SCIENCEFORECAST
Connecting Research to Drive Scientific Innovation

Research Article
Published: 26 Jan, 2018

SF Journal of Pharmaceutical and Analytical Chemistry

Implementing a Quality by Design Approach in Chromatographic Determination of Some Antidiabetic Drugs

Ahmed RM[1], Ibrahim A[2], El-Gendy AE[1] and Hadad GM[3]*

[1]*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Misr International University, Cairo, Egypt*

[2]*Department of pharmacy Practice and Science, School of Pharmacy, University of Maryland, Baltimore, USA*

[3]*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt*

Abstract

This article aims to explain the steps for application of quality by design (QbD) concept to analytical method development and validation, by using an example of simultaneous determination of four antidiabetic drugs (Metformin HCl, Vildagliptin, Sitagliptin phosphate mono hydrate, and Pioglitazone HCl) in its pharmaceutical dosage form by RP-HPLC. By using QbD tools, enable earlier understanding and identification of variables affecting the method performance. Fractional factorial design and central composite design were used for screening the variables and optimization of chromatographic conditions with building the design space by using Minitab 15 software. A QbD guide is described from identification of analytical target profile to definition of control strategy. The optimized chromatographic method was carried out in 5minutes and was performed using 0.01M phosphate buffer (pH 6.7): methanol: acetonitrile 42:26.5:30.5 as mobile phase at a flow rate 1.0mL/min and UV detection at 223nm.

Keywords: Quality by design; Experimental design; Risk assessment; Design space; RP- HPLC; Antidiabetic mixture

OPEN ACCESS

**Correspondence:*
Ahmed RM, Misr International University, Faculty of Pharmacy, Misr-Ismailia Road, Cairo, Egypt.
Tel: +201223682995
E-mail: rasha_ahmed@miuegypt.edu.eg

Received Date: 13 Oct 2017
Accepted Date: 15 Jan 2018
Published Date: 26 Jan 2018

Citation: Ahmed RM, Ibrahim A, El-Gendy AE, Hadad GM. Implementing a Quality by Design Approach in Chromatographic Determination of Some Antidiabetic Drugs. SF J Pharm Anal Chem. 2018; 1(1): 1001.

ISSN 2643-8178

Copyright © 2018 Ahmed RM. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

The improvement of chromatographic techniques is still tedious and not under the control of analysts. This is because of the fact that the majority of the parameters to manage to obtain acceptable separation conditions have complex effects on the chromatogram. The problem becomes even more complicated when the matrix contains more than one components with different physico-chemical properties [1].

Quality by design (QbD) has been considered a very useful tool for the development of robust analytical method. By means of QbD, quality is embedded into the process from the beginning to the end of the process, thus counteracting the traditional approach, which tests the quality at the end of the process. Experimental design and risk assessment were used as QbD tools to define analytical target profiles (ATPs) and operational design ranges for analytical methods [2-5].

By focusing on process validation, identification of method variables is critical to develop robust analytical methods that are applicable throughout the life of the method instead of using the traditional one factor at a time (OFAT) method development. So using of QbD in analytical development will involve understanding how analytical results are influenced by variation in input parameters; evaluating multivariate interactions and significantly will reduce the effort and cost related to post-approval variation [6].

The application of science- and risk-management are required in analytical method development by using QbD concept, which involves assessing risk, defining a design space, establishing a control strategy, and continuously improving method robustness? This approach helps to identify and minimize sources of variability that may lead to poor performance of the method. It also ensures that the method meets its intended performance requirements by building the quality in the analytical method throughout the process life cycle to improve the separation and focus the experiments on the optimal range for each variable [7,8].

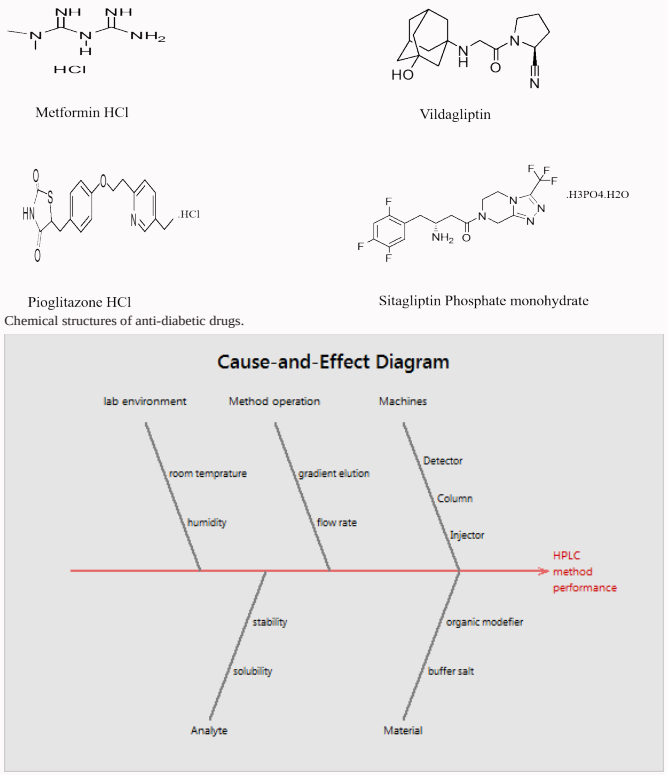

Figure 1: Ishikawa diagram for identification of critical process parameters.

