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A Quality by Design (QbD) Based Method Development and Validation of a High-Performance Liquid Chromatography for the Simultaneous Estimation of Metformin and Ertugliflozin

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ABSTRACT

This study explains about the Analytical Quality by Design approach for the optimization of a High-Performance Liquid Chromatography Method for the simultaneous estimation of Metformin and Ertugliflozin in pharmaceutical substance. The study aimed to optimize the High-Performance Liquid Chromatography (HPLC) by means of an analytical target profile in order to achieve good separation of compounds along with acceptable analysis time. Identification of risk factors for variables affects the method efficacy. This leads to the development of an accurate, precise, and economic method. The optimized conditions of the developed method were a stationary phase of a Discovery C18 250 x 4.6mm, 5m and a mobile phase of Orthophosphoric acid buffer (pH 2.2),ACN taken in the ratio 60:40 was selected as mobile phase and detection wavelength of 230nm. The flow rate was selected as 0.98ml/min at 29.15^{0}C column temperature. Using the central composite design (CCD) method was optimized. The method is showing the linearity over the concentration range of 25-150μg/ml for Metformin and 0.375-2.25μg/ml for Ertugliflozin. The intra-and inter-day precision were less than 2% of relative standard deviation. Accuracies between 99-102% of the true values. The LOD obtained for Metformin and Ertugliflozin were found to be 59 and 3.7, respectively. LOQ obtained for Metformin and Ertugliflozin were 77.6 and 5.2, respectively. Under accelerated conditions degradation percentage of the drug was found to be less than 10%, and the degradation product peak not affecting the system suitability of Metformin and Ertugliflozin.

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INTRODUCTION

Ertugliflozin is a drug used to treat type-2 diabetes. IUPAC name of Erthugliflozin is (1S,2S,3S,4R,5S)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-(hydroxymethyl) -6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol. Molecular weight is 436.89 g·mol^{-1} and the chemical formula is $C_{22}H_{25}ClO_7$. (Laxmi *et al.*, 2019; Cinti *et al.*, 2017) Structure of Ertugliflozin shown in Figure 1.

Metformin also used in the treatment of type-2 diabetes, especially in obese patients. This a first-line medication. IUPAC name of Metformin is N,N-Dimethylimidodicarbonimidicdiamide. The molec-

ular formula is $C_4H_{11}N_5$and the molecular weight is 129.167 g·mol^{-1}. (Rao *et al.*, 2019; Setter *et al.*, 2003) Structure of Metformin shown in Figure 2.

AQbD is a process of risk assessment, proactive and methodical approach to develop an analytical method. Which focus on the robustness of the method to minimize the source of variability. Hence developed method reaches requirements throughout the method and product lifecycle (Reid *et al.*, 2013; Peraman *et al.*, 2015). In this study efficiency of the developed method was determined according to Analytical Target Profile (ATP). (Raman *et al.*, 2015)

The current research work aimed to develop a Quality by Design based HPLC method for the estimation of Metformin and Ertugliflozin in pharmaceutical substance.

The developed method was validated for validation parameters like accuracy, precision, linearity, specificity, the limit of detection (LOD), and limit of quantification (LOQ), robustness and stability according to ICH guidelines.

According to International Conference on Harmonization (ICH) Q8 (R2), ICH Q9 guidelines, effects of various method input variables on method performance or response was evaluated (ICH Q8 (R2), 2009; ICH Q9, 2005).

According to literature Review (Kumari and Bandhakavi, 2020; Shafaat *et al.*, 2020), there were few studies were published on an estimation of Metformin and Ertugliflozin using chromatography methods (Nizami *et al.*, 2018). There is no data available on the Quality by Design based method. This made me to work on present research work.

