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An optimized Box-Behnken design RP-HPLC method for the quantification of Umiclinidinium Bromide and Vilanterol Trifenate in inhalation powder

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A liquid chromatographic method with enhanced efficacy has been proposed for the simultaneous quantification of Vilanterol Trifenate and Umiclinidinium Bromide in pharmaceutical formulation. This method is straightforward, sensitive, accurate, precise, and dependable. The RP-HPLC method's experimental parameters were multivariately optimized using the design of experiments (DoE). Mathematical models were created using three independent variables: the percentage of acetonitrile, the strength of the buffer, and the pH of the mobile phase. In order to examine the response surface methodology and the effects of these independent factors, a Box-Behnken Design (BBD) was utilized. The optimized and forecasted data from the contour diagram utilized a mixture of acetonitrile (58.6% v/v) and phosphate buffer (12.6 mM) with a pH of 5.1. The flow rate was adjusted to 1 milliliter per minute and the analytical wavelength employed was 272 nm. The pharmaceuticals were successfully separated with high resolution in less than 6 minutes under ideal conditions, and the validation process was conducted in accordance with the requirements set by the International Council for Harmonisation (ICH). The findings indicated that the Quality by Design technique was effective in optimizing the RP-HPLC method for quantifying Vilanterol Trifenate and Umiclinidinium Bromide.

Keywords: Vilanterol Trifenate; Umiclinidinium Bromide; Quality by design; RP-HPLC

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Introduction

Umeclidinium bromide (Figure 1) is a compound with the chemical formula 1-[2-(benzyloxy)ethyl]-4-(hydroxydiphenylmethyl)-1-azoniabicyclo[2.2.2]octane bromide. Vilanterol trifenatate (Figure 1) is a compound with the chemical formula triphenylaceticacid-4-{(1R)-2-[(6-{2-[(2,6 dicholorobenzyl) oxy] ethoxy} hexyl) amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol(1:1).
Umeclidinium is a pharmacological compound that acts as a long-lasting inhibitor of muscarinic receptors and is commonly referred to as an anticholinergic agent.

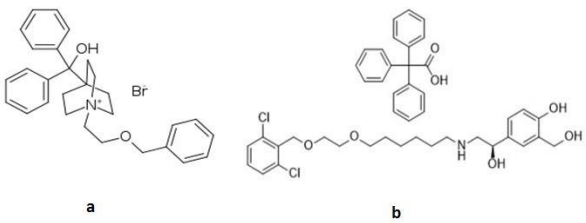

Figure 1. Structure of Umeclidinium bromide (a) and Vilanterol trifenatate (b)

This compound is a quinuclidine derivative that exhibits activity at multiple subtypes of muscarinic receptors. Umeclidinium functions as a competitive inhibitor, preventing acetylcholine from binding to muscarinic receptors on airway smooth muscle. This inhibition leads to bronchodilation, or the widening of the airways (1-3). It exhibits a delayed ability to reverse its effects at the M3 muscarinic receptor subtype in human cells grown in a laboratory setting. Additionally, when directly delivered to the lungs in animal models used for testing before human trials, it has a longer-lasting effect. Vilanterol is a specific type of drug that activates a certain receptor in the body called the beta2-adrenergic receptor. It has the ability to be active in the body for a longer period of time compared to other drugs. It activates intracellular adenylate cyclase, an enzyme that facilitates the conversion of adenosine triphosphate (ATP) into cyclic-3′,5′-adenosine monophosphate (cyclic AMP). This conversion is responsible for the pharmacological actions of these drugs. Bronchial smooth muscle undergoes relaxation in response to increased levels of cyclic AMP, which concurrently inhibit the release of mediators of acute hypersensitivity, particularly mast cells (4-6). Umeclidinium bromide/vilanterol trifenatate inhalation powder is approved by FDA indicated for the long-term, once-daily maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema. The

administration of umeclidinium and vilanterol (7,8) may provide advantageous effects for the management of asthma or COPD, including emphysema and chronic bronchitis. The current composition of umeclidinium combined with vilanterol is a dry powder designed for inhalation. It is contained in a plastic inhaler that is light grey and red in color and consists of two foil blister strips. Umeclidinium is contained within a single strip, whereas vilanterol is contained within the other strip (7,8). There is presently no published RP HPLC method for estimating Umeclidinium bromide and Vilanterol trifenatate individually or together in bulk or pharmaceutical dose forms using an experimental design approach. The literature has described the measurement of umeclidinium bromide and vilanterol trifenatate in pharmaceutical formulations using several analytical techniques, including UV spectrophotometric (9) and RP-HPLC (10,11). Based on the latest recommendations from the FDA, these techniques failed to accurately define the design space. It is recommended to employ "Quality by Design" (QbD) or "Design of Experiments" (DoE) to ensure the reliability of analytical technique validation through monitoring statistical quality control and researching factors that have a detrimental impact on quality in pharmaceutical analysis. The conventional approaches for method development involve employing trial and error, along with modifying one element at a time. Several factors, such as scarcity of the chromatographic column, the use of solvents and chemicals, and the important physicochemical properties of the analyte, often hinder the achievement of reliable chromatographic conditions in this approach. A few new drug applications have recently been approved by the FDA that include analytical techniques such as HPLC (12) and UV Spectrophotometry, together with the QbD methodology. This allows for flexibility in the defined method operable design region (MODR) while still complying with regulations. Quality by Design (QbD) has had a significant impact on the development of pharmaceutical goods and has enhanced the reliability of the manufacturing process since its approval by the FDA (13). An evaluation of all factors that exert the most significant influence on the outcomes of the HPLC technique is vital for a contemporary Quality by Design (QbD) approach to ensure the method's stability and reliability. Experimentally verifying numerous factors simultaneously is impractical, arduous, and cost-prohibitive. Comprehending how the quality of the system reacts to its parameters, leading to the creation of a design space for the approach, is essential for overcoming challenges and minimizing the need for experimentation. Consequently, we successfully developed a straightforward and accurate RP HPLC method for analysing Umeclidinium bromide and Vilanterol trifenatate. We utilized DoE to aid in the development process and employed Box Behnken design (BBD) to assess the method's robustness. Furthermore, we utilized response surface methodology (RSM) to interpret the data graphically.