The four anti-diabetic drugs (Metformin HCl, Vildagliptin, Pioglitazone, and Sitagliptin) were determined separately and in combination by using different techniques [9- 22]. In literature, no chromatographic method has been presented yet for the assay of these anti-diabetic drugs in the same mixture. The presented work is designed to develop new simple, robust method and economic with short time of analysis of four anti-diabetic drugs by applying the QbD approach to RPHPLC.

Experimental

Materials

Authentic standards of Metformin HCl, Pioglitazone, Vildagliptin and Sitagliptin were provided by AMG Pharm and Egyphar pharm. companies. All standards were certified to have a purity between 99.7 -100% (w/w) on dried basis. Januvia® tablets (Merck Sharp & Dohme, Cairo, Egypt), Galvus® tablets (Novartis Cairo, Egypt), Glucovage® tablets (Mina pharm Cairo, Egypt), Higlitazone® tablets (Hi pharm, Cairo, Egypt), Galvus Met® tablets (Novartis Cairo, Egypt), and Janumet® tablets (Merck Sharp & Dohme Cairo, Egypt) were purchased from local pharmacies.

All solvents used were HPLC grade; Methanol and acetonitrile were obtained from Sigma- Aldrich (Germany). Analytical grade of potassium dihydrogen phosphate (Adwic, Egypt), sodium hydroxide, hydrochloric acid and phosphoric acid were purchased from Sigma – Aldrich (Germany).

HPLC system and chromatographic conditions

Chromatographic measurements were carried out using Agilent HPLC (1200 series) with Quaternary pump(Model G1311A) , Manual injector(Model G1328B)and VWD (Model G1314) fitted with 20µL loop and connected with Agilent C18 column (250mm x 4.6mm i.d., 5µm particle size).

The optimum condition of mobile phase derived from the Minitab software consisted of 0.01M phosphate buffer (pH 6.7): methanol: Acetonitrile 42:26.5:30.5 and 223nm wavelength was selected for detection at 1mL/min.

Software

Experimental design set up, data analysis and desirability function calculations were performed by using Minitab 15 and Microsoft Excel 2010.

Stock solutions

Stock solutions of the studied compounds were prepared separately by dissolving Metformin HCl and Sitagliptin in water while Vildagliptin and Pioglitazone in methanol to obtain a concentration of 100µg/ ml. Calibration curves were established in a range of 0.2-20µg/mL for Metformin HCl, 0.2 -2.5µg/mL for Pioglitazone, 1.0 -20µg/mL for Vildagliptin and Sitagliptin separately.

Pharmaceutical dosage form preparation

Twenty tablets of each pharmaceutical product were separately weighed and finely powdered. A portion of the powder of each pharmaceutical product equivalent to 10mg of Metformin HCl and Pioglitazone, Vildagliptin and Sitagliptin was accurately weighed, separately transferred to 100ml volumetric flasks and dissolved in about 50ml methanol using ultrasonic bath (15min) and cooled to room temperature. The solutions were diluted to volume with the same solvent and then filtered through 0.45µm membrane filters (Millipore, Milford, MA). The first portions of the filtrates were discarded and the remainders were used as stock sample solutions. Further dilutions of the prepared solutions were carried out using the initial mobile phase composition to reach the linearity range specified

Table 1: Screening of knowledge space: fractional factorial design.

Exp no.	pH of buffer	Methanol	Acetonitrile	Wavelength	Symm	R_S	T
1	+1	-1	-1	1	1.45	1.98	35.80
2	0	0	0	0	1.24	1.40	14.80
3	+1	+1	-1	-1	1.75	2.46	13.65
4	-1	-1	1	1	1.33	1.76	16.64
5	0	0	0	0	1.24	1.43	13.62
6	-1	+1	+1	-1	0.71	2.83	10.27
7	+1	+1	+1	+1	0.59	1.70	4.89
8	+1	-1	+1	-1	1.9	2.49	8.06
9	-1	+1	-1	+1	0.91	2.71	31.00
10	-1	-1	-1	-1	1.05	2.93	73.10
11	0	0	0	0	1.26	1.40	14.23

Symm, metformin symmetry; R_s, resolution between metformin & Vildagliptin; T: Time of analysis

for each drug in the calibration graphs. The general procedures described under calibration were followed and the concentration of each drug was calculated.

Experiments for modeling

Initial data were acquired under the following conditions: pH values of 0.01M phosphate buffer of 5, 6, and 7 were selected. UV wavelength of 215, 220, and 225nm were selected. Methanol percentages of 15, 20, and 25 were used. Acetonitrile percentages of 25, 30, and 35 were used. Experimental runs were performed by using fractional factorial design and central composite design as screening and optimizations designs respectively.

Method validation

Linearity and range: The linearity of the method was evaluated by analyzing a series of different concentrations of each compound. Concentrations were chosen between the range of 0.2-20µg/mL for metformin HCl, 0.2 -2.5µg/mL for Pioglitazone, 1.0 -20µg/mL for Vildagliptin and Sitagliptin separately.

Precision: Repeatability of the method was tested by choosing three concentration levels for each compound and analyzing them as mentioned before under experimental section and they were repeated three times within a day (intra-day precision). While intermediate precision was tested by analyzing the three concentration levels three times on three consecutive days (inter day precision). The mean and relative standard deviation values for Metformin HCl, Pioglitazone, Vildagliptin and Sitagliptin were calculated.

Accuracy: The accuracy study was performed by applying the standard addition technique. This technique was carried out by adding different concentration of standards to definite concentration from the dosage form of each drug. The resulting mixtures were assayed and the mean percentage recoveries and standard deviation results were obtained for Metformin HCl, Pioglitazone, Vildagliptin and Sitagliptin.