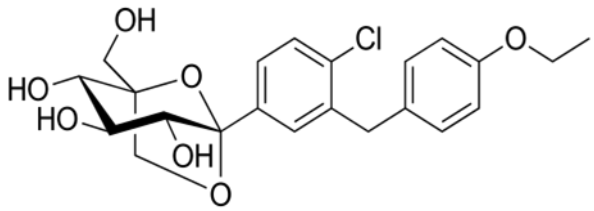


Figure 1: Ertugliflozin

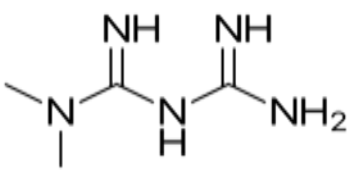


Figure 2: Metformin

MATERIALS AND METHODS

Chemicals and Reagents

Pure Metformin and Ertugliflozin were procured from Spectrum pharmaPvt Ltd (Hyderabad). Hydrochloric acid AR grade (HCL) and sodium hydroxide AR grade (NAOH) were obtained from Merck India Pvt Ltd. Hydrogen Peroxide (H_2O_2) was purchased from Qauligens. Acetic acid AR grade was purchased from Fisher scientific, India and S.D. Fine chem Ltd. Respectively. HPLC grade Acetonitrile (ACN) was purchased from Fischer scientific. HPLC grade water used throughout the analysis was obtained from the Merck milli-Q water purification unit.

Equipment

The LC system HPLC is used for method development and method validation. Detection was done by Waters with a diode array detector (model: 2996 detector 2487 separation module). The output signal was supervised and studied using Waters Empower 2 Software. Mettler Toledo balance was used to perform weighing. Other equipments used throughout the experimental work are a hot air oven (Yorco scientific), thermostat dry air equipment, Thermo scientific and pH meter (Eutech instruments pH tutor, pH meter, India).

Chromatographic Conditions

Various trials were conducted to select the mobile phase and stationary phase of a Discovery C18 250 x 4.6mm, 5m. Orthophosphoric acid buffer (pH 2.2) and ACN taken in the ratio 60:40 was selected as mobile phase. Using the central composite design (CCD) method was optimized.

Preparation of solutions

Preparation of Standard Solution

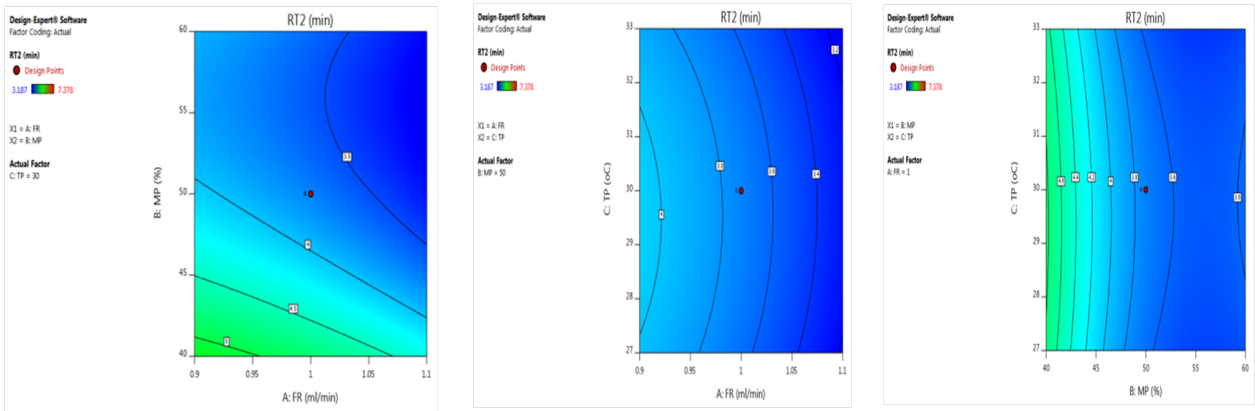
50mg of Metformin and 0.75mg of Ertugliflozin were weighed accurately and transferred to 50 ml clean dry graduation flasks separately. A 10ml of diluent (Water: Acetonitrile (50:50)) was added and sonicated for 10 minutes.