Experimental

Chemicals and reagents

Both medication standards were presented as donations from the pharmaceutical sector with Vilanterol trifenatate (purity≥99.23% and Umeclidinium bromide (purity ≥ 99.45%), Methanol, water, and acetonitrile (LC grade) were purchased from Merck India Ltd, Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid were purchased from SD Fine chemicals, located in Mumbai. Vilanterol trifenatate and Umeclidinium bromide (in the form of inhalation powder) were purchased at a nearby pharmacy with labeled claim 74.2 mg and 44 mg, respectively.

HPLC instrumental condition

The method was developed using an Acquity HPLC system (Waters, Milford, MA, USA) equipped with a model 2996 PDA detector and Empower-2 software. The separation of the two analytes was achieved using a mixture of acetonitrile and phosphate buffer (12.6 mM) in a ratio of 58.6:41.4 (v/v), maintaining a 1 $mLmin^{-1}$ flow rate at ambient temperature, in isocratic mode. An analysis and separation were conducted using a Phenomenex Gemini C18 column of 250 mm x 4.6 mm with a particle size of 5 µm. The chromatographic runs were performed in a controlled atmosphere at a constant temperature of 25 °C. The two drugs were examined at a wavelength of 272 nm utilizing the PDA detector. Prior to utilization, the solvents underwent degassing in an ultrasonic bath and were subsequently filtered through a 0.22 µm membrane filter. The diluent used was the mobile phase.

Software

Design-Expert version 12 was used to do calculations for the experimental design (BBD), desirability function, and data analysis.

Preparation of buffer solution

Weighed 0.11 grams of potassium dihydrogen orthophosphate and 1.41 grams of monobasic potassium phosphate. Then, placed them in a 1000 ml beaker and filled the beaker with HPLC water until it reached the 1000 ml mark.

Preparation of standard solutions

The 25 mg sample of each medicine was measured and transferred into a 25 ml volumetric flask. Following the addition of 15 ml of acetonitrile, the mixture was subjected to sonication for a duration of 15 minutes. The volume was adjusted with acetonitrile to reach the desired level, resulting in a stock solution with a concentration of 1000 μgml^{-1}. In order to prepare standard solutions with a concentration of 100 μgml^{-1}, 2.5 ml of the standard stock solutions are extracted and transferred to 25 ml volumetric flasks. Subsequently, the remaining volume was filled up to the suitable level using the mobile phase.

Preparation of pharmaceutical samples

A portion of the powder from the strip, weighing 74.2 mg of umeclidinium bromide and 44 mg of vilanterol trifenatate, was added to a 100 mL volumetric flask. In order to dissolve the drugs, a volume of 20 mL of acetonitrile (HPLC) grade was added and subjected to sonication for a duration of 5 minutes. The contents of the flask were maintained at ambient temperature for a duration of 10 minutes prior to being mixed with acetonitrile of high-performance liquid chromatography (HPLC) quality. This resulted in a concentration of umeclidinium at 742 μgmL^{-1} and vilanterol trifenatate at 440 μgmL^{-1}. The afore mentioned solution underwent filtration using a membrane filter with a pore size of 0.2 µm. A volume of 0.5 mL of the filtered solution was moved into a 10 mL flask and mixed with the mobile phase to achieve concentrations of 37.1 μgmL^{-1} for umeclidinium bromide and 22 μgmL^{-1} for vilanterol trifenatate.