Selectivity: Method selectivity was achieved by preparing different mixtures of Metformin HCl, Pioglitazone, Vildagliptin and Sitagliptin within the linearity range concentration. The laboratory prepared mixtures were analyzed according to the previous procedure described under the proposed method and the mean percentage recoveries and standard deviation results were calculated for them.

Robustness: The robustness of analytical procedure is a measure

Table 2: The coded and uncoded values of factors.

Coded Value	Decoded Value			
	pH	Methanol	Acetonitrile	Wavelength
-1.4	4.6	13	23	213nm
-1	5	15	25	215nm
0	6	20	30	220nm
+1	7	25	35	225nm
+1.4	7.4	27	37	227nm

of its capacity to remain unaffected by small but deliberate variation in method parameters. The design of experiments was done for testing of the robustness of method. The fractional factorial design was used to test the robustness of the method by changing the levels of factors (pH, methanol%, acetonitrile%, and wavelength) within the design space.

Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines to test the sensitivity of the method.

Results and Discussion

The considered analytes present in the study have different physicochemical properties, so it is difficult to find a method for their separation. There are many factors should be optimized to separate the compounds.

Method development was carried out within analytical Quality by design (AQbD) framework by implementation of key steps of QbD.

Analytical target profile and method scouting

The analytical target profile was defined as development of RP-HPLC for simultaneous determination of Metformin HCl, Vildagliptin, Sitagliptin, and Pioglitazone in bulk and in dosage form with complete resolution of peaks within short analysis time. The method should fulfill the general performance criteria as described in ICH guidelines. The scouting step should be carried out by analyzing different buffers, different pH, different organic modifiers, wave length, different columns, and different flow rates. The obtained chromatogram pointed out the use of buffers from slightly acidic to neutral pH to be away from the pKa of the analytes and using methanol and acetonitrile to allow the separation of Metformin and Vildagliptin. But during the scouting phase, there was non-significant effect of using different flow rate on resolution of peaks or the time of analysis. So, the starting point for screening to reach the knowledge space based on using phosphate buffers in pH range (5-7) with methanol and acetonitrile in different ratio at different wavelength.

Critical quality attributes, critical process parameters, and quality risk assessment

The critical quality attributes (CQA) are response variable, which are defined to evaluate the performance of the method. General CQAs of separation of analytical methods were symmetry of first peak, resolution between Metformin and Vildagliptin, and time of analysis. The analysis of time was considered as CQA due to high observed values in screening design.

Critical process parameters (CPP) are defined as factors that have effects on CQA and therefore should be monitored to ensure the method performance. They were selected by quality risk assessment tool as Ishikawa diagram and Pareto chart as shown in Figure 1. According to the trials and preliminary experiments, the flow rate,

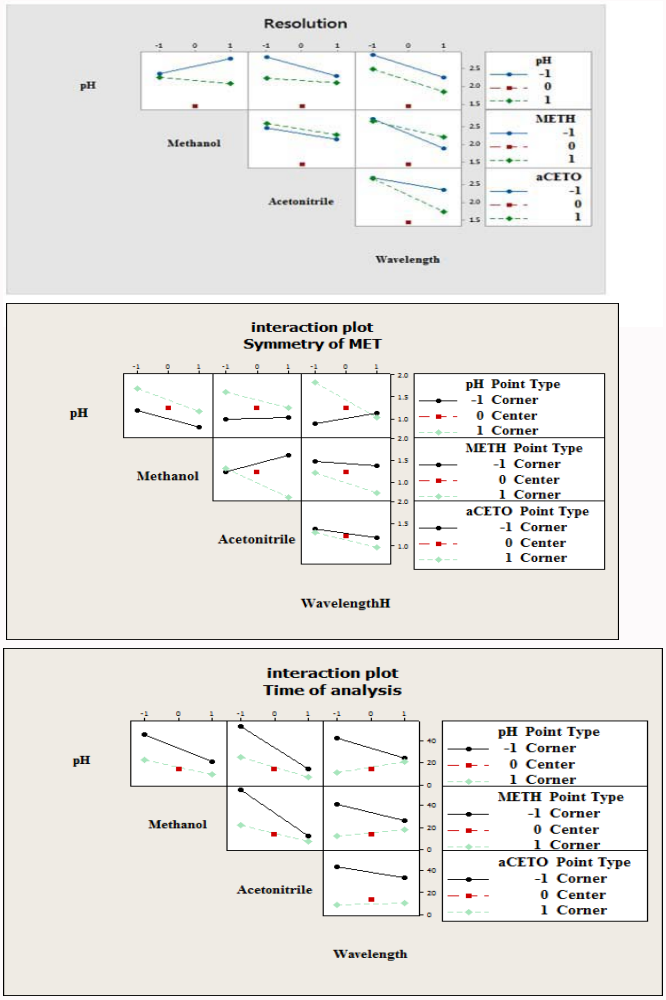

Figure 2: Interaction plots between the factors and their effects on the retention time of the last peak as a response.

type of buffer, and its concentration were being fixed in order to achieve the performance criteria.

The CPPs to be successfully evaluated, design of experiments should be used to allow the analysis of data. The CPPs should be entered in the designs were, pH of phosphate buffer, percentages of methanol, acetonitrile and wavelength.

Design of experiments and knowledge space

By using experimental design, screening design and specifically fractional factorial design (2^{4-1}) was established to obtain a data by performing a minimum number of experiments as shown in Table 1 which was defined as knowledge space. From the screening design it can be concluded that the curvature and the four factors are significant in the model since the p value ≤ 0.05, this imply that quadratic model should be considered by using central composite design. As shown in Figure 2, the interaction plots illustrate the relationship between factors and how these factors have an effect on the performance of the method and the coded and uncoded values in the design presented in Table 2.

