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Enhancing Glycemic Control through QbD-Optimized Bilayer Tablet with Saxagliptin and Metformin for Type 2 Diabetes Treatment

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KEYWORDS

Diabetes, Saxagliptin, Metformin, Bilayer Tablet, Quality by Design

ABSTRACT:
In the realm of healthcare, the age-related scourge of mortality finds a significant relationship with diabetes mellitus (DM), notably the insidious type 2 diabetes (T2DM). This study focuses on age-related mortality, primarily associated with diabetes mellitus (DM), particularly type 2 diabetes (T2DM). The pathogenesis of T2DM involves insulin resistance, pancreatic beta-cell dysfunction, incretin deficit, and gastrointestinal incretin resistance. To address these factors and enhance treatment efficacy, a combination of medications is often required. The oral hypoglycemic drug Saxagliptin, inhibiting dipeptidyl peptidase-4 (DPP-4), was added to Metformin to improve glycemic control. Quality by Design (QbD) was employed as a systematic, scientific, risk-based, and proactive approach to develop an optimized bilayer tablet containing Saxagliptin and Metformin. The study involved preformulation studies to assess compatibility, a validated RP-HPLC technique for quantitative analysis, and QbD in four steps to design the bilayer tablet. Physicochemical properties were evaluated in accelerated conditions, confirming compatibility. RP-HPLC analysis demonstrated accuracy, linearity, precision, and specificity. QbD steps included defining Critical Quality Attributes (CQAs), creating a Quality Target Product Profile (QTPP), optimizing layers, and preparing the final bilayer tablet. CQAs such as flow property, content uniformity, and drug release were crucial. Optimization involved risk assessment, Taguchi design, and Box-Behnken design. For the immediate-release layer, key variables were kneading time, lubrication time, and magnesium stearate. The sustained-release layer optimization considered HPMC level, compritol level, and MS level. The design space was established based on dissolution criteria. The bilayer tablet was formed by compressing optimized layers. Dissolution studies revealed drug release patterns fitting Korsmeyer-Peppas model, indicating Fickian and super case II transport for Saxagliptin and Metformin, respectively. Comparisons with commercial tablets showed similar dissolution profiles. Stability analysis over six months demonstrated minimal changes, complying with ICH requirements.

Introduction

Type 2 Diabetes Mellitus (T2DM) represents a formidable global health challenge, with age-related mortality primarily attributed to its complications[1]. As a multifaceted metabolic disorder, T2DM is characterized by insulin resistance, pancreatic beta-cell dysfunction, and disturbances in incretin hormones[2]. The pathogenesis of T2DM underscores the need for comprehensive treatment strategies that address various facets of its etiology. In recent years, the integration of multiple medications has emerged as an effective approach to manage T2DM, aiming to optimize glycemic control and mitigate associated complications[3]. Among the therapeutic agents, Saxagliptin, an inhibitor of dipeptidyl peptidase-4 (DPP-4), has shown promise in enhancing the incretin system's function. When combined with Metformin, a longstanding and clinically proven anti-diabetic medication, this dual therapy holds potential for synergistic effects, offering a more robust and comprehensive approach to T2DM management[4].

Quality by Design (QbD), a systematic and proactive approach towards pharmaceutical development, has gained prominence for its ability to optimize

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formulations, ensuring the delivery of safe and effective drug products[5]. The present study embarks on a QbD-driven exploration, seeking to develop an optimized bilayer tablet containing Saxagliptin and Metformin. The objective is to not only improve the pharmacological efficacy of the individual drugs but also to enhance patient compliance through a streamlined and effective treatment regimen[6]. This research endeavors to contribute to the growing body of knowledge surrounding T2DM management by integrating Saxagliptin and Metformin into a single, QbD-optimized formulation. Through a meticulous exploration of critical quality attributes (CQAs), formulation parameters, and dissolution profiles, we aim to establish a robust foundation for the development of a bilayer tablet that ensures both immediate and sustained release of the therapeutic agents[7]. The potential benefits of this approach extend beyond pharmacological efficacy, encompassing cost-effectiveness and improved patient adherence, thereby addressing critical challenges in the current landscape of diabetes treatment[8].

Material And method

Material

Saxagliptin and Metformin hydrochloride were obtained as gift samples. All chemicals were used in their analytical grade.

