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QbD Based Analytical Method Development and Validation for the Estimation of Remogliflozin etabonate and Vildagliptin in Bulk and in Their Dosage Form

Darade R. B., Pekamwar S. S., Gholve R. B.

School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded.

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KEYWORDS

Quality by design, HPLC, Remogliflozin etabonate, Vildagliptin, Box Behnken Design.

ABSTRACT:

Introduction: QbD based simple, accurate, precise, sensitive, economic and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Remogliflozin etabonate and Vildagliptin in bulk and in tablet dosage forms. Linearity, detection limit, quantitation limit, accuracy, precision, robustness was considered for development and validation of HPLC method for Remogliflozin etabonate (RMO) and Vildagliptin (VLD) in bulk and in tablet dosage form.

Objectives: The objective of present research work is to apply Quality by Design (QbD) approach for developing a simple, economic, accurate, precise and reproducible analytical method and validate the performance methods as per ICH guidelines by using chromatographic technique (RP HPLC).

Methods: Reversed phase chromatography was carried out by using High Performance Liquid Chromatographic System (Analytical Technologies ltd, HPLC 3000 series) equipped with UV detector controlled by HPLC workstation software, using Cosmosil C 18 (250 mm x 4.6 mm; 5µm) HPLC Column. The chromatographic separation was carried out using mobile phase comprised of 10 mM KH_2PO_4 buffer pH 3 and methanol (10:90 %v/v) with flow rate 0.8 ml/min and response recorded by UV detector at 216 nm. Design expert used as software for evaluation of experimental design study (Stat-Ease Inc., Minneapolis, USA, Version 13.0). Due to high competence with a limited number of runs, Box Behnken Design (BBD) and response surface methodology model is used for present study. Three factors, two levels and five center points are selected for BBD, leads to 17 experimental runs, which were carried out. Standard and sample prepared and injected in to chromatographic system. Retention time, theoretical plates, and peak asymmetry, peak area, resolution were measured as responses. For coefficients and nature of the robustness was evaluated by ANOVA.

Results: Data of ANOVA analysis for selected responses, having P value less than 0.05 and F value more than 2.5 signifies the results of proposed approach. Also, the % RSD values were less than 2.0 for method repeatability and intermediate precision results, indicating high degree of precision of the method. The detection limits and quantitation limits were very low, which is indicate method is sensitive.

Conclusions: Experiments were conducted in HPLC and peak resolution was evaluated. All three variables selected found critical for peak separation. QbD based RP-HPLC methods for simultaneous estimation of Remogliflozin etabonate and Vildagliptin was developed and validated as per ICH guidelines. Experimental results proved that the HPLC methods are linear in the proposed working concentration range as well as specific, sensitive, accurate, precise and robust. The percent recovery results of dosage forms showing that the excipients have no interference in the determination. The proposed method can be applied to the routine analysis of Remogliflozin etabonate and Vildagliptin in quality control department of pharmaceutical industry.

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1. Introduction

A Quality by Design (QbD) is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management[1].

Diabetes is a chronic, metabolic disease characterized by disorder characterized by hyperglycaemia, glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. About 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.5 million deaths are directly attributed to diabetes each year. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades.[2,3]

Diabetes mellitus is a chronic disease that is associated with long-term complications and requires life-long pharmacological and non-pharmacological management. Several new agents have been approved for diabetes mellitus management in recent years.[4]

Remogliflozin etabonate (RE) is a prodrug of remogliflozin, a sodium-glucose co-transporter type 2 (SGLT2) inhibitor anti-hyperglycemic agents. These drugs lower blood glucose by increasing urinary glucose excretion.[5]

Vildagliptin is an orally active potent and selective DPP-4 inhibitor that improves glycaemic control in patients with type 2 diabetes primarily by enhancing pancreatic (α and β) islet function. Vildagliptin has been shown both to improve insulin secretion and to suppress the inappropriate glucagon secretion seen in patients with T2DM.[6]

2. Objectives

Literature survey reveals that various methods have been reported for the determination of RMO and VLD in combination[7-10]. However, yet no HPLC method was reported using quality by design approach for simultaneous estimation of RMO and VLD as individual drug or in combination.