In fractional factorial design, the ANOVA and model fit statistics indicate significant models with p value 0.0008 and 0.003 for time of analysis and symmetry of Metformin simultaneously and non-significant model for resolution as its p value 0.07. But good model fit with adjusted R^2 0.996, 0.999, and 0.902 for Metformin symmetry, time of analysis and resolution simultaneously.

The equations of models derived from Minitab software were

Y(symm) = 1.22+0.211pH-0.22 Methanol- 0.07 acetonitrile -0.014 wavelength-0.03 pH* methanol – 0.098 pH*acetonitrile- 0.26 pH* wavelength.

Y(T) = 24.19-8.58 pH-9.24 Methanol- 14.2 acetonitrile -2.10 wavelength+2.9 pH* methanol +5.09 pH* acetonitrile +6.85 pH* wavelength.

$Y(R_s)$ = 2.54-0.022pH+0.25 Methanol+0.015acetonitrile -0.14 wavelength + 0.032 pH* methanol +0.28 pH* acetonitrile +0.18 pH* wavelength.

Pareto charts reported the effects of CPPs on the performance of the method and categorize the factors according to their effects on CQA. So, using the RSM approach to optimize the factors affecting the method should be advised, to get the optimum condition by using desirability functions and to perform the design space.

Response surface methodology and design space

The experimental domain for CPPs to be further studied by central composite design was the following, pH of phosphate buffer (4.6-7.4), Methanol percentage (13-27), Acetonitrile percentage (23-37), and wavelength (213-227). In order to estimate the coefficients, 31 runs in central composite design was planned including 7 runs

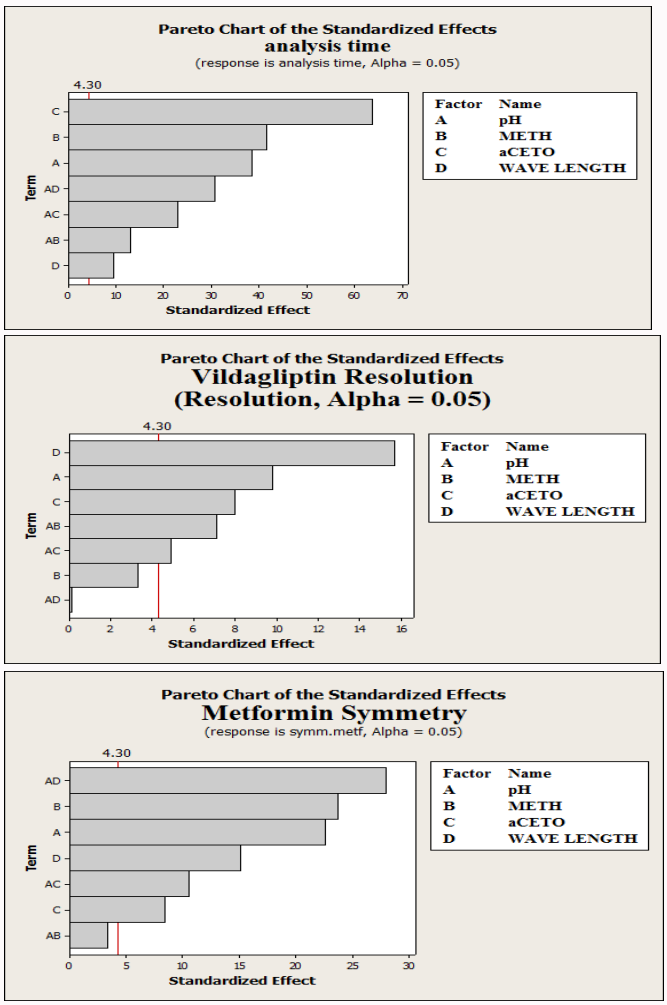

Figure 3: Pareto charts of the parameters and their effects on the response.

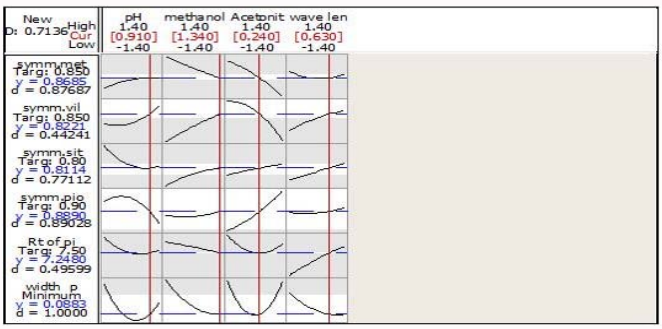

Figure 4: Desirability plot which displays the optimum conditions of the method.

of center point to estimate the experimental error with taking into account the interactions between parameters. The experimental design was reported in Table 3 with the measured responses.

The polynomial quadratic equations are

Y(T) = 14.21- 5.99 pH -5.84 methanol- 11.26 acetonitrile- 2.2 wavelength+ 2.97 pH*pH+ 0.13 methanol*methanol+ 4.4 Acetonitrile*Acetonitrile- 0.28 wavelength*wavelength+ 0.3pH* methanol+ 2.62 pH*acetonitrile+ 3.3pH* wavelength+ 3.5 methanol*acetonitrile+ 2.46 methanol* wavelength+ 2.547 acetonitrile*wavelength.