Development and Validation of HPLC Method for Simultaneous Estimation of Saxagliptin and Metformin Hydrochloride

Preparation of PBS (pH 3.0)

Potassium dihydrogen phosphate (1.3609 g) was precisely weighed and transferred to a 1000 ml volumetric flask, dissolved in water, and pH adjusted to 3.0 using ortho-phosphoric acid (10%). The solution was then filtered through a 0.45-membrane filter after reaching a volume of 1000 ml[9].

Preparation of Standard Solution

Saxagliptin (5 mg) and Metformin hydrochloride (equivalent to 50 mg MET) were dissolved in 50 ml of methanol. The standard stock solution was diluted with methanol to create the working standard solution [10].

Method Validation

System Suitability

System suitability tests were conducted to ensure instrument duplicability. RSD (%) of retention times, theoretical plate, and tailing factor were calculated based on six injection repetitions [11].

Linearity

The least squares linear regression approach was used to determine linearity by creating a calibration plot between peak area and concentration for Metformin hydrochloride and Saxagliptin.

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

LOD and LOQ were determined using the calibration curve method with the formulas mentioned, incorporating standard deviation and slope.

Accuracy

According to ICH recommendations, three drug concentration levels (80%, 100%, and 120%) were added in triplicate, and the percentage recovery was calculated[12].

Precision

Intra-day and inter-day precision were determined at three different concentrations within the linearity range for three replications at three different times on each of the three days. RSD (%) was calculated.

Robustness

Robustness was evaluated by varying PBS pH, flow rate, column temperature (±5°C), and the ratio of acetonitrile (±5) in the mobile phase[14].

Development of Bilayered Tablet

Preparation of Bilayer Tablet

Optimization was performed for both the SR and IR layer formulations of the bilayer tablets. The die cavity was pressurized with low pressure after placing the optimized IR grains. In the subsequent phase, the upper punch was raised, and the optimized SR granules were poured over the previously compressed IR layer in the die cavity. Proper compression was applied to achieve the desired hardness[15].

Evaluation of Bilayer Tablet

Physical Characteristics

Thickness: Vernier calipers were employed to measure the tablet's thickness.

Hardness: The tablet's hardness was determined using a Monsanto hardness tester, where pressure was applied

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between two plungers, and the tablet's breaking point was noted.

Friability: Ten tablets were accurately weighed, added to the Roche friability apparatus, rotated for 4 minutes at 25 RPM, and then reweighed[16,17]. Friability was computed using the formula:

Friability (%) = [(W1 - W2) / W1] × 100.

Weight Variation: Twenty tablets were individually weighed, and weight variation was calculated using the formula:

Weight Variation (%) = [(Individual Unit Weight - Average Weight) / Average Weight] × 100

Drug Content Analysis

Twenty tablets were broken, powdered, and equivalent to 500 mg of Metformin hydrochloride or 2.5 mg of Saxagliptin. The resulting solution, diluted in pH 6.8 PBS, was analyzed using an HPLC method after filtration [18,19] .

In vitro Dissolution Studies of Bilayer Tablet

In vitro dissolution tests were conducted using the USP Apparatus I at 37±0.5°C and 100 rpm with PBS (pH 6.8). Five-milliliter samples were drawn periodically up to 12 hours and analyzed using the HPLC technique to determine Saxagliptin and Metformin hydrochloride percentage releases.

Drug Release Kinetic Modeling

The drug release kinetics were determined using curve fitting. Various kinetic models' equations were fitted to the cumulative drug release data, and the model with the highest R2 value was considered the most appropriate [20].

If the two dissolution profiles are identical, the f2 value is 100; a value of zero indicates dissimilarity. According to SUPAC-IR rules, dissolution profiles are considered similar if the f2 value falls between 50 and 100[21,22].

Results and discussion

Development and validation of HPLC method for simultaneous estimation of Metformin hydrochloride and Saxagliptin

For the simultaneous determination of Metformin hydrochloride and Saxagliptin, a straightforward HPLC technique with stability indicators was developed and validated. To achieve the best separation and resolution between Metformin hydrochloride and Saxagliptin, various stationary and mobile phases were initially tested. The Hyperclone C18 column and a mobile phase consisting of acetonitrile, PBS (pH 3.0), and water were found to be most effective for separating Metformin hydrochloride and Saxagliptin at a flow rate of 0.8 mL/min. Wavelength selection for Saxagliptin and Metformin hydrochloride solutions involved scanning the range of 200–400 nm. The isobestic points for Saxagliptin and Metformin hydrochloride were identified at 220 nm and 250 nm, respectively. In this study, a wavelength of 220 nm was discovered to be suitable for the simultaneous estimation of both drugs.