Made up the volume to 50ml with diluent to produce 1000μg/ml Metformin and 15μg/ml Ertugliflozin solution. 1ml of each solution were taken into a 10ml volumetric flask and made up to 10ml with diluent to get 100μg/ml Metformin and 1.5μg/ml Ertugliflozin solution.

Preparation of buffer

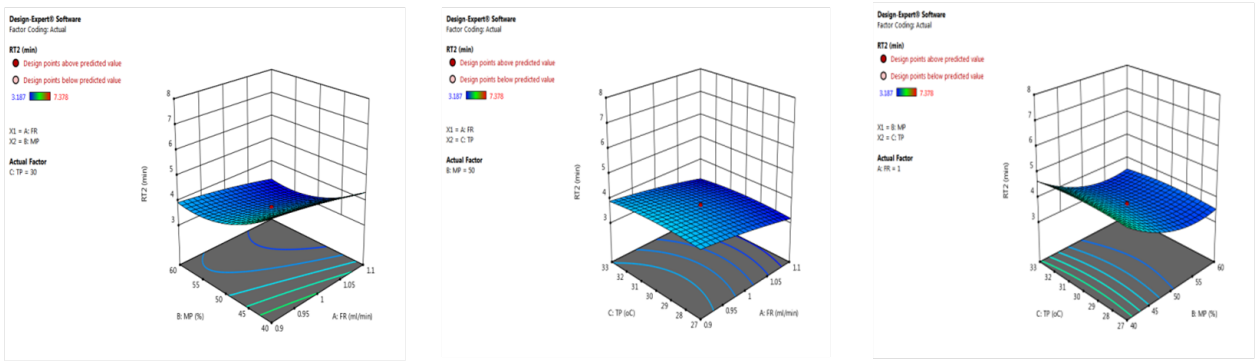
Buffer: 0.1% Orthophosphoric acid

1ml of orthophosphoric acid taken into a volumetric flask and diluted to 1000ml with HPLC grade water (pH 2.2).



A B C

Figure 3: 2D contour plots of retention time as a function of FR, Column temperature and organic ratio



A B C

Figure 4: 3D contour plots of retention time as a function of FR (a), Column temperature (b) and organic ratio(c)