Y (R_s) = 2.27+0.23 pH +0.33 methanol+0.04 acetonitrile+ 0.03 wavelength+ 0.72 pH*pH-0.27methanol*methanol+0.32 Acetonitrile*Acetonitrile-0.19 wavelength*wavelength+ 0.086pH* methanol+ 0.24 pH*acetonitrile+ 0.02pH* wavelength+ 0.16 methanol*acetonitrile+ 0.038 methanol* wavelength+0.054 acetonitrile*wavelength.

Y(Symm) = 1.15+ 0.12 pH -0.16 methanol- 0.04 acetonitrile- 0.011 wavelength- 0.079 pH*pH+ 0.012 methanol*methanol -0.07 Acetonitrile*Acetonitrile+0.11 wavelength*wavelength- 0.11pH*methanol-0.04pH*acetonitrile+0.009pH*wavelength- 0.288methanol*acetonitrile-0.036methanol*wavelength-0.036

Table 3: Response surface methodology: central composite design.

Exp no.	pH	Methanol	acetonitrile	Wave length	symm	T	Rs
1	0.000	1.400	0.000	0.000	0.68	7.59	4
2	-1.400	0.000	0.000	0.000	0.74	27.09	3.3
3	-1.000	-1.000	-1.000	1.000	1.02	28.16	2.88
4	-1.000	1.000	-1.000	-1.000	0.89	32.99	2.57
5	-1.000	1.000	1.000	1.000	0.71	9.58	2.64
6	1.000	-1.000	-1.000	-1.000	0.83	31.65	1.91
7	1.000	-1.000	1.000	1.000	1.81	8.16	2.23
8	1.000	1.000	-1.000	-1.000	1.75	13.65	2.46
9	0.000	0.000	0.000	0.000	1.21	13.64	1.62
10	0.000	0.000	0.000	0.000	1.26	14.00	1.40
11	0.000	0.000	0.000	0.000	1.25	14.50	1.50
12	0.000	0.000	0.000	0.000	1.25	14.10	1.40
13	0.000	0.000	1.400	0.000	0.71	6.14	4.59
14	-1.000	1.000	-1.000	1.000	0.91	31.0	2.71
15	1.000	-1.000	1.000	-1.000	1.90	8.07	2.49
16	0.000	0.000	0.000	-1.400	1.24	13.11	2.99
17	0.000	0.000	-1.400	0.000	0.95	37.73	3.90
18	0.000	0.000	0.000	1.400	1.16	13.34	3.50
19	0.000	0.000	0.000	0.000	1.23	13.9	1.50
20	-1.000	-1.000	-1.000	-1.000	1.05	73.16	2.93
21	0.000	0.000	0.000	0.000	1.22	13.90	1.40
22	1.000	1.000	-1.000	1.000	1.61	13.94	2.68
23	-1.000	1.000	1.000	-1.000	0.71	10.27	2.83
24	1.000	1.000	1.000	-1.000	0.58	4.89	3.04
25	-1.000	-1.000	1.000	-1.000	1.20	17.82	1.87
26	1.400	0.000	0.000	0.000	0.91	12.13	6.76
27	1.000	1.000	1.000	1.000	0.58	4.89	3.12
28	0.000	0.000	0.000	0.000	1.26	14.00	1.50
29	1.000	-1.000	-1.000	1.000	0.91	35.84	1.98
30	-1.000	-1.000	1.000	1.000	1.33	16.64	1.76
31	0.000	-1.400	0.000	0.000	1.32	20.57	2.20

acetonitrile*wavelength.

The role of RSM, finding a way to reach a complete description of the problem and consequently can be effectively applied to define the design space. In RSM, the ANOVA and model fit statistics indicate the validity and significance of the two models except the resolution model was valid but non-significant [23-26].

By applying central composite design (CCD), we can estimate the optimization of the method which is approach to search along response surface for optimal range of input variables to satisfy a goal such as maximizing/minimizing/targeting a response variable [27,28].

The aim from determining the design space of an analytical procedure is providing information on how often the analytical procedure will meet its critical quality attributes in order to provide reliable data. To start building the design space all historical information and previously developed methods related to this method are used them in the risk assessment step to get the important factors

Table 4: Robustness design fractional factorial design.

Exp no.	pH	methanol	acetonitrile	Wave length	symm	T	Rs
1	1.00	1.00	1.00	1.00	0.71	5.21	2.30
2	0.00	0.00	0.00	0.00	0.69	5.25	2.40
3	0.00	0.00	0.00	0.00	0.68	5.20	2.41
4	1.00	-1.00	-1.00	1.00	0.69	5.30	2.45
5	1.00	-1.00	1.00	-1.00	0.68	5.25	2.50
6	0.00	0.00	0.00	0.00	0.66	5.19	2.35
7	-1.00	-1.00	-1.00	-1.00	0.67	5.35	2.20
8	-1.00	1.00	1.00	-1.00	0.71	5.15	2.31
9	1.00	1.00	-1.00	-1.00	0.71	5.22	2.20
10	-1.00	-1.00	1.00	1.00	0.62	5.15	2.36
11	-1.00	1.00	-1.00	1.00	0.65	5.19	2.33

Table 5: The results of p-values in robustness design.

Item	Symmetry	Resolution	Time of analysis
Model	0.19	0.09	0.18
pH of buffer	0.08	0.11	0.26
methanol	0.11	0.06	0.09
acetonitrile	1.00	0.09	0.08
wavelength	0.15	0.13	0.31
pH*methanol	0.69	0.03	0.70
pH*acetonitrile	0.69	0.92	0.19
pH*wavelength	0.11	0.29	0.16

will affect our method. After establishing the central composite design the knowledge area can be used to define the design space [29,30].

