

# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF UMECLIDINIUM AND VILANTEROL IN PHARMACEUTICAL DOSAGE FORM

## ABSTRACT

A Sensitive, fast, linear and accurate LC technique was developed for the simultaneous determination of UMEC/VI in Powder dosage form. The estimation was carried out using Phenomenex C<sub>18</sub> column (150 × 4.6 mm, 5μ) with ammonium acetate: acetonitrile taken in the ratio 60:40 as mobile phase and pumped at a flow rate of 0.9 ml/min at 30°C. Detection wavelength selected was 245 nm. Retention times of UMEC/VI were found to be 2.219 min and 2.794 min. The method was validated in terms of linearity, precision, accuracy, LOD, LOQ as per ICH guidelines. Degradation studies performed indicated the stability of the drug. All of these analytical validation parameters were evaluated, and the percent RSD was calculated, indicating the method's suitability for determination of UMEC/VI in pharmaceutical dosage form.

**Keywords:** Umeclidinium, Vilanterol, RP – HPLC, Stability – indicating, Anoro Ellipta.

## 1. INTRODUCTION

Vilanterol (VI), chemically, 4-[(1R)-2-[(6-{2-[(2, 6-dichlorophenyl) methoxy] ethoxy}hexyl)amino]-1-hydroxyethyl]-2-(hydroxymethyl)phenol (Fig. 1(A)) is a long-acting, selective beta2-adrenergic agonist (LABA) with intrinsic 24-hour action for once daily COPD and asthma diagnosis. The activation of intracellular adenylyl cyclase, which catalyses the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate, is responsible for its pharmacological action (cAMP). Increases in cyclic AMP are linked to relaxation of the bronchial smooth muscle and suppression of the release of hypersensitivity mediators from mast cells in the lungs [1].

Umeclidinium (UMEC) (Fig.1 (B)) is a long-acting muscarinic antagonist (LAMA), used to treat COPD symptoms as a preventive treatment. Chemically, it is 1-[2-(benzyloxy)ethyl]-4-(hydroxydiphenylmethyl)-1-azabicyclo [2-2-2] octan-1-ium bromide. It comes as a single-dose inhalation monotherapy or as a combination of a fixed-dose medication with VI, a long-acting beta2-agonist [2]. COPD is a chronic obstructive pulmonary disease characterised by shortness of breath, cough, sputum production, and persistently reduced airflow (less than 80% FEV1 in 1 second). UMEC inhibits acetylcholine binding and therefore opens the airways by

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inhibiting bronchoconstriction by blocking the M3 muscarinic receptor, which is abundantly expressed in the airway smooth lung muscle.

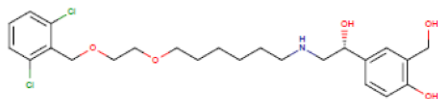


Fig 1(A): Chemical Structure of VI

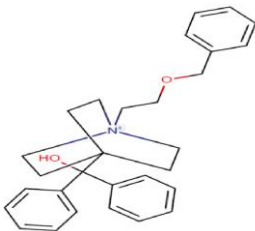


Fig 1(B): Chemical structure of UMEC

The literature survey conducted revealed some analytical studies[3-7] performed for the drugs, the key idea is to develop an easy, sensitive and accurate method for estimation of UMEC/VI in pharmaceutical dosage form employing RP-HPLC. In terms of linearity and range, precision, and accuracy, the suggested approach was verified using criteria from the International Conference on Harmonization [8-10].

## 2. EXPERIMENTAL

### 2.1 MATERIALS AND METHODS

**2.1.1 Chromatographic Instrumentation:** The analysis was performed using WATERS HPLC 2695 SYSTEM fitted with quaternary pumps, Photo Diode Array Detector and Auto Sampler integrated with Empower 2 software. Column used for separation was Phenomenex C<sub>18</sub> (150 x 4.6 mm, 5 $\mu$ ). Ammonium acetate: acetonitrile taken in the ratio 60:40 as mobile phase and pumped through column at a flow rate of 0.9 ml/min at 30°C. Optimized wavelength selected was 245 nm.