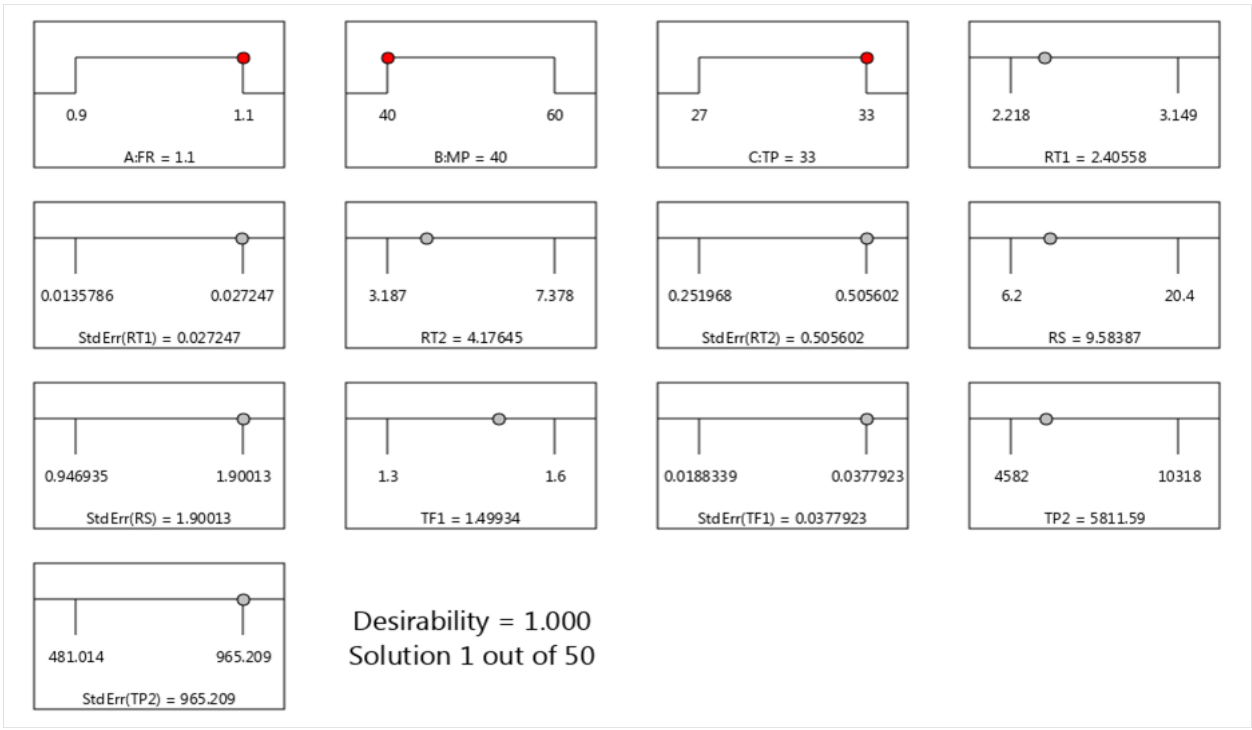


Figure 5: Overall desirability of the final method

Method validation

The method validation was performed as per ICH guidelines.

To conduct system suitability studies, six replicate samples were injected into the system to calculate the retention time, area, theoretical plates, SD and %RSD. In linearity studies conducted by injecting different concentrations range of 25-150μg/ml of Metformin and 0.375-2.25μg/ml of Ertugliflozin. By taking concentration on X-axis and peak area on the Y-axis, a linearity plot was plotted. The correlation co-efficient (R^2) should be less than 1. Accuracy performed to determine percentage recovery by injecting 50%, 100% and 150% of concentrations of Metformin and 80%, 100% and 120% of concentrations of Ertugliflozin standard in triplicate. After performing repeatability (intra-day) and intermediate precision (inter-day), method precision was determined. In precision, % RSD should be less than 2. Limit of detection (LOD)and Limit of Quantification (LOQ) refers to the lowest concentration level resulting in a peak area of three times and ten times the baseline noise, respectively. Robustness referred to as the extent of a method to remain unaltered by small or deliberate changes in chromatographic conditions like an organic solution in mobile phase ratio ($\pm$ 10), flow rate ($\pm$ 10) and temperature ($\pm$ 10).

Stress studies

To conduct acid hydrolysis, base hydrolysis and neutral hydrolysis, 1ml of stock solution added 1ml of 2N HCl, 1ml of stock solution and 1ml of 2N NaOH solution and 1ml of stock solution and 1ml of water individual volumetric flask, respectively. Three solutions were shaken in Radley apparatus 70ºC for 1 hr, then neutralized and diluted to 10ml. In oxidative degradation, to 1ml of stock solution, a 1ml of 20% H_2O_2 solution was added and kept in the dark area at room temperature for 24 hrs and diluted to 10ml. In thermal degradation, 50mg of Metformin and 0.75mg of Ertugliflozin were kept in a petri dish and placed in a hot air oven at 70ºC for 24hrs. The sample was collected at different points o time and dissolved in the diluent to produce 10ml. In photodegradation, 50mg of Metformin and 0.75mg of Ertugliflozin were applied uniformly over a petri dish and allowed to fall sunlight for 24hrs. Samples were collected at multiple time points and dissolved in the diluent to produce 10ml.