Table 1. Experimental design domain (BBD) for each run with responses

Run	Factors			Responses		
	% ACN (v/v) X_1	Buffer(mM) X_2	pH Buffer X_3	Vt Rt Y_1	Rt Um Y_2	Rs Y_3
1	60	15	6.0	2.165	3.886	4.235
2	60	10	5.0	1.254	2.765	4.034
3	50	20	4.0	2.664	3.431	2.443
4	50	20	6.0.	2.875	3.672	2.453
5	40	20	5.0	1.934	3.99	5.12
6	60	15	4.0	3.321	4.032	2.44
7	50	15	5.0	2.543	3.562	3.02
8	50	15	5.0	2.543	3.562	3.02
9	50	15	5.0	2.543	3.562	3.02
10	40	15	4.0	2.905	3.122	1.43
11	50	15	5.0	2.543	3.562	3.04
12	50	10	4.0	2.123	3.121	2.883
13	50	10	6.0	3.032	3.903	2.663
14	40	10	5.0	2.932	3.123	2.823
15	60	20	5.0	2.674	4.452	4.434
16	40	15	6.0	2.872	4.132	3.52
17	50	15	5.0	2.543	4.123	4.21

ACN: acetonitrile; Vt Rt: Vilanterol trifenatate Retention time; Um Rt: Umeclidinium bromide Retention Time; Rs: Resolution.

Software aided method optimization

The response surface quadratic methodology was employed to analyse and understand the response behaviour in the vicinity of optimized factor values, with the aim of achieving optimal system performance. The significance of the model was assessed using analysis of variance (ANOVA). Using this optimized approach, specific circumstances were chosen and then tested through a series of first experiments. These tests were conducted through trial and error in order to acquire a more profound comprehension of the performance of the technique and to determine the crucial independent elements and their impact on the dependent variables. The chromatogram was recorded using different combinations and proportions of mobile phase to effectively separate the drugs umeclidinium bromide and vilanterol trifenatate. This was achieved by using solvents such as acetonitrile and methanol, as well as buffers like potassium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate with varying pH levels. The development of an HPLC technique using AQbD relies on selecting key parameters and responses, which is facilitated by initial testing and risk assessments. Besides conducting exploratory testing, the Ishikawa (fishbone) diagram was employed to identify and assess crucial factors that contribute to the overall risk of

the procedure's efficacy. The chromatographic conditions were optimized to a greater extent by implementing the Box-Behnken Design (BBD). The term "Box-Behnken Design" denotes a design for a multivariate response surface comprising centre replicates and points positioned at the midpoints of each edge of a cube with multiple dimensions. A Box-Behnken Design was employed, consisting of a fixed set of 3 variables and 2 levels. This resulted in the creation of 17 chromatographic conditions, including 5 centre points, as shown in Table 1. The implementation of randomization in experimental trials reduced the influence of uncontrolled variables on bias. After conducting a thorough investigation, three key parameters were identified as the most significant: the percentage of acetonitrile, the molarity of the buffer, and the pH of the buffer. In separative analytical techniques such as chromatography, critical quality attributes (CQAs) may be linked to factors related to the suitability of the system, such as retention time and resolution, as well as the accuracy and robustness of the method. The specified values for the three parameters, namely the percentage of acetonitrile, the molarity of the buffer, and the pH of the buffer, were 50% v/v, 15 mM, and 5.0, respectively. The concentration of acetonitrile (A) in this configuration was maintained at a fixed range of 40 to 60% v/v. Similarly, the lower and upper limits of the molarity content of buffer (B) were established at 10 and 20 mM, respectively. Similarly, the buffer pH (C) was selected to have a minimum volume of 4.0 and a maximum volume of 6.0. The data was evaluated using the statistical software “Design Expert” (Version 12.0.1, Stat-Ease Inc., Minneapolis, MN, USA). Verification is required to assess the performance of a method, namely in terms of accuracy, precision (with a maximum of 2% relative standard deviation), and resilience as the desired outcome.

Method validation

The chromatographic technique underwent validation in accordance with the Q2R (1) guidelines established by the International Conference on Harmonization (ICH). These guidelines evaluate various aspects of the method, including precision, accuracy, specificity, robustness, linearity, limit of detection, and limit of quantitation (14).

System suitability test

Prior to sample analysis, six replicates of standard solutions containing 100 µgmL^{-1} of umeclidinium bromide and 100 µgmL^{-1} of vilanterol trifenatate were injected to assess system suitability characteristics, including the number of theoretical plates, resolution, and tailing factor. The relative standard deviation must be below 2% in all cases. Acceptability requirements for standards in system appropriateness were set in each chromatogram.

Linearity

The linearity of the devised method was established using six concentrations ranging from 15-120 µgmL^{-1} for umeclidinium bromide and 5-40 µgmL^{-1} for vilanterol trifenatate. The linearity solution, which included the relevant sample concentrations, was injected three times in succession. The calibration curve was generated using the utilization of linear regression analysis, wherein the peak area was plotted against the concentration.

Accuracy and precision

The accuracy of the sample solution for each drug at 50, 100, and 150% levels was determined by adding a known quantity of standard in triplicate. The samples were then tested using the optimized method. Subsequently, the recovery percentage for both medications was computed. The acceptance criteria for the mean recovery of the target concentrations were defined as 100 ± 2%. The precision of the optimized technique was assessed by measuring the repeatability and intermediate precision. Intermediate precision accounts for discrepancies that may arise within laboratories due to factors such as variations in time, personnel, and equipment. Inter-assay precision is a synonym for intermediate precision. Repeatability refers to the precision achieved when the same operating circumstances are maintained over a short period of time. To assess the accuracy of the technique, six uniform samples of umeclidinium bromide and vilanterol trifenatate were examined.

The limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of umeclidinium bromide and vilanterol trifenatate were assessed using the standard deviation method. The limit of detection (LOD) was determined as 3.3 times the standard deviation of the response (σ) divided by the slope of the calibration curve (S). Similarly, the limit of quantification (LOQ) was determined as 10 times the standard deviation of the response (σ) divided by the slope of the calibration curve (S).

Robustness

The resilience of a method pertains to its capacity to remain unaltered by modest or deliberate modifications to its parameters. The effectiveness of the optimized method was evaluated by introducing small modifications to the chromatographic parameters, including the fraction of solvent in the mobile phase, the flow rate of the mobile phase, and the analytical wavelength, in the system suitability solution. The % relative standard deviation was employed for the calculation.

Results and Discussion

Preliminary studies and factor selection

A preliminary inquiry was carried out to find a straightforward, reliable, and economical RP-HPLC technique for determining the quantities of umeclidinium bromide and vilanterol trifenatate when combined. The selection of the critical chromatographic parameters was predicated on literature reviews and preliminary

investigations. The research conducted to determine the factor levels for screening and optimization studies demonstrated that the mobile phase conditions needed to be fine-tuned to achieve a rapid separation of umeclidinium bromide and vilanterol trifenatate. A mobile phase composition consisting of phosphate buffer pH, buffer strength, and% acetonitrile was found to be more suitable for the simultaneous estimation of both medications. This led to a significant alteration in retention time. As a result, it is considered one of the most crucial factors for the creation of methods.

Table 2. ANOVA regression analysis for models and responses

Response	Mean	[a]SD	[b]%CV	[c]PV	[d]R^2	Adj. R^2	Predict. R^2	[e]AP	[f]SS	[g]d	[h]MS	[i]F	P
Vt Rt (Y_1)	2.56	0.3945	15.43	7.99	0.9537	0.9221	0.9121	5.990	2.17	6	0.3616	4.48	0.0036
UM Rt (Y_2)	3.65	0.3715	10.19	3.51	0.9947	0.9199	0.9629	6.27	1.39	3	0.4618	4.35	0.0423
Rs (Y_3)	3.91	0.8281	25.69	60.58	0.7607	0.5454	-0.6273	6.1743	8.78	9	0.9761	4.53	0.0310

Vt Rt: Vilanterol trifenatate Retetion time; Um Rt: Umeclidinium bromide Retetion Time. [a]Standard deviation, [b]Coefficient of variations; [c]Press Value; [d]Coefficient of Regression, Adjust R^2, Predict R^2; [e]Adequate Precision; [f]Sum of squares; [g]Degrees of freedom; [h]Mean sum of squares; [i]Fischer's ratio.

Table 3. Results for validation studies

Parameters	**vilanterol trifenatate**	**umeclidinium bromide**
Linearity	5-40 μgmL^{-1}	15-120 μgmL^{-1}
Sensitivity		
LOD	0.12 μgmL^{-1}	0.18 μgmL^{-1}
LOQ	0.36 μgmL^{-1}	0.54 μgmL^{-1}
Precision[a]		
Repeatability(% RSD)	0.412-1.013	0.219-0.987
Intermediate precision (% RSD)	1.021-1.459	0.877-0.912
Accuracy[b]		
Recovery studies	99.80-100.38	99.62-101.50
Robustness		
Change in flow rate(% RSD)	1.12	0.88
Change in mobile phase composition (% RSD)	0.89	0.72
Change in wavelength(% RSD)	1.11	1.01

Method development

A Box-Behnken design (BBD) was employed in the current study to optimize the analytical procedure. The experimental design presented illustrates a comprehensive and efficient approach, utilizing systematic sampling to evaluate three crucial elements of the RP-HPLC method: the percentage of acetonitrile, buffer strength, and buffer pH. To assess the strength of the RP-HPLC method, a multivariate approach called Design of Experiments (DoE) with Box-Behnken Design (BBD) was employed. This approach allowed for the simultaneous investigation of how different parameters affect the responses of interest, namely the retention times of umeclidinium bromide (Y2), vilanterol trifenatate (Y1), and resolution (Y3). By analysing the impact of three elements on replies and evaluating the obtained data, it was feasible to construct mathematical models that investigated the correlation between the studied components and the desired responses. The response surface quadratic model exhibited the highest level of accuracy for BBD. The model was validated using ANOVA with the assistance of Design Expert software. The anticipated R-squared values for the retention period of umeclidinium bromide (Y2) and vilanterol trifenatate (Y1) were quite consistent with the adjusted R-squared values, with a difference of less than 0.2. A negative predicted R-squared value for resolution (Y3) suggests that the current model is not a good predictor of the response, and that the overall mean might be a more reliable predictor. Adequate precision assesses the ratio of the signal to the noise. A ratio exceeding 4 is considered favourable, and the obtained responses for Y1, Y2, and Y3 were 5.990, 6.27, and 6.17, respectively, indicating satisfactory precision. The quadratic model can be utilized to explore the design space. The model's F-values for the