**2.1.2 Chemicals and reagent:** HPLC grade Acetonitrile, Methanol, Distilled Water, Ortho – phosphoric acid buffer, Ammonium Acetate were obtained from Rankem. UMEC/VI pure drugs

(API), UMEC/VI dosage form (Anoro Ellipta) were received from Spectrum Pharma Research Solution, Hyderabad.

**2.1.3 Preparation of Diluent:** Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in 50:50 ratio

**2.1.4 Preparation of Standard stock solutions:** 2.5mg VI and 6.25mg UMEC were accurately weighed and added to a 10ml volumetric flask, together with 3/4th of the diluents, then sonicated for 10 minutes. Standard stock solution was created by filling flasks with diluents and labeling them as such. (VI - 250 µg/mL, UMEC - 625 µg/mL)

**2.1.5 Preparation of Standard working solutions (100% solution):** 1 mL of each stock solution was transferred into a 10 mL volumetric flask, which was then diluted. (VI - 25 µg/ml, UMEC - 62.5 µg/ml)

**2.1.6 Preparation of Test solutions:** In a 50 ml volumetric flask, the contents of nasal spray administered by 50 actuations (25 and 62.5 µg each) were collected. Then 20ml acetonitrile was added, and it was sonicated for 25 minutes before being filled to the mark, yielding 1250 & 3125µg/ml. It was centrifuged for 20 minutes. Finally, the supernatant was gathered and filtered through 0.45 µm filters (Millipore, Milford and PVDF). 2ml of the sample stock solution was pumped into a 10ml volumetric flask, which was then filled with diluent. (VI - 25 µg/ml, UMEC - 62.5 µg/ml).

## **2.2 Validation:**

### **2.2.1 System suitability parameters:**

The parameters, peak tailing, resolution and USP plate count were defined by preparing standard solutions of VI (25ppm) and UMEC (62.5ppm) and the solutions were injected six times.

### **2.2.2 Specificity:**

In the presence of elements, specificity refers to the ability to measure the analyte unambiguously. In this approach we do not consider interfering peaks of these drugs in blank and placebo at retention times. So, the method is said to be accurate.

### **2.2.3 Precision:**

The method was checked for both intra-day and inter-day precision.

#### **2.2.4 Linearity:**

Appropriate aliquots of UMEC and VI standard stock solutions were placed in separate 10 mL volumetric flasks and diluted up to the mark with mobile phases to achieve final concentrations of 15.625 - 93.75µg/ml for UMEC and 6.25 - 37.5µg/ml for VI. The solutions were then injected into the system and chromatograms were registered.

#### **2.2.5 Accuracy:**

Recovery trials were used to determine the method's accuracy. For this experiment, a standard addition approach was used. A known amount of UMEC and VI was introduced, equivalent to 50%, 100%, 150% of the drug's label claim, respectively. Each addition set was performed three times. The accuracy was calculated as a percentage of recovered analytes.

#### **2.2.6 Robustness:**

Tiny subtle improvements are made in methods such as flow rate, mobile phase ratio, and temperature, but the effects have not been recognized and are within the limits of ICH Guidelines. Robustness conditions such as Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) & temperature plus (35°C) was established and samples were double-injected. System suitability parameters were not much affected & all parameters were passed. %RSD was within the limit.

#### **2.2.7 LOD:**

The lowest amount of analyte that can be detected is referred to as the LOD. LOD values are derived from calibration curve using standard deviation (SD) and slope of calibration curve. The formula for determining LOD is,

$$\text{LOD} = 3.3 \times \text{avg SD/slope}$$

#### **2.2.8 LOQ:**

The smallest quantity of analyte that can be measured is referred as LOQ. LOQ is measured from calibration curve using standard deviation (SD) and slope of calibration curve. The formula for determining LOQ is,