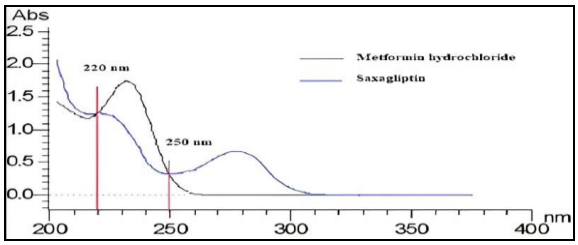

Figure 1: Overlaid spectra of Metformin hydrochloride and Saxagliptin: two isobestic point at 220nm and 250 nm.

Method validation

The proposed method was validated as per ICH guidelines.

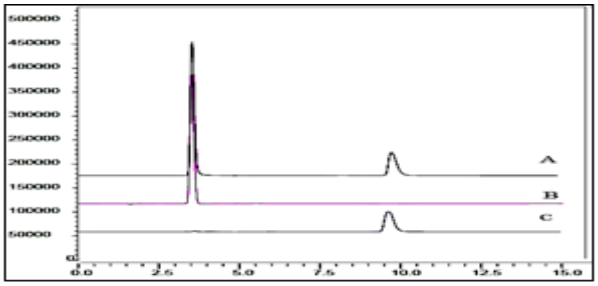

Figure 2: Overlay of chromatograms; A: mixture of Metformin hydrochloride and Saxagliptin, B: Metformin hydrochloride, C: Saxagliptin

System suitability

The adequacy of the system was evaluated by calculating factors such as the tailing factor, the number of theoretical plates, resolution, and repeatability. All parameters in Table 18 meet the USP limit for system appropriateness. Repeatability, measured using the RSD (percentage of response), was found to be less than 2.

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The number of theoretical plates for both peaks was discovered to be higher than 2000, and the tailing factor was under 2.

Table 1: System suitability parameter for method (n=6)

Parameter	Metformin			Saxagliptin		
	Mean	SD	% RSD	Mean	SD	% RSD
Area (mV)	11850108.17	69808.09	0.59	637241.50	5952.53	0.93
Theoretical plates#	3661.83	30.36	0.83	6959.33	102.83	1.48
Tailingfactor	1.22	0.01	0.66	1.64	0.02	1.48
Retention time (min)	3.54	0.04	1.16	9.71	0.08	0.86
Resolution	17.92	0.05	0.30	16.16	0.29	1.79

Linearity

By examining six solutions with concentrations of Metformin hydrochloride and Saxagliptin ranging from 20 to 120 g/mL and 2 to 12 g/mL, respectively, linearity of the proposed technique was assessed. Utilising concentration versus peak area, the calibration curve was built.

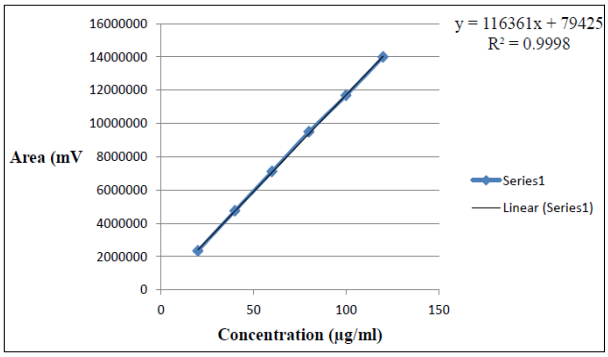

Figure 3: Calibration plot for Metformin hydrochloride.

Table 2: Linearity data of method (n=3)

Parameter	Metformin	Saxagliptin
Range (µg/ml)	20-120	2-12
Correlation coefficient	0.9998	0.9995
Slope	116361	60215
Intercept	794225	31816

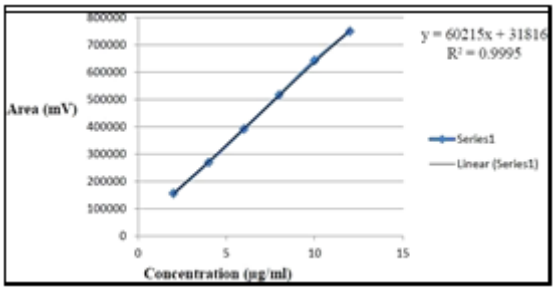

Figure 4: Calibration plot for Saxagliptin

For Metformin hydrochloride and Saxagliptin, good linearity was demonstrated over the investigated concentration range. For both medicines, the coefficient of determination from the linear regression analysis was found to be closer to one.