retention time of umeclidinium bromide (Y2), retention time of vilanterol trifenatate (Y1), and resolution (Y3) were 4.48, 4.35, and 4.53, respectively. These values indicate that the model is statistically significant. The probability of occurrence is 0.36% for Y1 and 0.19% for Y2, whereas the probability for resolution (Y3) is 3.10% compared to an F-value, suggesting that this might be attributed to noise. Therefore, the significant responses exhibited a p-value of less than 0.05, indicating that the model terms are statistically significant. The adjusted R-square value and minimal standard deviation indicate a significant relationship between the fitted models and the experimental data. The data was provided in Table 2. The equations expressed in terms of coded factors can be utilized to produce accurate forecasts of the response for specific levels of each element. This equation is valuable for determining the relative influence of the elements by comparing their coefficient. The ultimate equations for Y1, Y2, and Y3 are:

vilanterol trifenate (Y1) = $+2.56 - 0.536X_1 +0.1008X2-0.0086X_3+0.6045X_1X_2 -0.2808X_1X_3-0.1745X_2X_3$

umeclidinium bromide: (Y2) = $+3.65 + 0.0960X_1 + 0.3291X_2 + 0.2359X_3$

Resolution: (Y3) = $+3.26 + 0.2813X_1 + 0.2559X_2 + 0.4594X_3 -0.4743X_1X_2 + 0.0738X_1X_3 + 0.0575X_2X_3 + 0.5863X_1^2 + 0.2725X_2^2 - 0.9240X_3^2$

Based on the coefficients and signs of the equations, it is evident that the factor % acetonitrile (X1) has a positive impact on the retention time of umeclidinium bromide (Y2) and resolution factor (Y3), whereas it has a negative impact on the retention time of vilanterol trifenatate (Y1). The increase in buffer concentration (X2) has a beneficial impact on all responses. Conversely, the pH of the buffer (X3) negatively affects the retention time of vilanterol trifenatate, but positively affects Y2 and Y3. As pH increases the vilanterol becomes ionised and elute fast and retention time decreases. The interactions between X1 and X2 positively influenced Y1 and negatively influenced Y3. Similarly, X2 and X3 had a good impact on Y2 and a negative impact on Y1. Lastly, X1 and X3 had a positive effect on Y3 and a negative effect on Y1. The impact of X32 on Y3 was detrimental, while X12 and X22 had a beneficial influence on Y3.

The analysis involved examining response surface and contour plots to visually represent the impact of the factors and their interactions on the response variable. The contour plots exhibited curvature, revealing a non-linear impact of factors on responses. Figures 2 and 3 provide 2D (A) and 3D (B) contour graphs illustrating the impact of acetonitrile volume (X1) and buffer strength (X2) on the retention time of umeclidinium bromide (Y1) and vilanterol trifenatate (Y2). An inverse relationship was observed between the volume of acetonitrile (X1) and the retention time of umeclidinium bromide (Y1), as well as vilanterol trifenatate (Y2). Similarly, there was an inverse relationship between the concentration of the buffer (X2) and the retention time of umeclidinium bromide (Y1), as well as vilanterol trifenatate (Y2). Consequently, it was advised to have elevated concentrations of X1 and X2 in order to get the desired retention duration for umeclidinium bromide (Y1) and vilanterol trifenatate (Y2). The analysis of the 3D and 2D contour plots depicted in Figure 4 revealed the impact of the acetonitrile volume (X1) and buffer concentration (X2) on resolution, specifically in terms of curvature effects. Both X1 and X2 exhibited a growing trend of curvature, resulting in enhanced resolution at elevated levels. Hence, it was advised to optimize the levels of X1 and X2 in order to attain resolution.