$$\text{LOQ} = 10 \times \text{avg SD/slope}$$

### 2.3 Degradation studies:

Degradation study was carried out by treating samples with acid, alkali, hydrogen peroxide (oxidation), neutral (water), thermal condition, and UV application. Acid and alkali degradation was carried using 2N HCl and 2N NaOH respectively. Oxidation degradation was performed using 20% H<sub>2</sub>O<sub>2</sub> solution. Acid, alkali and oxidation degradation studies were kept at 60<sup>o</sup> for 30 mins. Stress testing within neutral conditions were studied by refluxing the sample in H<sub>2</sub>O for 1hr at 60<sup>o</sup>. To study dry heat degradation, the standard drug solution were placed in the oven at 105 ° C for 1 hr. The drug's UV degradation stability were also studied by exposing the 250µg/ml VI & 625µg/ml UMEC solution to UV Light by placing the beaker in the UV chamber for 1day or 200wt hours/m<sup>2</sup> in the photo stability chamber. The resulting solution were diluted 10 µl were injected into the system and the sample stability was evaluated by the chromatograms.

## 3. RESULTS AND DISCUSSION

### 3.1 Optimization of Chromatographic Condition (Method Development)

Method development was done by changing various, mobile phase ratios, and buffers etc. methanol: water, methanol: OPA, acetonitrile: ammonium acetate in various ratios were tried as mobile phase. Finally, acetonitrile: ammonium acetate (40:60 v/v) was selected for the optimization of chromatographic condition. With reasonable resolution, UMEC/VI were elucidated at 2.219 min and 2.794 min. Optimized chromatogram is depicted in fig. 2.

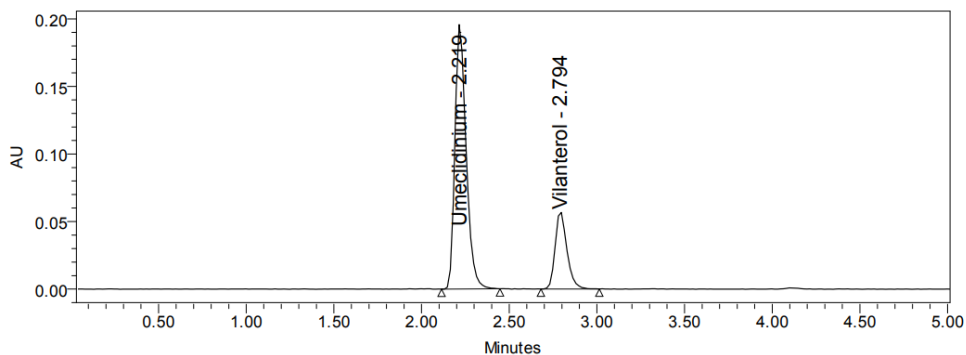


Fig 2: Optimized trial chromatogram of UMEC/VI

### 3.2. Method Validation

#### 3.2.1 System suitability parameter

All system suitability parameters, peak tailing, resolution and USP plate count were within range according to ICH rules. The resolution was found to be 4.66. System suitability parameter and acceptance limit are shown in table 1.

Table 1: system suitability parameter of UMEC/VI

Parameters	UMEC	VI	Acceptance limit
USP plate count	5595.5	8254	>2000
Tailing factor	1.256	1.206	<2
R <sub>t</sub> (min)	2.219	2.794	≥2

#### 3.2.2 Specificity

No intrusive peaks at retention time of main drug were observed after injecting blank and placebo, so the method was said to be specific. Chromatograms are depicted in fig. 3-5.