LOD and LOQ

For Metformin hydrochloride, the LOD and LOQ values were determined to be 1.9 and 6 g/mL, respectively. Saxagliptin had a LOD and LOQ of 0.33 g/mL and 0.99 g/mL, respectively.

Accuracy

The proposed approach was used to analyse the sample solution containing the standard medication at 80, 100, and 120% accuracy levels. Table 20 displays the RSD (%) and the mean percentage recovery. Both medications' recovery range and RSD were determined to be between 98.75 and 101.8% and 0.15 and 0.71 percent, respectively, which was within the acceptable range.

Table 3: Accuracy data for the method (n=3)

Drug	Pre-analyzed Concentration (mcg/ml)	Amount added (mcg/ml)	% Recovery	% RSD
Metformin Hydrochloride	50	40	101.63	0.33
		50	101.88	0.35
		60	101.02	0.71
Saxagliptin	5	4	98.75	0.39
		5	101.80	0.15
		6	101.17	0.24

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Precision

By choosing three concentrations from the linearity range and evaluating them in triplicate on the same day for intra-day and three different days for inter-day, the precision of the proposed approach was assessed. In accordance with the permissible range, the% RSD for both intra-day and inter-day was found to be less than 2 (table 21). As a result, the suggested method is accurate for estimating both medicines.

Table 4: Precision data for method

Drug	Amount added (mcg/ml)	Amount found			
		Intra-day		Inter-day	
		% Found	% RSD	% Found	% RSD
Metformin Hydrochloride	40	100.27±0.18	0.18	99.19±0.39	0.39
	80	101.53±0.38	0.37	100.96±0.19	0.19
	120	99.72±0.08	0.08	99.13±0.61	0.62
Saxagliptin	4	99.22±1.28	1.29	98.61±0.29	0.29
	8	100.39±1.01	1.01	100.87±0.79	0.78
	12	99.25±0.14	0.15	98.23±0.25	0.26

Robustness

By adjusting chromatographic parameters including buffer pH (0.1), flow rate (0.1 ml/min), column temperature (5^0 C), and the ratio of acetonitrile (1 : 5) in the mobile phase, the robustness of the approach was evaluated. In each circumstance, the solution was injected, and Saxagliptin and Metformin hydrochloride levels were measured. Tables 22 and 24, respectively, indicate the mean, standard deviation, and RSD for Metformin hydrochloride and Saxagliptin. ANOVA was used to statistically analyse the data (tables 23 and 25). There was no difference between the groups, as indicated by the estimated value of F being less than its tabulated value. As a result, it was determined that the approach was reliable for analyzing both substances to the extent that chromatographic settings could be changed.

Table 5: Robustness for Metformin hydrochloride

S.No	Assay of Metformin hydrochloride (µg/ml)								
	Run1	Run2	Run3	Run4	Run5	Run6	Run7	Run8	Run9
1	49.62	50.98	49.49	50.24	48.93	50.94	50.83	49.27	50.35
2	50.12	50.21	50.38	49.83	49.64	49.52	49.03	49.74	50.92
3	50.94	50.56	49.92	50.52	50.27	49.91	49.62	50.38	50.03
Mean	50.23	50.58	49.93	50.20	49.61	50.12	49.83	49.80	50.43
SD	0.67	0.39	0.45	0.35	0.67	0.73	0.92	0.56	0.45
RSD	1.33	0.76	0.89	0.69	1.35	1.46	1.84	1.12	0.89

Run 1: Control sample (flow rate 0.8 ml/min, acetonitrile:buffer:water in ratio of 20:30:50 %v/v/v, temperature 35°C and pH 3.0).Run 2: Sample (pH 2.9),Run 3: Sample (pH 3.1),Run 4: Sample (flow rate 0.7 ml/min),Run 5: Sample (flow rate 0.9 ml/min), Run 6: Sample (Column Temperature 30°C), Run 7: Sample (Column Temperature 40°C),Run 8: Sample (acetonitrile:buffer:water in ratio of 25:25:50) Run 9: Sample (acetonitrile:buffer:water in ratio of 15:35:50)

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Table 6: ANOVA table for deviation (Metformin hydrochloride)

Source of Variation	**SS**	**df**	**MS**	**F**	*P-value*	F crit
BetweenGroups	2.39973	8	0.29996	0.82948	0.58827	2.51015
WithinGroups	6.50933	18	0.36163			
Total	8.90906	26				