The objective of present research work is to apply Quality by Design (QbD) approach for developing a simple, economic, accurate, rapid, reproducible analytical method and identification, optimization of the chromatographic factors that had significant effects on separation attributes and validate the performance of developed analytical methods as per ICH guidelines by using chromatographic technique (RP HPLC).

3. Methods

Material: High Performance Liquid Chromatographic System (Analytical Technologies ltd, HPLC 3000 series) equipped with UV detector and Cosmosil C 18 (250 mm x 4.6 mm; 5µm) Column was used for the chromatographic analysis.

HPLC Method Development by QbD Approach:

Selection of Analytical Target Profile (ATP):

An ATP consist of a description of the intended purpose, appropriate details on the product attributes to be measured and relevant performance characteristics with associated performance criteria. The ATP includes the performance requirement for asingle attribute or a set of quality attributes. The retention time, theoretical plates, and peak asymmetry were identified as ATP for proposed method[11].

Determine critical quality attributes (CQA)

The CQAs are the method parameters that are directly affect the ATP. The mobile phase ratio, flow rate and wavelength were critical method parameters required to be controlled to maintain the acceptable response range of ATP.

Selection of Design (DoE):

After defining the ATP and CQAs, the Box-Behnken experimental design was applied to optimization of HPLC method. The various interaction effects and quadratic effects of the mobile phase ratio, flow rate and wavelength on the retention time, theoretical plates, and peak asymmetry, peak area, resolution was studied using Box-Behnken statistical screening design.

A three factor mobile phase composition, flow rate and wavelength were selected at three different

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levels; design was made with Design Expert® (Version 13.0, Stat-Ease Inc., and M M).

As independent variables, mobile phase ratio, flow rate and wavelength were chosen and shown in Table 6. The dependent variables were retention time, theoretical plates, and peak asymmetry, peak area, resolution for proposed independent variables.

Box-Behnken design was selected for this study and following experiments were obtained from DoE. Five center points were considered; 17 runs were generated by design expert.

Selection of Solvent (diluent):

The mobile phase mixture comprised of 10 mM phosphate buffer pH 3 and methanol in the ratio of 10:90 was prepared and mixed well. This mixture was selected as solvent (diluent) based on the solubility study.

Selection of Detection wavelength:

The separately prepared standard solutions of Remogliflozin etabonate (10 µg/mL) and Vildagliptin (10 µg/mL) were scanned between 400 nm to 200 nm by using diluent as blank and recorded the overlain spectrum of both drugs (Figure 2). The optimal absorbance for both drugs was observed at a wavelength of 216 nm. Hence, this wavelength was selected for detection of Remogliflozin etabonate and Vildagliptin.

Preparation of Standard Solution:

The 1000 µg/ml standard stock solution was prepared by dissolving an accurately weighed 10 mg of Remogliflozin etabonate (RMO) and Vildagliptin (VLD) in 10 ml diluent. The stock solution was further diluted to a sub-stock 100 µg/ml. The 10 µg/ml solution was prepared by diluting 1 ml of sub-stock solution to 10 ml with diluent.

Preparation of Sample Solution:

Twenty Remogliflozin etabonate and Vildagliptin Tablets 100/50 mg (Remo-V) were weighed and crushed, and 544.3 mg powder (equivalent to 100 mg RMO and 50 mg VLD) was transferred into a 100 ml dry volumetric flask. Added 50 ml diluent and sonicated for 20 min with intermediate shaking to dissolve the content completely. Subsequently, the solution was kept on bench top to reach room temperature and made up to the volume up to the mark with mobile phase and mixed thoroughly. 0.45 µ membrane filter was used to filter this solution and then the sample was analyzed by HPLC.