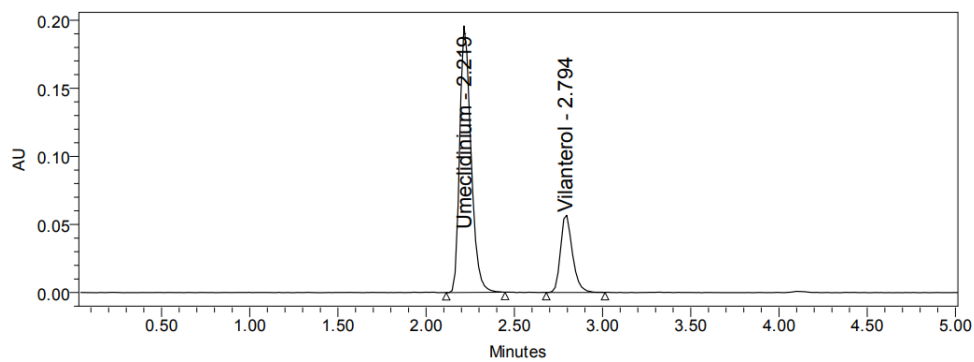


Fig 3: Optimized Chromatogram of UMEC/VI

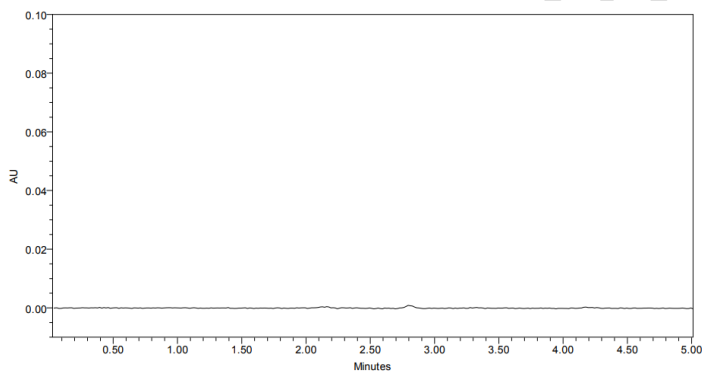


Fig. 4: Chromatogram of Blank



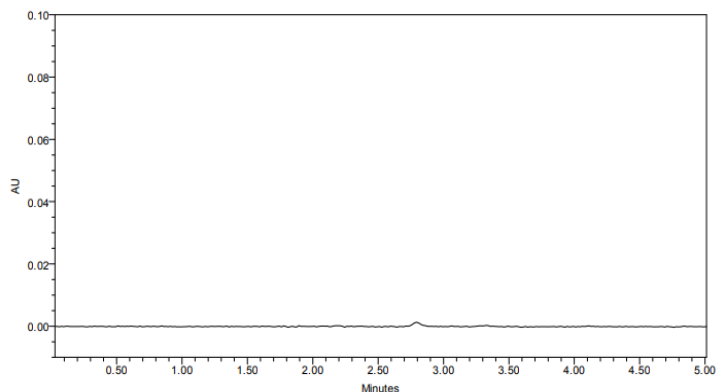


Fig. 5: Chromatogram of Placebo

### 3.2.3 Precision

The precision was tested using varied drug concentrations and the solutions were made from stock solutions and injected in interday and intraday intervals to assess precision. For intraday experiments, the concentrations were generated at six separate times during the day. **Interday and interday precision** were demonstrated in the Table 2. The percentage RSD of System precision was found to be 1.0 and 0.9 for UMEC/VI.

Table 2: Precision data of UMEC/VI

Precision type	%RSD	
	UMEC	VI
Intra – day	0.8	0.6
Inter – day	1.6	1.1

### 3.2.4 Linearity

The method was linear over the range 15.625 - 93.75 $\mu$ g/ml and 6.25 - 37.5 $\mu$ g/ml for UMEC/VI. The calibration curve was constructed by plotting the response factor against a concentration of drugs (Fig 6, Fig 7). The slope and intercept value for calibration curve was  $y = 16315x + 10672$ ,  $R^2 = 0.9992$  for UMEC and  $y = 12261x + 4146.2$ ,  $R^2 = 0.999$  for VI. The results show an excellent correlation between the response factor and the concentration of drugs.