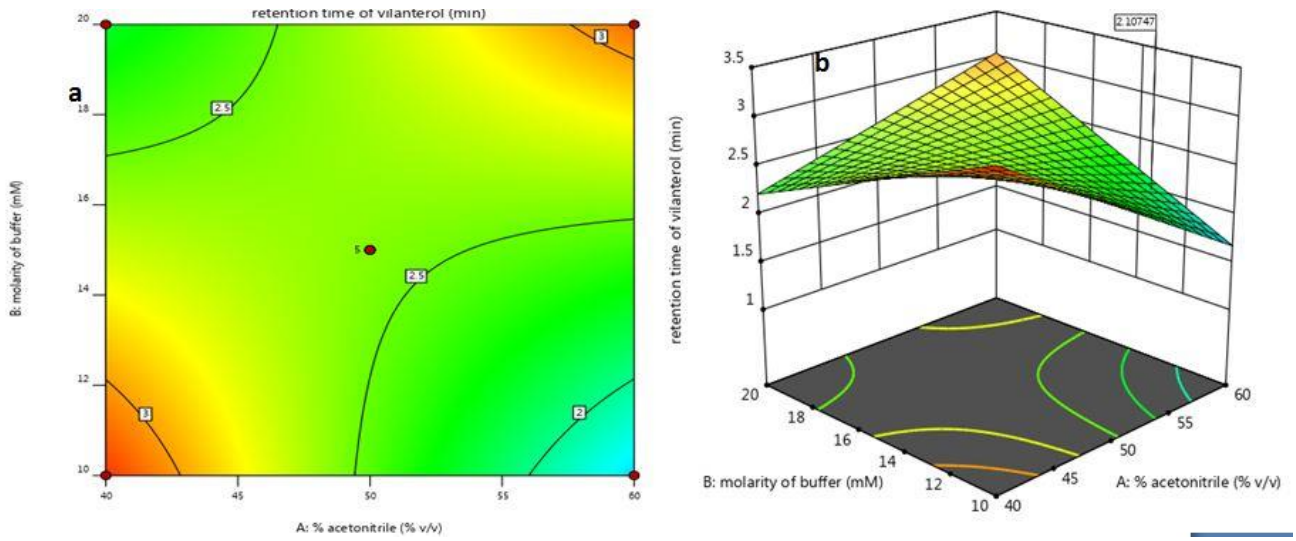

Figure 2. 2D design plot (a) and 3D design plot (b) to study the effect of factors on retention time of vilanterol trifenatate (Y1)

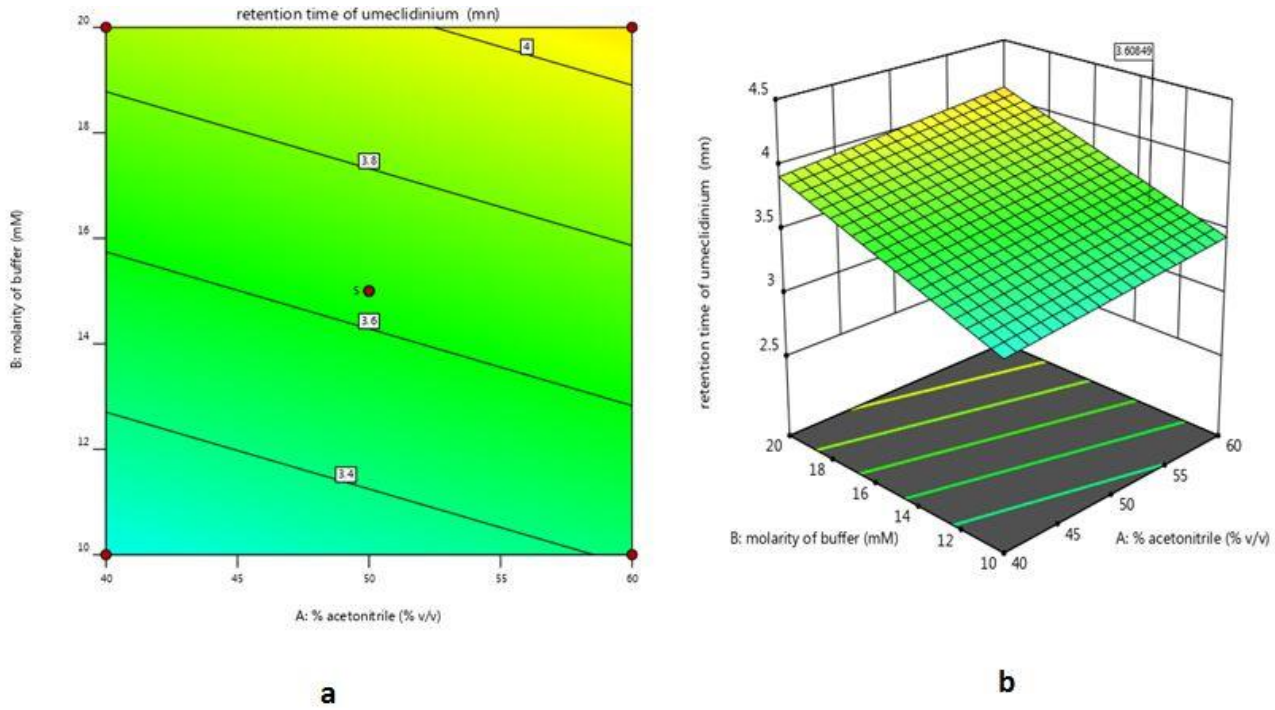

Figure 3. 2D design plot (a) and 3D design plot (b) to study the effect of factors on retention time of umeclidinium bromide (Y2)