Table 7: Robustness for Saxagliptin

S.No	**Assay of Saxagliptin (µg/ml)**								
	Run1	**Run2**	**Run3**	**Run4**	**Run5**	**Run6**	**Run7**	**Run8**	**Run9**
1	5.02	4.87	5.02	5.05	4.98	5.07	5.13	4.96	5.02
2	4.96	4.92	4.98	4.93	5.08	4.95	4.89	5.03	5.17
3	4.87	4.96	4.86	5.09	5.03	4.93	4.93	4.87	5.13
Mean	4.95	4.92	4.95	5.02	5.03	4.98	4.98	4.95	5.11
SD	0.08	0.05	0.08	0.08	0.05	0.08	0.13	0.08	0.08
RSD	1.53	0.92	1.68	1.66	0.99	1.52	2.58	1.62	1.52

Table 8: ANOVA table for deviation (Saxagliptin)

Source ofVariation	**SS**	**df**	**MS**	**F**	***P-value***	**F crit**
BetweenGroups	0.0782	8	0.009775	1.495326	0.226935	2.510158
WithinGroups	0.117667	18	0.006537			
Total	0.195867	26				

Preparation and Evaluation of Optimised bilayer Tablet

To create a flat surface, optimised IR grains were maintained in the die chamber and squeezed gently. Pour the optimised SR over the pressed optimised IR layer to create the second layer. Finally, the desired pressure for the desired hardness was applied to both layers.

Post Compression properties of bilayer tablet

Bilayer tablet's post-compression qualities were assessed and found to be acceptable.

Table 9: Post compression properties of bilayer tablet

Avg Wt(mg)	**Hardness (kg/cm^2)**	**Friability (%)**	**Size (mm)**	**Thickness (mm)**	**Assay (%)**	
					MET HCL	**Saxagliptin**
1109.52 ±4.23	6.2 ± 0.05	0.06	18 ± 0.01	7.50 ± 0.07	98.63 ± 2.4	97.23 ±1.81

Dissolution Study:

A dissolution study was conducted for 12 hours in PBS at pH 6.8. The in vitro drug release profiles of Saxagliptin and Metformin HCl from bilayer tablets are depicted in Figures 5 and 6, respectively.

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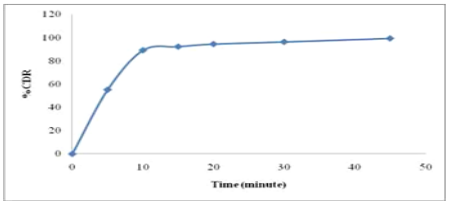

Figure 5: *In vitro* drug release of Saxagliptin from optimized bilayer tablet

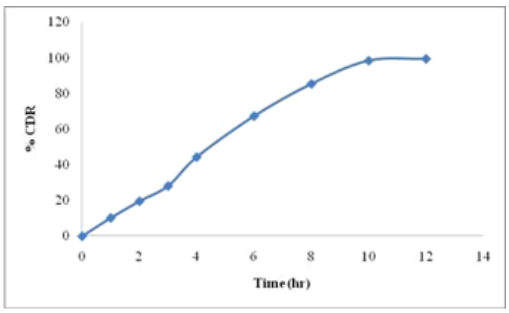

Figure 6: *In vitro* drug release of Metformin HCl from optimized bilayer tablet

Drug release kinetic study

The results of fitting Saxagliptin drug release data from an OPT bilayer tablet into several kinetic models are shown in Fig. 7. The outcome demonstrates that Saxagliptin release from the OPT bilayer tablet was best described by the first-order model (R=0.930), and the diffusional release exponent (n) was found to be less than 0.45, indicating severely Fickian behavior. The drug release data for Metformin hydrochloride from the OPT-bilayer tablet, as shown in Fig. 8, were possibly fit using various kinetic models. The outcome showed that the Korsmeyer-Peppas model (R=0.986) best fits the release of Metformin hydrochloride from the OPT-bilayer tablet, and the diffusional release exponent was found to be 1.53 (n > 0.85), confirming super case II transport.