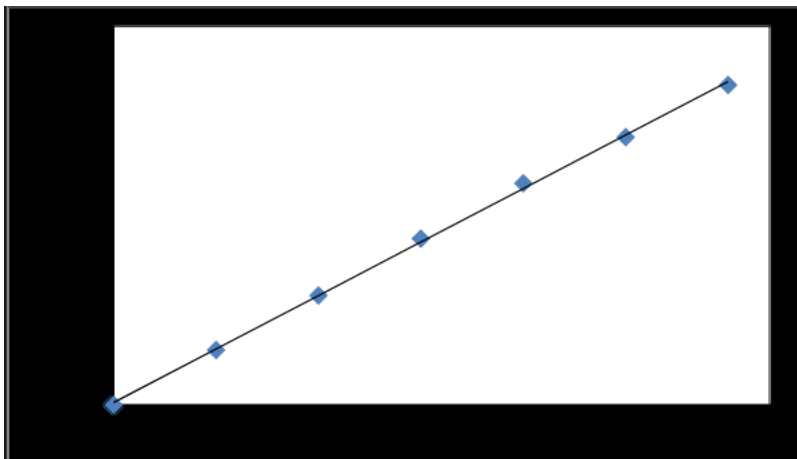


Fig 6: Calibration curve of UMEC

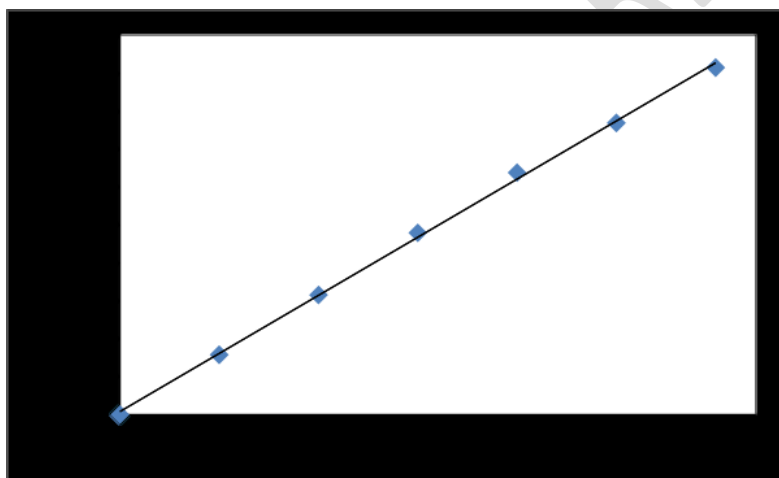


Fig 7: Calibration curve of VI

### 3.2.5 Accuracy

The approach's accuracy was tested three times using the conventional addition technique, each time in triplicate. The proportion of standard drug recovered by the recovery study was then used to calculate the accuracy. Table 3 shows the mean UMEC/VI recovery rates from the combined

formulation. Accuracy is measured in terms of % Recovery. %Recovery was obtained as 100.09% and 99.94% for Umeclidinium and vilanterol.

**Table 3: Accuracy data of proposed study of UMEC/VI**

Recovery Range	% Recovery	
	UMEC	VI
50%	100.51	100.35
100%	100.20	98.98
150%	99.54	100.49
Mean % Recovery	100.09%	99.94%

### 3.2.6 Robustness

Robustness of the method was determined by changing three HPLC variables, like flow rate (0.8 and 1.0 mL min<sup>-1</sup>), mobile phase ratio (65B: 35A and 55B:45A) and column temperature (25 and 35°C). The effects of these changes on the % RSD of both the substances (UMEC/VI) were determined. The value of percentage standard deviation was within the range, as shown in Table 4, which indicates that the developed method is robust and none of the variables possess significant effects on the method performance.

**Table 4: Robustness Data of UMEC/VI**

Parameter	Condition	% RSD	
		UMEC	VI
Change in flow rate ( $\pm$ 0.1 ml/ min)	0.8 mL min <sup>-1</sup>	1.1	1.4
	1.0 mL min <sup>-1</sup>	0.7	0.7
Change in mobile phase composition acetonitrile: ammonium acetate ( $\pm$ 5 mL)	65B:35A	0.6	0.4
	55B:45A	0.4	0.3
Change in column temperature ( $\pm$ 5 °C)	25 °C	1.1	1.6
	35°C	1.2	1.0

### 3.2.7 LOD and LOQ

Table 5 shows the minimum detection limit and minimum quantitation limit of UMEC/VI. LOD of UMEC/VI were found to be 0.33 and 0.15. LOQ of UMEC/VI were found to be 1.01 and 0.45, respectively.

