.86	
150%	37.5	37.65	100.41		150%	93.75	93.01	99.21	
	37.5	37.24	99.30			93.75	94.51	100.82	
	37.5	37.90	101.08			93.75	93.10	99.31	

Table 3: Inter-day precision of vilanterol and umeclidinium

Drug	Sample Weight(mg)	Inter-day precision	
		SD	%RSD
Vilanterol	2.5	99.92	0.6
Umeclidinium	6.25	98.97	0.8

Table 4: Assay result of tablet dosage formulation

Drug	Label strength (mcg)	Amount found (mg)	% Assay
Vilanterol	25	9.947	99.41%
Umeclidinium	62.5	9.99	100.08%

Table 5: Forced degradation studies of vilanterol and umeclidinium

Type of degradation	Vilanterol			Umeclidinium		
	Area	%recovered	%Degraded	Area	%Recovered	% Degraded
Acid	238989	94.71	5.29	531288	94.47	5.53
Base	237927	94.29	5.71	530606	94.35	5.65
Peroxide	241812	95.83	4.17	540969	96.19	3.81
Thermal	247099	97.92	2.08	552288	98.20	1.80
Uv	249630	98.93	1.07	554606	98.62	1.38
Water	250513	99.28	0.72	557428	98.62	1.38

REFERENCES

1) https://www.drugbank.ca/salts/DBSALT001113
2) https://www.drugbank.ca/drugs/DB09082
3) Siva Kishore Masimukku1, Rambabu Chintala. Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Vilanterol And Fluticasone Furoate In Pharmaceutical Formulations. Asian J Pharm Clin Res, Vol 10, Issue 4, 2017, 302-305.
4) Dennis L. Kelleher, Rashmi S. Mehta. Safety, Tolerability, Pharmacodynamics and Pharmacokinetics of Umeclidinium and Vilanterol Alone and in Combination: A Randomized Crossover Trial. PLOS One. 2012; 7(12): e50716.
5) T Penne Dumitrescu, LL Santos. A Novel Method for Studying the Pharmacokinetics of [14C] Umeclidinium After Application to the Axilla or Palm of Healthy Male Subjects. Clin Transl Sci. 2016 Aug; 9(4): 183–191.