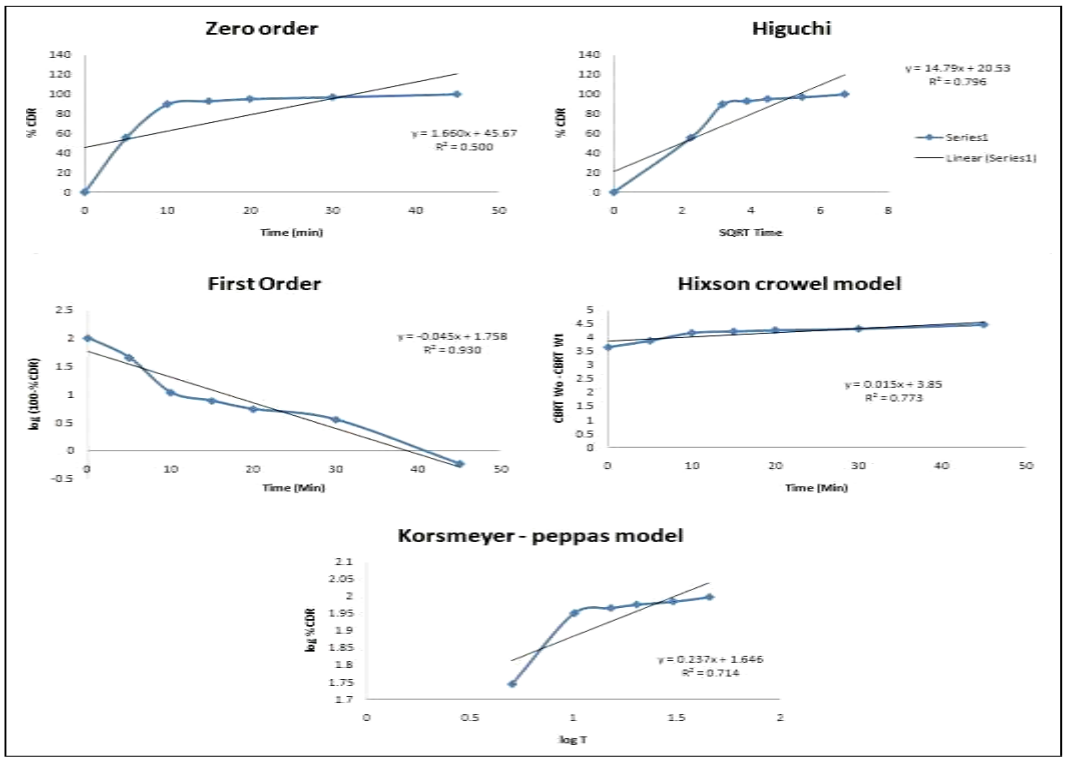

Figure 7: Kinetic models for Saxagliptin release from bilayer tablet

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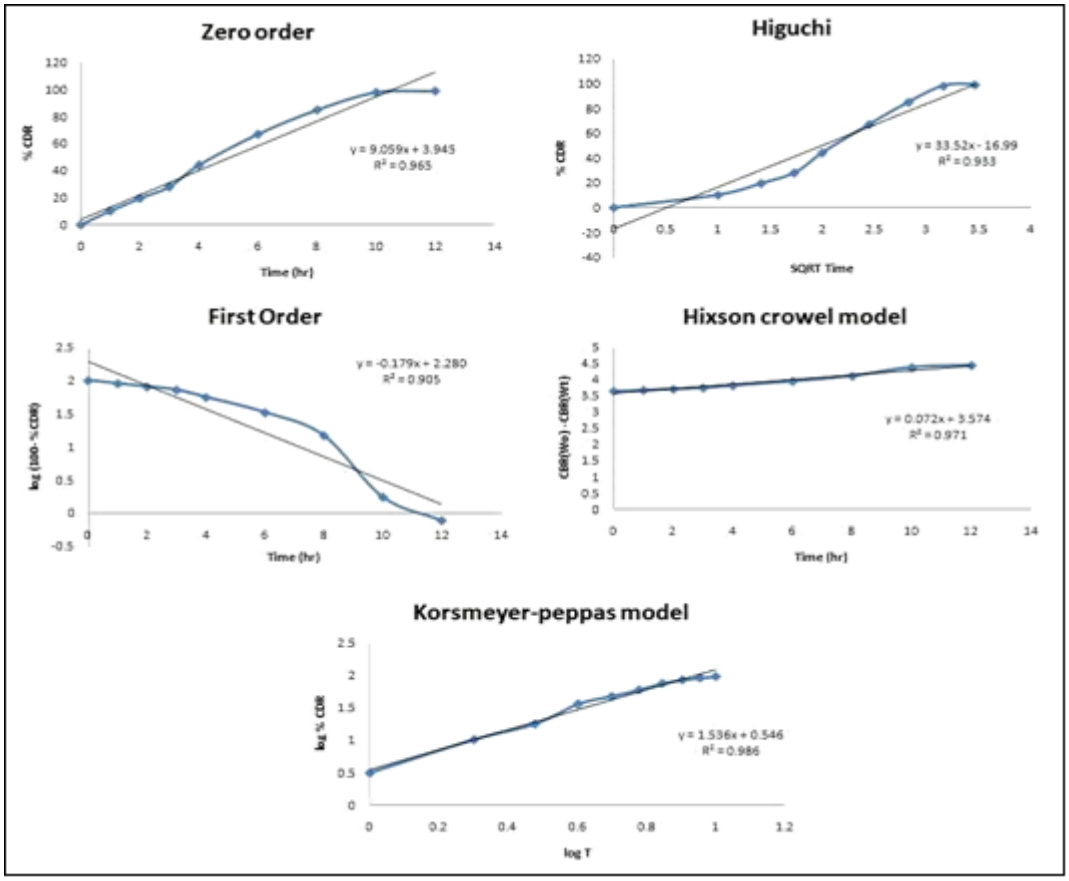