In term of specification for CQA for Metformin symmetry, a desired value above 0.7, for analysis time, the desired value less than 8min. for resolution, the desired value more than 1.5. The search for global solution was performed by optimization in Minitab 15 as represented in Figure 4. These plots made it possible to high light the combination leading to the desired values. By using contour plots as presented in Figure 5 the behavior of each pair of parameters to reach the optimum condition and fixing the other factors at certain values. Some aspects should be taken into accounts for selecting the design space, by using of high percentage of methanol helped in high results in resolution of Vildagliptin and short the time of analysis.

In this study, the design space calculated as a multi-dimensional combination between CPPs where CQAs corresponded to higher performance criteria. The design space calculated from the original set point that derived from response optimizer [pH of buffer 6.8, methanol percentage 26.5%, acetonitrile 30.6%, and wavelength 223nm] to obtain the desirability of Metformin symmetry in a range 0.7-0.9, Vildagliptin resolution equal or more than 1.5, and time of analysis between 5 min to 8 minutes. As shown in overlay contour plot in Figure 6, the design space colored in white corresponding to the following values: pH of 0.01M phosphate buffer (6.1-6.9), methanol percentage (18.6-27), Acetonitrile % (30.75- 31.75), wavelength (222-223nm).

Robustness

It is a method to prove that all factors will not affect the method performance upon minor change. DS can be used as an area of theoretical robustness as any change of parameters conditions will

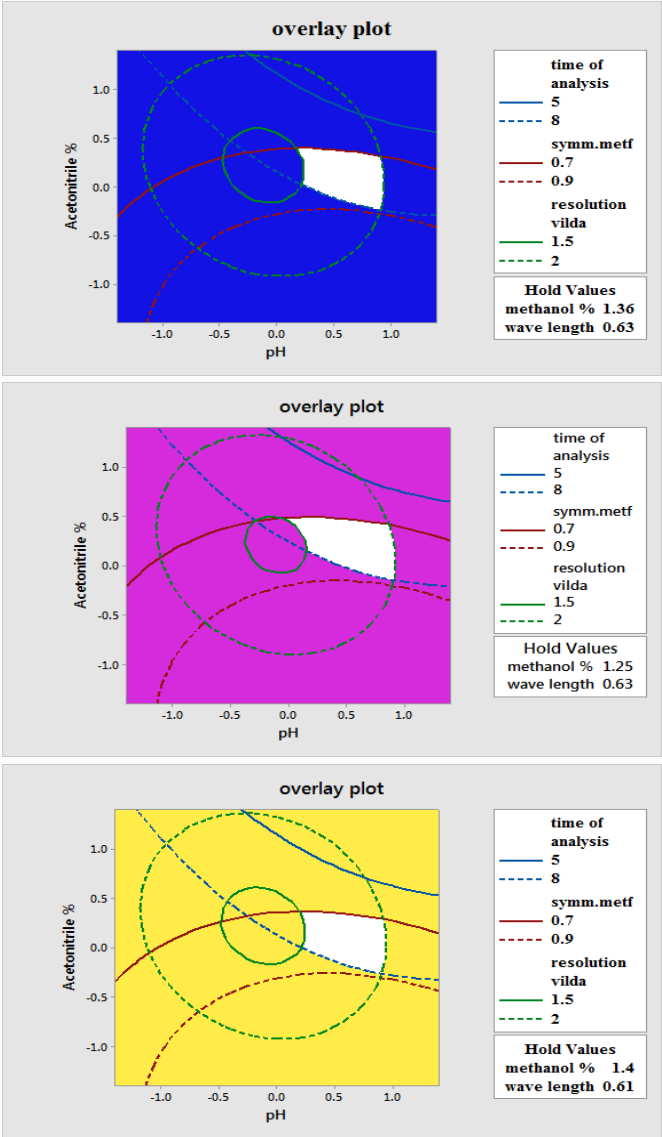

Figure 5: Overlay contour plots between factors to get time of analysis ≤ 8 minutes, R_s ≥ 1.5 and Symmetry ≥ 0.7.

not affect the CQAs. So, to achieve the robustness step, new fractional factorial design was performed between pH of buffer, methanol percentage, acetonitrile percentage and wavelength around their optimum conditions [pH of buffer 6.7, methanol percentage 26.5%, acetonitrile 30.6%, and wavelength 223nm] as shown in Table 4. By analyzing the p values of all parameters, this test proved their non-significant effect on CQAs as their values above 0.05 as shown in Table 5 except the p- value between methanol and pH of buffer was significant with a value 0.028 that should be counted in consideration.

Method control

According to ICH guidelines, method control defined as a method is performed to ensure that a product of required quality will be produced consistently. This method was achieved by verifying the system suitability parameters. These parameters were determined by the resolution , symmetry , time of analysis during the validation step as the resolution of all peaks should be not less than 1.5, the symmetry of peaks should not less than 0.7 with a time of analysis not more than 8 minutes and the chromatogram obtained according to these results was shown in Figure 6. So, it was found that the method can be controlled and robust to changing in pH of phosphate buffer (6.1-6.9), percentage of methanol (25-27), acetonitrile% (30.75 - 31.75), wavelength (222-225nm) to get accepted range of CQAs as Symmetry of Metformin (0.85-0.95), Resolution of Metformin and Vildagliptin (1.95-2.5), and time of analysis (4.85-6).