Optimization of chromatographic conditions

The aim of analytical method development was to achieve satisfactory resolution of analytes of interest in the shortest possible time. The main focus was on the mobile phase and its composition selection along with other parameters such as detection wavelength, selection of diluent, buffer pH, flow rate, and selection of organic solvent.

Initially, the mobile phase comprised of methanol and 10 mM KH_2PO_4 buffer pH 3.0 (80 : 20%v/v) was tried to estimate both drugs. During initial trial each drug injected separately, followed by drugs mixture.

During optimization of the method, Cosmosil C18 (ID: 250mm x 4.6mm, Particle size: 5 µm) and Sapphirus C18 HP Classic (ID: 250mm x 4.6mm, Particle size: 5 µm) column were used.

Subsequently, tried a number of mobile phase (eluent) compositions by using KH_2PO_4 buffer (10 mM or 20 mM) at different pH level (2.3, 2.5, 3.0) with organic solvent (Methanol), different flow rates and with different columns to resolve the peaks of both drugs and improve their peak shape.

After the several experimental trials, it was concluded that eluent comprised of 10 mM KH_2PO_4 buffer pH 3 and methanol (10:90 %v/v), flow rate: 0.8 ml/min gave better peak shapes with satisfactory resolution in short time. The chromatogram of optimized chromatographic condition is shown in Figure 1.

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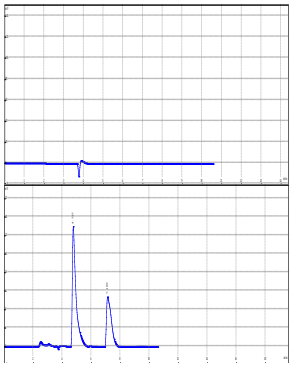

Figure: 1. Chromatogram of blank and optimized chromatographic condition for RMO and VLD

Method Validation:

The optimized RP HPLC method was validated for linearity, accuracy, precision, robustness, detection limit and quantitation limit as per ICH guidelines[12].

Table 1: Linearity results

Linearity level	Concentration (µg/mL)		Peak area response	
	RMO	VLD	RMO	VLD
1	10	5	637977	260971
2	20	10	928359	441208
3	30	15	1206014	610768
4	40	20	1528984	820361
5	50	25	1832709	983445

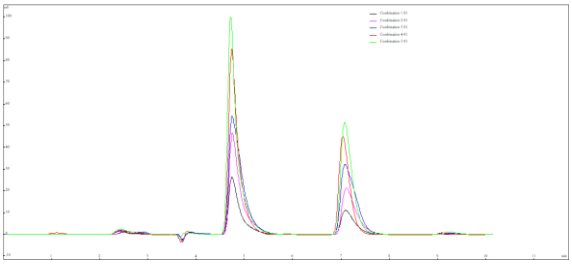

Figure 2. Overlay HPLC chromatogram for linearity of RMO and VLD.

4. Results

Experiments were conducted in HPLC and peak resolution was evaluated. All the three variables selected found critical for peak separation. The proposed model was evaluated by ANOVA. The analysis provides 2 D (contour plot), 3 dimensional representations (3D response surface) by plotting the response against other two factors, and the third one kept constant at a desired level, and the 3 D representation of as a response as shown in figure 6-14, the probability values and F- values were recorded for each factor. The above study signifies the robust nature of the proposed method conditions at extended variations.

Design expert used as software for evaluation of experimental design study (Stat-Ease Inc.,Minneapolis, USA, Version 13.0). Due to high competence with a limited number of runs, Box Behnken Design(BBD) and response surface methodology a model used for study. Three factors, three levels and five center points are selected for BBD, leads to 17 experimental runs,which were carried out. Standard and sample prepared and injected into chromatographic system. Retention time, theoretical plates, and peak asymmetry, peak area, resolution were measured as responses. For coefficients and nature of the robustness was evaluated by ANOVA with a linear approach. The significance and contribution of the factors were estimated by statistical ANOVA. The significance of the model was evaluated by the P values and F-value. Data of ANOVA analysis for selected responses, having P value less than 0.05 and F value more than 2.5 signifies the results of proposed method.