Figure 8: Kinetic models for Metformin hydrochloride release from bilayer tablet

***In vitro* dissolution comparison**"Model-independent factors, such as the similarity factor (f2), difference factor (f1), dissolution efficiency (DE%), and mean dissolution time (MDT) for the optimized bilayer tablet and the marketed formulation, are presented in Table 36. This comparison of dissolution profiles is illustrated in Figures 9 and 10 for the optimized bilayer tablet and the marketed tablet, respectively.

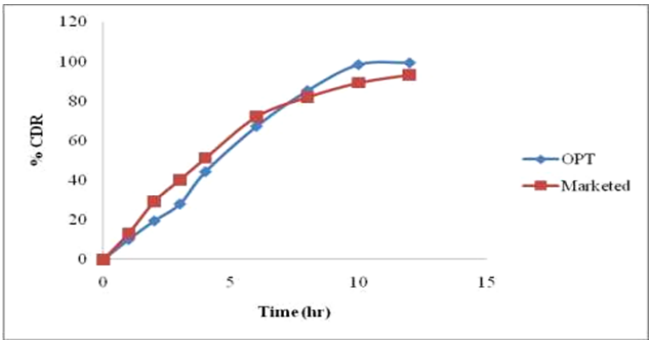

Figure 9: Comparison of dissolution profile of Metformin hydrochloride

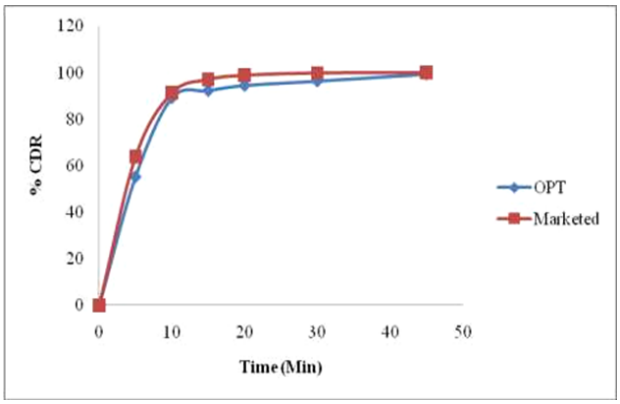

Figure 10: Comparison of dissolution profile of Saxagliptin

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When dissolution profiles were compared, it was discovered that f2 had a value greater than 50 and f1 had a value less than 15, indicating that the dissolution profiles of the produced bilayer tablet and the tablet that is currently on the market are comparable.

Table 10: Model independent parameters comparison for Optimized bilayer tablet (OBLT) and marketed formulation

Parameter	**Saxagliptin**		**Metformin hydrochloride**	
	OBLT	**Marketed**	**OBLT**	**Marketed**
DE (%)	85.44	88.88	60.42	61.67
MDT	6.3 Min	5.0 Min	4.69 hr	4.06 hr
f1	4.27		11.72	
f2	67.75		78.09	

Stability Study

Based on the results of rapid stability testing conducted in compliance with ICH criteria, the tablets exhibited no significant changes in hardness, weight variation, and friability. The assay results and content uniformity also remained within acceptable limits. Figures 11 and 12 illustrate that there are no significant differences in the cumulative release percentage compared to 0 months at 1, 3, and 6 months ($p > 0.05$). The stability of the optimized bilayer tablet is well demonstrated by the minimal changes observed in all the aforementioned characteristics.