Table 5: LOD and LOQ Data of UMEC/VI

Molecule	LOD	LOQ
UMEC	0.33	1.01
VI	0.15	0.45

### 3.2.8 Assay:

The label containing **Anoro Ellipta** specifies UMEC 62.5 mcg, VI 25mcg. Assay with dosage type was performed. The mean percentage of assays obtained for UMEC and VI were 99.64 and 100.79 percent respectively.

### 3.3 Degradation studies

Acidic, alkaline, oxidative, thermal, UV and hydrolytic degradation studies were performed on Umeclidinium and Vilanterol. Since the % degradation was under acceptance criteria, It represents the stability-indicating properties of the method. % degradation was demonstrated in table 6. The chromatograms of the sample are shown in Fig 8 – 13.

Comment [BAB1]: Water

Table 6: degradation study data of UMEC/VI

Stress condition	% Degradation	
	UMEC	VI
Acidic (2N HCl)	5.80	5.76
Alkaline (2N NaOH)	5.09	5.01
Oxidative (20% H <sub>2</sub> O <sub>2</sub> )	4.41	4.48
Thermal (105°C)	2.18	2.57

UV	1.31	1.74
Neutral (Water)	0.99	0.88

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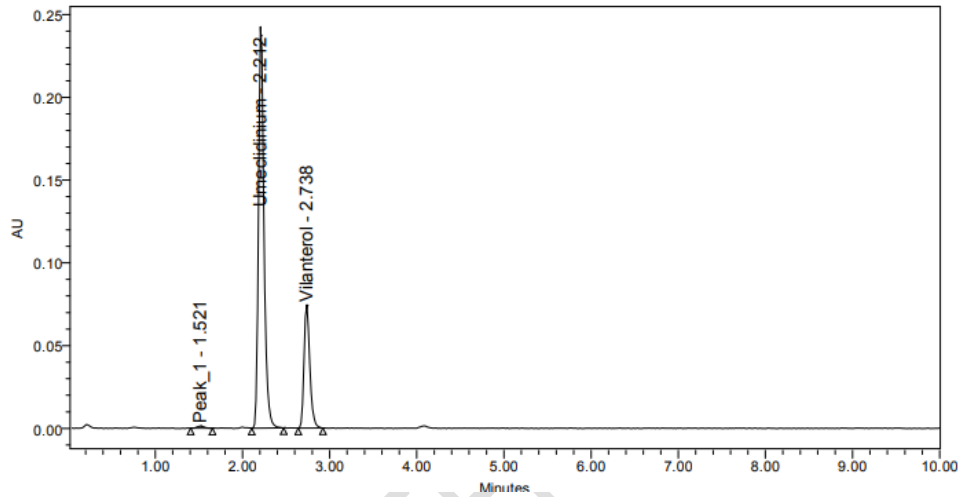