Method validation and application to dosage form

Range & linearity: The linearity of the method was evaluated by analyzing a series of different concentrations of each compound. Concentrations were chosen between the range of 0.2-20µg/mL for metformin HCl, 0.2 -2.5µg/mL for Pioglitazone, 1.0-20µg/mL for Vildagliptin and Sitagliptin separately. The assay was performed according to experimental conditions previously mentioned and each concentration was repeated three times. Linear regression analysis was performed and regression parameters were calculated and presented in Table S1. High correlation coefficients were found 0.9991, 0.9994, 0.9993, and 0.9998 for Metformin HCl, Pioglitazone HCl, Vildagliptin and Sitagliptin, respectively.

Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH

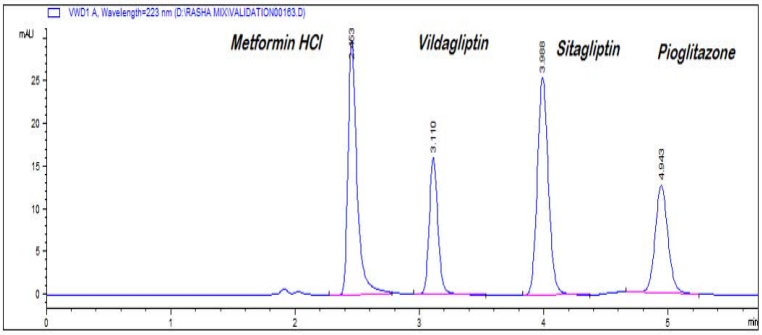

Figure 6: HPLC chromatogram for separation of metformin HCl, Vildagliptin, Pioglitazone, and Sitagliptin.

guidelines to test the sensitivity of the method as shown in Table S1. Therefore the proper way to evaluate the limits of the method is curve fitting and statistical methods

LOD = 3.3σ /S and LOQ = 10σ /S

Where σ is the standard deviation of residuals and S is the slope of the calibration curve.

Precision: Repeatability of the method was tested by choosing three concentration levels for each compound and analyzing them as mentioned before under experimental section and they were repeated three times within a day (intra-day precision).The RSD of the calculated intraday precision were found 0.84, 0.84, 0.47, and 1.03 for metformin HCl, Vildagliptin, Sitagliptin, and Pioglitazone HCl respectively. While intermediate precision was tested by analyzing the three concentration levels three times on three consecutive days (inter day precision).the values of RSD were found 1.24, 0.74, 0.96, and 0.75 for Metformin HCl, Vildagliptin, Sitagliptin, and Pioglitazone HCl respectively. So the method is precise as the relative standard deviation of inter-day and intra-day is less than 2% as shown in Table S1.

Accuracy: The accuracy study was performed by applying the standard addition technique, the resulting mixtures were assayed and determined and presented in Table S2. The mean percentage recoveries and relative standard deviation results were found to be 99.33±1.21, 100.13±1.34, 100.37±1.55, and99.90±1.70 for Metformin HCl, Pioglitazone HCl, Vildagliptin and Sitagliptin respectively. Therefore, a good recovery results were obtained with small standard deviation

Specificity: Method selectivity was achieved by preparing different mixtures of Metformin HCl, Pioglitazone, Vildagliptin and Sitagliptin within the linearity range concentration. The laboratory prepared mixtures were analyzed according to the previous procedure described under the proposed method and the mean percentage recoveries and standard deviation results were calculated for them and described in Table S3. So, high selectivity was carried out by obtaining good results of mean and standard deviations.

Application: The proposed method was applied to different single and combined dosage forms. These samples were analyzed according to the applied analytical method and the percentage recoveries for Metformin HCl, Pioglitazone HCl, Vildagliptin and Sitagliptin were determined and satisfactory results were obtained for each compound in good agreement with label claims for each one and presented in Table S4.

Conclusion

The great advantage of implementation of quality by design to analytical methods is providing an organized approach, with which it is possible to address both simply and tricky experimental problems. Also by means of design of experiments, the analyst obtains more useful and more precise information about the studied method. By understating the analytical target profile, evaluation of traditional methodologies, and formation a design space, will lead to perform and design appropriate method that meet the ATP requirements. Thus applying QbD to analytical method development and validation, the method can analyze the mixture of the studied compounds in synthetic mixtures and in their dosage forms with good separation between them within a reasonable short time (less than 5 minutes) by using UV-HPLC.

References

1. Ayre, P Mane, K Ghude, M Nemade, P Gide. Implementing Quality by Design-A methodical approach in the RP-HPLC method development process. Int. J. Adv. Pharm. Anal. 2014; 4: 1–6.
2. F Krier, M Brion, B Debrus, P Lebrun, A Driesen, E Ziemons, et al. Optimisation and validation of a fast HPLC method for the quantification of sulindac and its related impurities. J. Pharm. Biomed. Anal. 2011; 54: 694–700.
3. S Orlandini, B Pasquini, M Stocchero, S Pinzauti, S Furlanetto. An integrated quality by design and mixture-process variable approach in the development of a capillary electrophoresis method for the analysis of almotriptan and its impurities. J. Chromatogr. A. 2014; 1339: 200–209.
4. J Huang, G Kaul, C Cai, R Chatlapalli, P Hernandez-Abad, K Ghosh, et al. Quality by design case study: an integrated multivariate approach to drug product and process development. Int J Pharm. 2009; 382 : 23-32.
5. S Orlandini, B Pasquini, R Gotti, A Giuffrida, F Paternostro, S Furlanetto. Analytical quality by design in the development of a cyclodextrin-modified capillary electrophoresis method for the assay of metformin and its related substances. Electrophoresis. 2014; 35: 2538–2545.
6. E Rozet, P Lebrun, P Hubert, B Debrus, B Boulanger. Design Spaces for analytical methods. TrAC - Trends Anal. Chem. 2013; 42: 157–167.
7. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q9: Quality Risk Management, ICH, Geneva, Switzerland, 2005.
8. International conference on harmonization (ICH) of Technical Requirements for Registration of pharmaceuticals for humans Use, Topic Q9 (R2) Quality Risk Management, ICH, Geneva, Switzerland, 2005.
9. Madhukar, A Prince, R Vijay Kumar, Y Sanjeeva, K Jagadeeshwar, D Raghupratap. Simple and sensitive analytical method development and validation of metformin hydrochloride. Int. J. Pharm. Pharm. Sci. 2011;

3: 117–120.