Table 11: Physicochemical parameters of Optimized bilayer tablet (OBLT) at 0, 1, 3 and 6 months

Test		**0 month**	**1 month**	**3 months**	**6 months**
Description		Circular uncoated bilayer tablet	Circular uncoated bilayer tablet	Circular uncoated bilayer table	Circular uncoated bilayer tablet
Average Wt (mg)		1109.52±5.23	1109.23±6.81	1108.94±7.3	1108.98± 6.73
Hardness (kg/cm2)		6.2 ± 0.05	6.1 ± 0.07	6.2 ± 0.08	6.2 ± 0.02
Friability (%)		0.06	0.06	0.05	0.05
Assay	**Saxagliptin**	98.63 ± 2.4	98.02 ± 3.2	97.97 ± 2.8	97.84 ± 3.2
	MET HCl	97.23 ± 1.81	97.19 ± 1.86	97.06 ± 1.72	96.54 ± 2.06
CU (%RSD)	**Saxagliptin**	2.43	3.26	2.86	3.27
	MET HCl	1.86	1.91	1.77	2.13
Dissolution	**Saxagliptin**	NLT 80% at 30 minute	Complies	Complies	Complies
	MET HCl	Sustained release & NLT 85% at 10 hr	Complies	Complies	Complies

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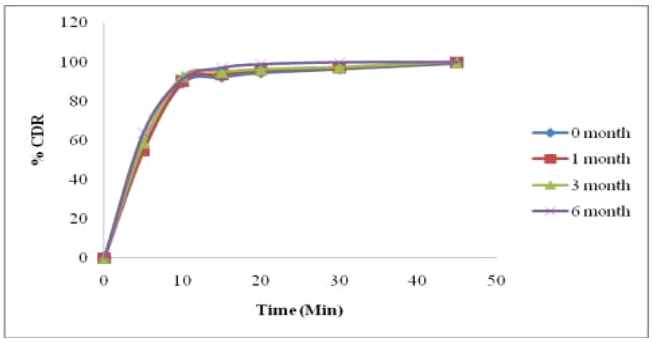

Figure 11: Dissolution profile of Saxagliptin from OBLT at 0, 1,3 and 6 months

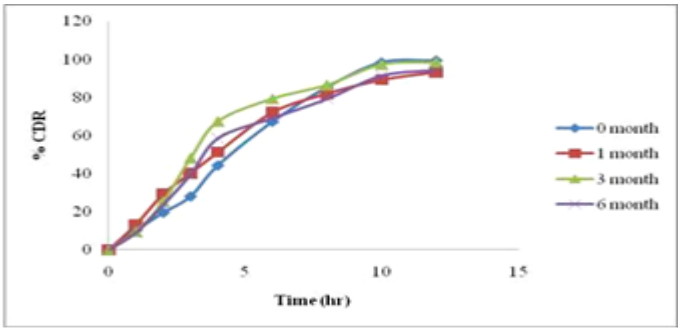

Figure 12: Dissolution profile of Metformin hydrochloride from OBLT at 0, 1,3 and 6 months

Conclusion

In this study, a bilayer tablet comprising an immediate-release (IR) Saxagliptin layer and a sustained-release (SR) Metformin hydrochloride layer was successfully developed using the Quality by Design (QbD) technique. Preformulation studies involved FTIR spectra analysis for Saxagliptin and Metformin hydrochloride, assessing melting temperatures, and evaluating physicochemical characteristics. Medicine-excipent compatibility was confirmed with no significant differences in FTIR spectra before and after 4 weeks of accelerated storage, indicating no interaction between excipients and model medicines. The creation of the bilayer tablet was divided into three stages. The immediate-release layer of Saxagliptin was optimized in the first step using risk assessment and factor screening studies. The second stage involved optimizing the sustained-release layer of Metformin hydrochloride. The final step included the preparation of bilayer tablets with both optimized layers, meeting set limits in pre and post compression parameters. Comparison of the optimized bilayer tablet's dissolution profile with a commercial formulation revealed their similarity. Stability studies over six months under accelerated conditions showed no discernible changes in physicochemical properties or dissolution profiles. In summary, this newly designed bilayer tablet effectively aids Type 2 Diabetes Mellitus (T2DM) patients in controlling blood sugar levels. Its once-daily dosage improves patient compliance, reducing both the financial burden and the quantity of pills needed compared to standard dosages.

Conflict of intereset

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