RESULTS AND DISCUSSION

Method Development

Initial trials were conducted to optimize the method according to the central composite design (CCD) method. HPLC studies were carried out using a Kromasil C18 (250$\times$4.6 mm, 5 μm) and 0.1%OPA: Methanol (61.2:38.8%) as mobile phase, at wavelength detection of 230nm. The flow rate was selected as 0.98ml/min at 29.15^{0}C column temperature. The retention time was found to be 2.4min and 4.037 min Metformin and Ertugliflozin, respectively. Using the central composite design (CCD) method was optimized. The factors viz; % Organic concentration, flow rate, Column temperature were taken, and counter and a 3D surface plot showing the effect of each parameter on Retention Time, Theoretical plates and Asymmetry (CQA) were generated. Results and ANOVA studies were shown in Tables 1 and 2, respectively. A desirability function applied to the optimized conditions to predict retention time, asymmetry and theoretical plates.

2D contour plot was developed as a function of 1% organic concentration, pH and buffer strength. To understand the results, 2D contour plots and 3D plot were generated from data using Design Expert® software (shown in Figures 3 and 4).

To get an optimum set of conditions, composite desirability was applied based on the specified goals and limits of each responses. If the response on the desirability scale is on 1 it is a fully desirable response and the response is on 0. It is an undesirable response. Responses based on specified goals and boundaries for retention time, area and asymmetry obtained desirability composite was 1. As shown in Figure 5.

Optimization of Chromatographic Conditions

Initial trials were performed to optimize the method according to the central composite design (CCD) method. HPLC studies were carried out using a Kromasil C18 (250$\times$4.6 mm, 5 μm) and 0.1%OPA: Methanol (61.2:38.8%) as mobile phase, at wavelength detection of 230nm. Flow rate was selected as 0.98ml/min at 29.15^{0}C column temperature. Peaks of Metformin and Ertugliflozin were developed at 2.4min and 4.037 min, respectively. An optimized chromatogram was shown in Figure 6.

Method validation

After optimization, the developed method was validated as per ICH Q2R1 guidelines requirements, and then the method was used for simultaneous determination of Metformin and Ertugliflozin

System Suitability

After injecting six replicate samples of Metformin and Ertugliflozin into the system, different parameters like retention time, area, theoretical plates, SD and %RSD were calculated. All the parameters were within the limits as per guidelines. Results were

Table 1: Central composite experimental design matrix with response

Std	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
	A: FR	B: MP	C: T	RT1	RT2	RS	TF1
	ml/min	%	0C	MIN	NUM	NUM	
4	1.1	60	27	2.323	3.261	6.3	1.4
14	1	50	35.0454	2.547	3.617	7.3	1.5
8	1.1	60	33	2.299	3.229	6.2	1.3
19	1	50	30	2.579	3.697	7.5	1.4
5	0.9	40	33	2.920	4.384	7.6	1.5
3	0.9	60	27	2.801	3.966	6.6	1.4
2	1.1	40	27	2.397	3.647	7.7	1.5
13	1	50	24.9546	2.601	3.783	7.9	1.5
20	1	50	30	2.589	3.714	7.6	1.4
11	1	33.1821	30	2.819	7.378	20.4	1.6
17	1	50	30	2.587	3.716	7.6	1.4
18	1	50	30	2.580	3.704	7.5	1.4
1	0.9	40	27	2.943	4.530	8.5	1.5
6	1.1	40	33	2.399	3.603	7.2	1.5
12	1	66.8179	30	2.514	3.533	6.8	1.5
7	0.9	60	33	2.827	3.971	6.2	1.4
15	1	50	30	2.564	3.698	7.7	1.5
16	1	50	30	2.580	3.730	8.1	1.5
9	0.831821	50	30	3.149	4.524	6.5	1.5
10	1.16818	50	30	2.218	3.187	6.5	1.4