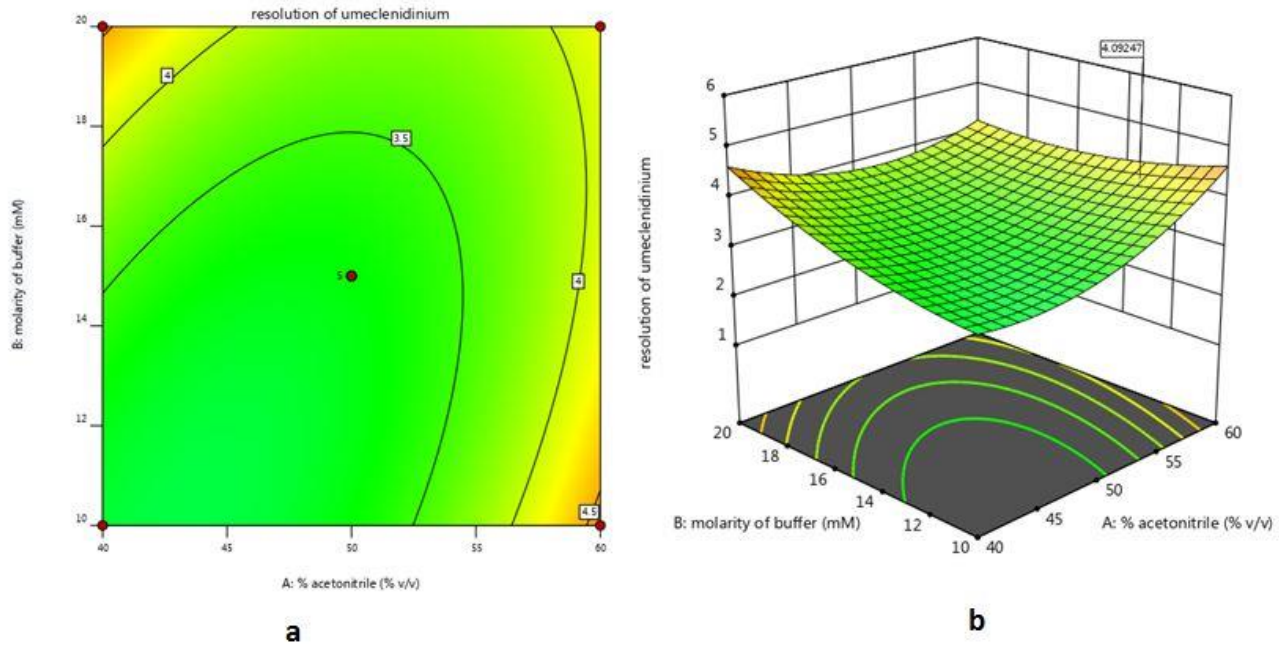

Figure 4. 2D design plot (a) and 3D design plot (b) to study the effect of factors on resolution factor of umeclidinium bromide (Y3)

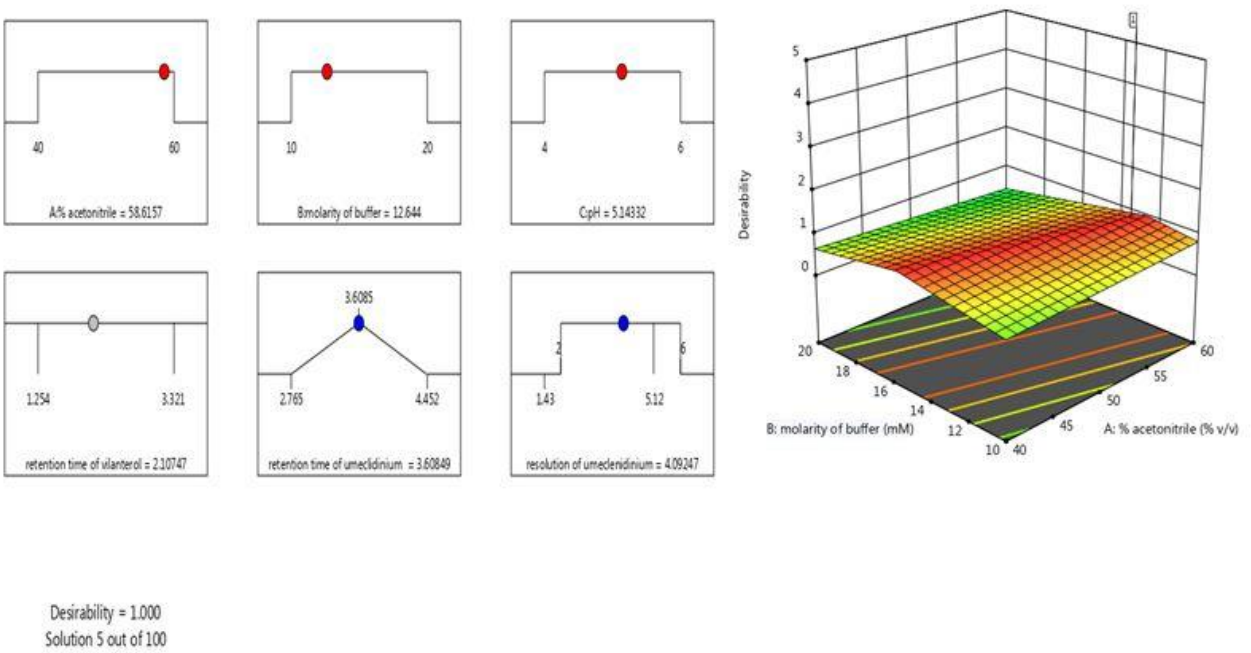

Figure 5. Desirability plot.