10. RI El-Bagary, EF Elkady, BM Ayoub. Liquid Chromatographic Methods for the Determination of Vildagliptin in the Presence of its Synthetic Intermediate and the Simultaneous Determination of Pioglitazone Hydrochloride and Metformin Hydrochloride. Int. J. Biomed. Sci. 2011; 7: 201–208.

11. MA Jiladia, SS Pandya, AG Jiladia. Estimation of pioglitazone in bulk and tablet dosage forms by HPLC method. Int. J. Pharm. Sci. 2010; 2: 386–389.

12. R Kommana, RS Devarapalli. Development and validation of HPLC and UV spectrophotometric methods for determination of pioglitazone hydrochloride in bulk and its formulations. Der Pharm. Lett. 2013; 5: 269–278.

13. E Souri, H Jalalizadeh, S Saremi. Development and validation of a simple and rapid HPLC method for determination of pioglitazone in human plasma and its application to a pharmacokinetic study. J. Chromatogr. Sci. 2008; 46: 809–812.

14. RR Mamdouh, MR Safa'a, YM Ghada, EA AbdelAleem. Simultaneous determination of pioglitazone and glimepiride in their pharmaceutical formulations. Scholars Research Library. 2010; 3: 176.

15. C Sreedhar, MK SRT, AHG S Kanagala, SVK. New Analytical Method Development and Validation of Some Oral Hypoglycemic Drugs. Research Journal of Pharmaceutical. Biological and Chemical Sciences. 2012; 3: 20-30.

16. BSC, PRA. Development and validation of spectrophotometric method for the determination of DPP4 inhibitor, Sitagliptin, in its pharmaceutical dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2: 138-142.

17. N Monila, R Pulla, H Shabad, V Swathi, J Rajsekhar, A Ramesh, et al. New extractive method development of sitagliptin phosphate in API and its unit dosage forms by spectrophotometry. IOSR J. Pharm. Biol. Sci. 2012; 1: 37–40.

18. T Raja, A Rao. Validated HPTLC Method for Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate in Bulk Drug and Formulation. Rasayan J. Chem. 2012; 5: 407–413.

19. RI El-Bagary, EF Elkady, BM Ayoub. Spectrophotometric Methods for the Determination of Sitagliptin and Vildagliptin in Bulk and Dosage forms. Int. J. Biomed. Sci. 2011; 7: 55–61.

20. Inamdar Hitesh P. A revised RP-HPLC method for simultaneous determination of Vildagliptin and Pioglitazone HCl – application to commercially available dosage forms. Int. J. Pharm. Sci. Res. 2013; 4: 847–855.

21. RD Latha, K Pushpa. Method Development and Validation for the simultaneous estimation of Vildagliptin and Metformin in tablet dosage formsby RP-HPLC. International journal of pharmacy and pharmaceuical sciences. 2013; 5: 459–463.

22. PSRCHNPV D, AL Rao, SC Dinda. Simultaneous Determination of Metformin and Vildagliptin in Solid Dosage Form By. Int. Res. J. Pharm. 2013; 4: 122–128.

23. H Wu, M White, MA Khan. Quality-by-Design (QbD): An integrated process analytical technology (PAT) approach for a dynamic pharmaceutical co-precipitation process characterization and process design space development. Int. J. Pharm. 2011; 405: 63–78.

24. MH Rafamantanana, B Debrus, GE Raoelison, E Rozet, P Lebrun, S Uverg-Ratsimamanga, et al. Application of design of experiments and design space methodology for the HPLC-UV separation optimization of aporphine alkaloids from leaves of Spirospermum penduliflorum Thouars. J. Pharm. Biomed. Anal. 2012; 62: 23–32.

25. S Roy. A holistic concept of building quality in pharmaceuticals. International Journal of pharmaceutical and Biomedical Research. 2012; 3: 100-106.

26. PF Gavin, BA Olsen. A quality by design approach to impurity method development for atomoxetine hydrochloride (LY139603). J. Pharm. Biomed. Anal. 2008; 46: 431–441.

27. F Krier, M Brion, B Debrus, P Lebrun, A Driesen, E Ziemons, et al. Optimisation and validation of a fast HPLC method for the quantification of sulindac and its related impurities. J. Pharm. Biomed. Anal. 2011; 54: 694–700.

28. KE Monks, H-J Rieger, I Molnár. Expanding the term "Design Space" in high performance liquid chromatography (I). J. Pharm. Biomed. Anal. 2011; 56: 874–879.

29. S Orlandini, B Pasquini, M Stocchero, S Pinzauti, S Furlanetto. An integrated quality by design and mixture-process variable approach in the development of a capillary electrophoresis method for the analysis of almotriptan and its impurities. J. Chromatogr. A. 2014; 1339: 200–209.

30. S Orlandini, S Pinzauti, S Furlanetto. Application of quality by design to the development of analytical separation methods. Anal. Bioanal. Chem. 2013; 405: 443–450.