Table 2: ANOVA table for Retention time using CCD

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1.06	9	0.1173	105.79	< 0.0001	significant
A-FR	0.9695	1	0.9695	874.66	< 0.0001	
B-MP	0.0622	1	0.0622	56.15	< 0.0001	
C-TP	0.0009	1	0.0009	0.7967	0.3931	
AB	0.0005	1	0.0005	0.4196	0.5317	
AC	0.0001	1	0.0001	0.0705	0.7960	
BC	0.0001	1	0.0001	0.0597	0.8120	
A^2	0.0130	1	0.0130	11.71	0.0065	
B^2	0.0083	1	0.0083	7.49	0.0209	
C^2	0.0011	1	0.0011	0.9833	0.3448	
Residual	0.0111	10	0.0011			
Lack of Fit	0.0107	5	0.0021	27.65	0.0012	significant
Pure Error	0.0004	5	0.0001			
Cor Total	1.07	19				

Table 3: Result of system suitability

S.No	Metformin		Ertugliflozin	
	RT	Area	RT	Area
1	2.515	2552371	1.38	239700
2	2.518	2530245	1.4	243723
3	2.52	2422701	1.44	245503
4	2.523	2526138	1.42	248543
5	2.527	2523971	1.43	242764
6	2.53	2508851	1.42	244180
Mean		2510713		244069
SD		45343.5		2932.4
%RSD		1.8		1.2

Table 4: Result of linearity

S. No	Metformin		Ertugliflozin	
	Conc. (ppm)	Area	Conc. (ppm)	Area
1	0	0	0	0
2	25	638163	0.375	61564
3	50	1286114	0.75	127241
4	75	1858023	1.125	185685
5	100	2543171	1.50	242332
6	125	3173970	1.875	304469
7	150	3755568	2.25	363582

Table 5: Results for Accuracy

Drug Name	%	Area	Total Conc	Added Conc	Std Conc	Amt Rec	AVG % Rec
Metformin	50%	3776096	149.8943	50	100	49.89	100.95
		3800845	150.8789	50	100	50.88	
		3795078	150.6494	50	100	50.65	
	100%	5058775	200.9238	100	100	100.92	100.7467
		5026299	199.6318	100	100	99.63	
		5078147	201.6945	100	100	101.69	
	150%	6238379	247.8527	150	100	147.85	99.12333
		6273225	249.239	150	100	149.24	
		6266469	248.9702	150	100	148.97	
Ertugliflozin	80%	363063	2.239213	0.75	1.5	0.74	100.1533
		366337	2.259535	0.75	1.5	0.76	
		365559	2.254706	0.75	1.5	0.75	
	100%	485915	3.001753	1.5	1.5	1.50	100.2633
		484964	2.99585	1.5	1.5	1.50	
		487929	3.014254	1.5	1.5	1.51	
	120%	601817	3.721154	2.25	1.5	2.22	98.50333
		600535	3.713197	2.25	1.5	2.21	
		600788	3.714767	2.25	1.5	2.21	

Table 6: Results for Precision

S.No	Metformin		Ertugliflozin	
	Intra-day Precision	Inter-day Precision	Intra-day Preci-sion	Inter-day Preci-sion
1	2544638	2438949	245566	232491
2	2524412	2456129	242421	228170
3	2469751	2397470	247505	236798
4	2547631	2424212	241610	240907
5	2530091	2431030	247226	235219
6	2482730	2441079	240309	233638
AVG	2516542	2431478	244106	234537
STDEV	32662.1	19825.3	3062.9	4282.2
%RSD	1.3	0.8	1.3	1.8

Table 7: Summary of degradation study

S. No.	Degradation Condition	Metfomin % degrada-tion	Ertugliflozin % degra-dation
1.	2N HCl, 8 hrs	7.99	7.37
2.	2N NaOH, 8hrs	6.18	5.46
3.	Neutral hydrolysis, 24 hrs	0.72	0.95
4.	Oxidative degradation, 24 hrs	4.16	4.21
5.	Thermal degradation, 3 days	2.37	2.60
6.	Photo degradation, 24 hrs	1.51	1.83