The ideal set of conditions for each response was determined using a composite desirability, taking into account the defined objectives and boundaries. The desirability function "R" with a value of one indicates the successful attainment of desired objectives within the given restrictions. The entire experimental region was thoroughly examined to identify compositions that best satisfied the criteria, as seen in Figure 5. The optimal values for the chromatographic conditions of RP-HPLC were determined as follows: the volume of acetonitrile was 58.61%, the buffer strength was 12.6 mM, and the buffer pH was 5.2. These conditions resulted in a retention time of 2.152±0.0162 min for vilanterol trifenatate, a retention time of 3.674±0.013 min for umeclidinium bromide, and a resolution of 4.12±0.021, as shown in Figure 6.

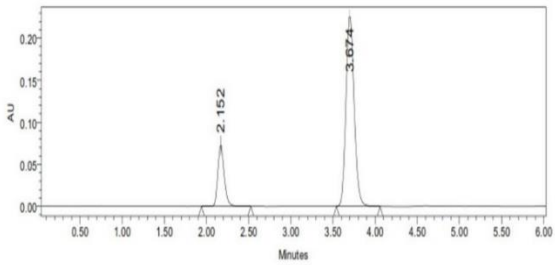

Figure 6. Chromatogram of vilanterol trifenatate and umeclidinium bromide from the optimized chromatographic conditions.

Table 4. Analysis of Vilanterol trifenatate and Umeclidinium in commercial formulation

Formulation	Labelled claim(mg)		Amount found*(mg)		%Recovery*±%RSD	
	Vilanterol trifenatate	Umeclidinium	Vilanterol trifenatate	Umeclidinium	Vilanterol trifenatate	Umeclidinium
ANORO ELLIPTA	44	74.2	43.99	74.17	99.99±0.12	99.96±0.09

*Average of three determinations

Method validation

According to the ICH, system suitability evaluations are a fundamental component of liquid chromatographic methods. The column efficiency, as determined by the number of theoretical plates for both medications, was greater than 2000, the resolution was 4.12, and the tailing was less than 2. For umeclidinium bromide at 30 μgmL^{-1} and vilanterol trifenatate at 10 μgmL^{-1}, the percent relative standard deviation for six replicate injections was 0.87 and

1.09, respectively. As % RSD was found to be less than 2%, it has shown good injection repeatability. The developed method's linearity was confirmed by plotting the linearity curve over concentration ranges of 15-120 µgmL^{-1} for umeclidinium bromide and 5-40 µgmL^{-1} for vilanterol trifenatate, with a correlation coefficient (r^2=0.999) for both medicines, as shown in Table 3. The obtained correlation coefficient (r^2=0.999) demonstrates excellent correlation between peak area and concentration. For the recovery study, different concentrations of samples (50, 100, and 150 %) of standard concentrations for both drugs were prepared and showed recovery of 99.62-101.50 % and 99.80-100.38 % for umeclidinium bromide and vilanterol trifenatate, respectively. Data is shown in Table 3, indicating that the developed method has high level of accuracy with % RSD 1.87 and 1.73 for umeclidinium bromide and vilanterol trifenatate, respectively. Intermediate precision and repeatability were carried out and the resultant data are given in Table 3. The precision values for both drugs were less than 2 %., indicating that the method was repeatable and precise. The LOD and LOQ were found to be 0.18 and 0.54 µgmL^{-1}, respectively, for umeclidinium bromide, and 0.12 and 0.36 µgmL^{-1}, respectively for vilanterol trifenatate. The robustness of the RP-HPLC method to such small changes in the optimum experimental modifications showed the insensitivity of the method to such small changes. The retention time of umeclidinium bromide and vilanterol trifenatate, as well as resolution, were significantly impacted by the mobile phase composition and buffer's pH. The proposed method was applied for the determination of vilanterol trifenatate and umeclidinium in marketed formulations available (ANORO ELLIPTA). The % recovery was found to be 99.99 ± 0.12 and 99.96 ± 0.09 for vilanterol trifenatate and umeclidinium respectively. The assay results are shown in Table 4.

Conclusions

The present study focuses on the systematic use of Quality by Design (QbD) principles to develop a novel RP-HPLC method for the concurrent estimation of umeclidinium bromide and vilanterol trifenatate. This approach is characterized by its speed, accuracy, and cost-effectiveness. The experimental design outlines the examination of key factors, including the buffer strength, volume of acetonitrile, and pH of the buffer. The utilization of modeling software facilitated a more comprehensive comprehension of all the variables that influence the optimization of the technique and the separation of umeclidinium bromide and vilanterol trifenatate. The BBD method was employed to enhance the response resolution between umeclidinium bromide and vilanterol trifenatate within a reasonably short timeframe (6 minutes). The optimized model demonstrates that an acetonitrile concentration of 58.6% v/v, a phosphate buffer concentration of 12.6 mM, and a buffer pH of 5.1 are suitable for estimating the amounts of umeclidinium bromide and vilanterol trifenatate. The validation study verified that the technique is able to accurately and precisely measure the desired parameters under certain conditions, thereby confirming its selectivity, specificity, accuracy, linearity, precision, and robustness. This supports the decision to choose the best conditions for the method. The implementation of the response surface technique enhances method development and robustness testing. This method has been developed based on the concept of design space and is appropriate for regulatory submission within the framework of regulatory flexibility.

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Conflict of interest

No conflict of interest

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