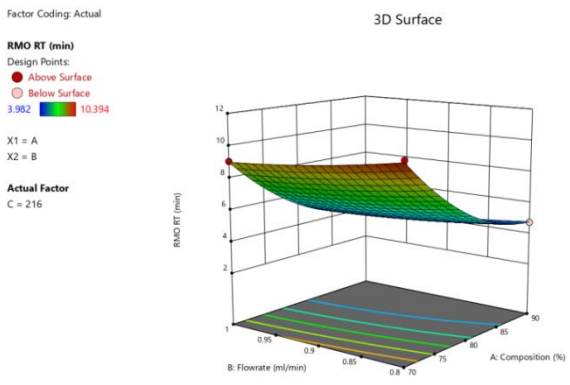

Figure: 3. 3D-response surface plot showing the effect of CQA (mobile phase composition and flow rate) on the Retention time of RMO.

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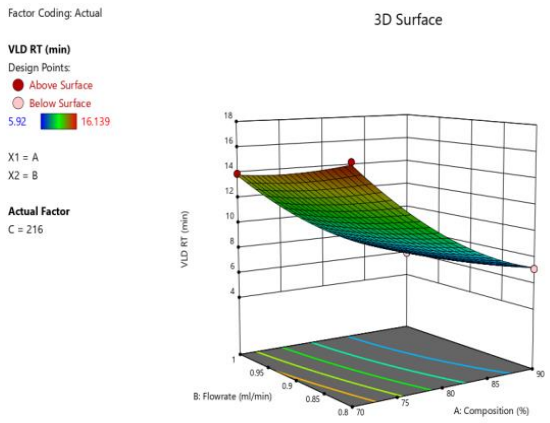

Figure: 4. 3D-response surface plot showing the effect of CQA (mobile phase composition and flow rate) on the Retention time of VLD.

The method was found to be suitable as all the system suitability criteria. Results of system suitability are summarized in Table **2.**

Table 2: System suitability results[13].

Parameters	**Results**		**Acceptance criteria**
	RMO	**VLD**	
Retention time (min)	4.72	7.06	NA
Tailing factor	1.31	1.25	NMT 2
Theoretical plate counts	7573	8500	NLT 2000
Resolution between RMO and VLD peak	_	3.84	NLT 2
% RSD for peak area of five replicate injections	0.34	1.22	NMT 2%

5. Discussion

The developed method was found to be linear in the concentration range of 10-50 µg/mL for Remogliflozin (R^2 = 0.999) and 5-25 µg/mL for Vildagliptin (R^2 = 0.998), showing the suitability of method for analysis in the studied concentration range. Linearity results for Remogliflozin and Vildagliptin are shown in Table 1. Overlay of HPLC chromatograms for linearity of RMO and VLD are shown in Figure 2.

The mean % recovery results obtained from triplicate samples at each level were found to be 100.22, 99.71, and 100.05 % for Remogliflozin etabonate and 100.08, 99.60, and 99.90 % for vildagliptin at 50, 100, and 150 % levels of nominal working concentration respectively. The accuracy (recovery) results were found within the acceptance criteria, signifying that the method is accurate and free from interference from the excipients in the estimation.

Method repeatability and intermediate precision results shows that the % RSD values less than 2.0 for RMO and VLD, signifying the method is precise and reproducible. Results of method repeatability and intermediate precision.

The DL was 0.0447 µg/mL for RMO and 0.07 µg/mL for VLD respectively, indicating that even small quantities of the RMO and VLD can be detected. The QL was 0.135 µg/mL for RMO and 0.217 µg/mL for VLD respectively, indicating that even small quantities of the RMO and VLD can be quantified.

The results of change in pH and wavelength for each changed chromatographic method parameter studied during robustness were met the acceptance criteria, indicating method robustness.

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