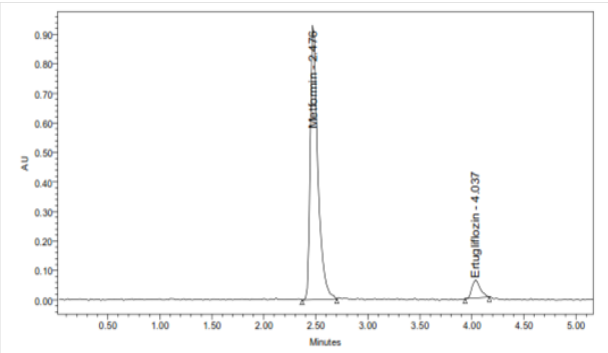


Figure 6: Optimized chromatogram

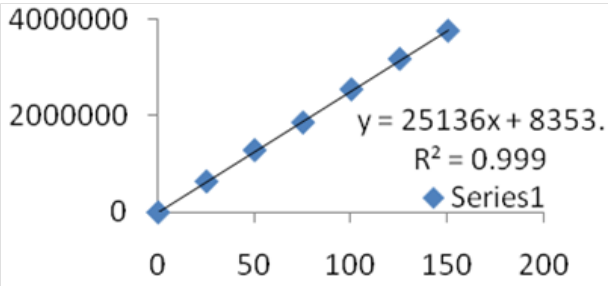


Figure 7: plot of Metformin

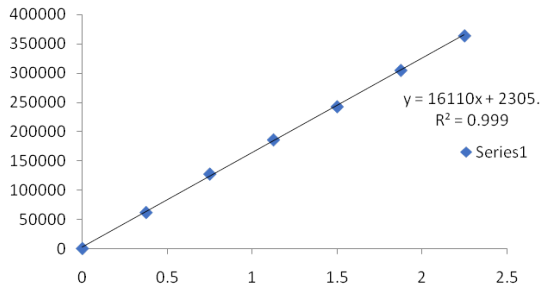


Figure 8: Linearity plot of Ertugliflozin Accuracy

shown in Table 3.

Linearity

The regression equation obtained was y = 25136x + 8353 and y = 16110x + 2305 for Metformin and Ertugliflozin respectively. r^2 (Correlation co-efficient) was found to be 0.999 for Metformin and Ertugliflozin . Results were shown in Figures 7 and 8 and Table 4. The accuracy of Metformin and Ertugliflozin showed good recovery, and the results were within the limit, i.e 99-102%W/V. Accuracy results are given in Table 5.

Precision

The % RSD of intraday and inter-day precisionswere

less than 2 for Metformin and Ertugliflozin. The results were within limits as per the guidelines and shown in Table 6.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were determined according to signal to noise ratio method. The LOD obtained for Metformin and Ertugliflozin were found to be 59 and 3.7, respectively. LOQ obtained for Metformin and Ertugliflozin were 77.6 and 5.2, respectively.

Robustness

To determine the robustness of the method, need to alter the parameters like column temperature, flow rate and % organic concentration. Studies were performed by changing the flow rate (+ 2 ml/min), column temperature (+5^{o}C) and mobile phase ratio. The % RSD of Metformin and Ertugliflozin were calculated and found that the results were within limits.

Stress Studies

Under accelerated conditions, stress studies were conducted. One significant degradation product was found in acid and base hydrolysis. There are no degradation products were found in of neutral hydrolysis, peroxide hydrolysis, photodegradation and thermal degradation conditions. From the obtained results degradation percentage of the drug was found to be less than 10%, and the degradation product peak not affecting the system suitability of Metformin and Ertugliflozin. Results were shown in Table 7.

CONCLUSIONS

Using Design Expert® software, a robust QbD method was developed for the simultaneous estimation of Metformin and Ertugliflozin. Method validation was performed as per the ICH guidelines, and the obtained results were within the limit. This stability-indicating method can able to separate drug substance from the degradation products.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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