Fig 8: Acid chromatogram of UMEC/VI

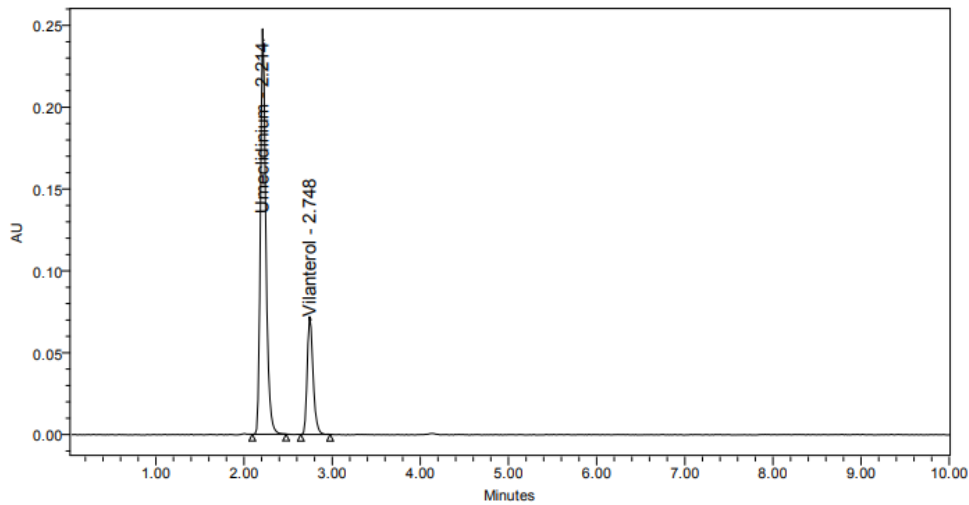


Fig 9: Base chromatogram of UMEC/VI

Comment [BAB3]: Alkaline

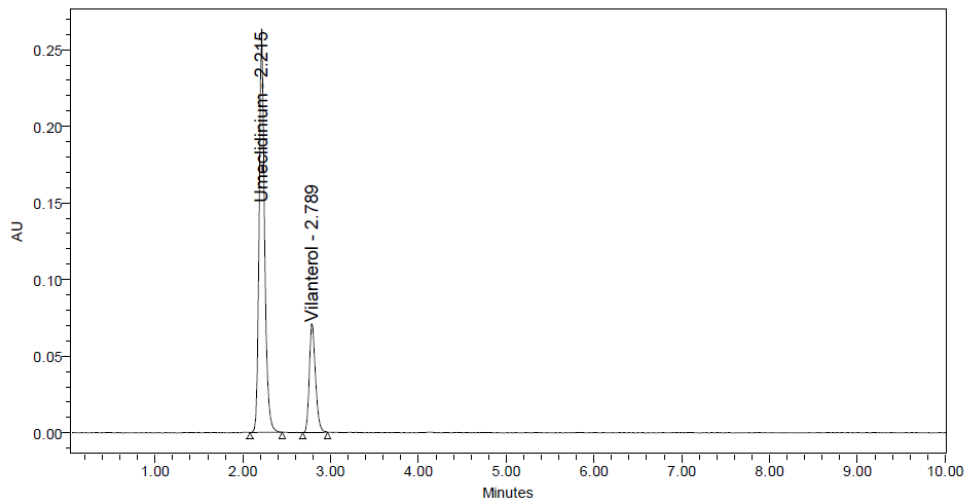


Fig 10: Peroxide chromatogram of UMEC/VI

Comment [BAB4]: Oxidative

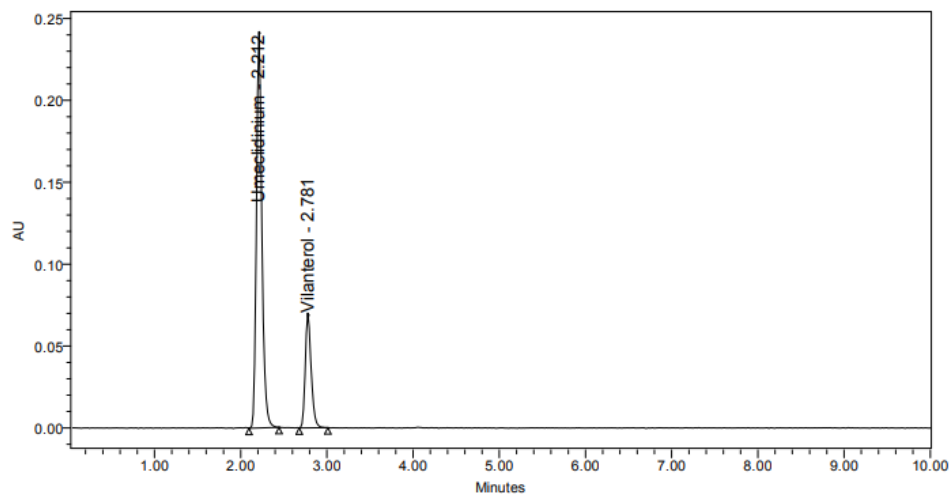


Fig 11: Thermal chromatogram of UMEC/VI

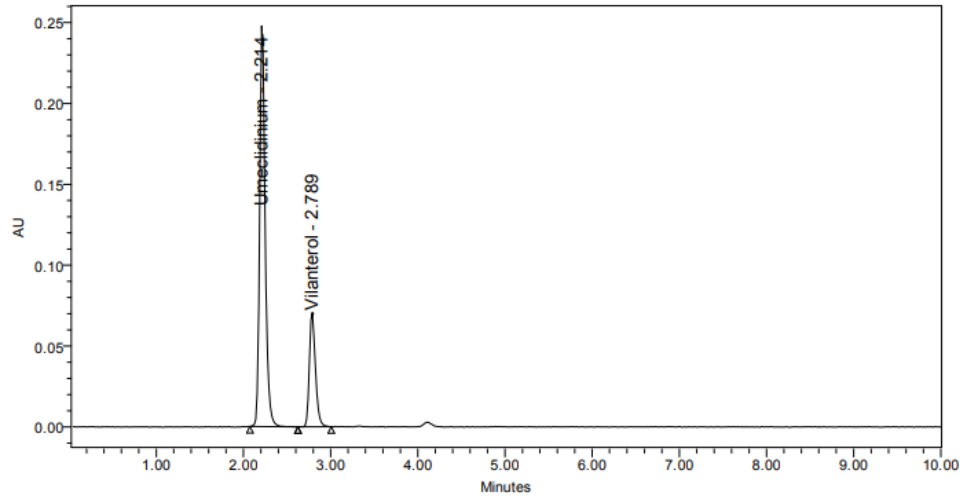


Fig 12: UV chromatogram of UMEC/VI

Comment [BAB5]: Photolytic degradation

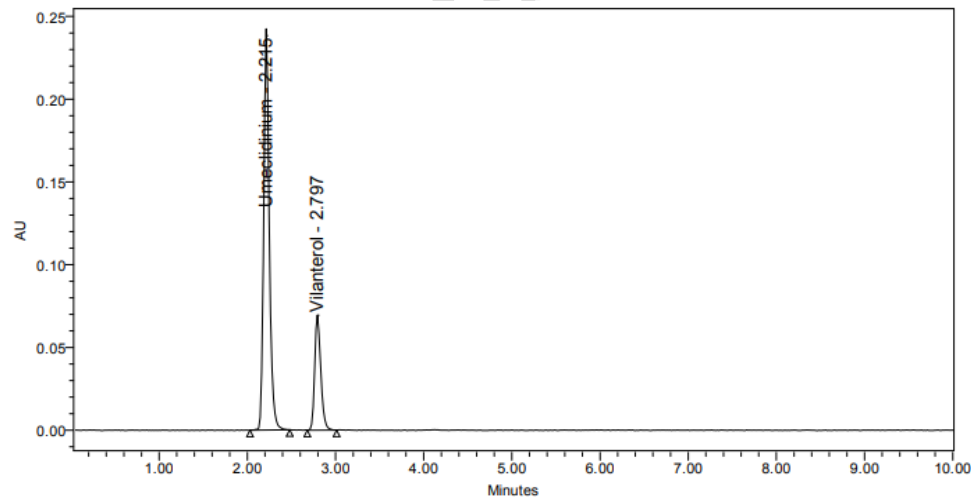


Fig 13: Water chromatogram of UMEC/VI

#### 4. CONCLUSION

For the simultaneous determination of UMEC/VI in powder dosage form a simple, linear, sensitive and accurate method was developed. The % RSD of UMEC/VI were found to be 1.0

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and 0.9. Percentage Recovery was obtained as 100.09% and 99.94% for UMEC/VI respectively. LOD, LOQ values obtained from regression equations of UMEC/VI were 0.33, 1.01 and 0.15, 0.45 respectively. Regression equation of UMEC is  $y = 16315x + 10672$ , and  $y = 12261x + 4146.2$  of VI. The mean percentages of assay for UMEC/VI were obtained as 99.64% and 100.79%. The percentage of degradation resulted from the stability studies were under the acceptance limit. The method developed was low cost, easy, sensitive and fast that can be adopt in regular Quality control test in companies.

#### CONSENT AND ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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