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Research Article

METHOD DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SELECTED ANTIDIABETIC DRUGS IN THE PRESENCE OF THEIR DEGRADATION PRODUCTS

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ABSTRACT

Objective: This study was designed to conduct forced degradation and validation studies for the simultaneous estimation of metformin, sitagliptin, pioglitazone, and glimepiride.

Methods: Analytes were separated on an Agilent XDB-C18, 150 × 4.6 mm, 5 µm column using an isocratic elution mode having mobile phase composition of 20 mM potassium dihydrogen phosphate buffer (pH 4.0):acetonitrile (65:35% v/v). Analytes were detected at a wavelength of 225 nm. The optimized method was validated as per the ICH Q2 guidelines.

Results: The retention times of metformin, sitagliptin, pioglitazone, and glimepiride were 3.47, 4.83, 5.83, and 9.44 min, respectively. The linearity was 25–100 µg/ml for metformin, 2.5–10 µg/ml for sitagliptin, 1–4 µg/ml for pioglitazone, and 0.75–3 µg/ml for glimepiride. The correlation coefficient for calibration curves was >0.99, and accuracy was between 98 and 102% for each analyte. Inter- and intra-day precisions were calculated <2% relative standard deviation for each analyte.

Conclusion: A significant degradation was observed in the presence of acidic, basic, neutral, oxidative, and photolytic stress conditions. The method is simple, precise, accurate, robust, and reproducible and was able to successfully separate and quantify metformin, sitagliptin, pioglitazone, and glimepiride in the presence of their degradation products.

Keywords: Metformin, Sitagliptin, Pioglitazone, Glimepiride, Stress testing, Degradation products, Stability-indicating method, Reversed-phase liquid chromatographic.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the most prevalent metabolic disease worldwide. Inadequate management and control of hyperglycemia in patients with T2DM may lead to the risk of developing complications over the long term due to chronic and progressive nature of the disease arising from pathophysiology of beta-cell dysfunction, insulin resistance, and increased hepatic glucose output. Patients with T2DM often require a combination of therapeutic agents to achieve glycemic control over the long term [1-6].

Fixed-dose combination (FDC) therapies have been shown to improve adherence by reducing costs, pill burden, and the complexity of treatment regimen [8-10]. A treatment approach with a FDC that includes a combination of antidiabetic medications could be used to obtain adequate glycemic control in patients with type 2 diabetes. Fig. 1a-d represents the structures of metformin, sitagliptin, pioglitazone, and glimepiride, respectively [14-17]. A combined formulation consisting of metformin, sitagliptin, and pioglitazone or glimepiride in a single tablet would potentially offer increased patient convenience and subsequent potential for increased therapeutic compliance and can be studied for the treatment of adults with inadequately controlled T2DM to improve glycemic control. A clinical trial was conducted for the evaluation of sitagliptin in combination with metformin and sulfonylurea [13]. The aim of that clinical trial protocol was to determine the non-inferiority of the effectiveness of sitagliptin compared to a control group of patients treated with thiazolidinedione as add-on therapy, in low-income ethnic minority type 2 diabetic patients who are failing to maintain adequate control with maximal doses of metformin and a sulfonylurea agent.

Advantages of simultaneous stability studies are the identification of new degradation products, to understand mutual induction and/or inhibition of rates of degradation and to analyze the degradation products of both drugs. As per the literature survey, all the reported ultraviolet (UV) spectroscopic and high-performance liquid chromatographic (HPLC) assay methods were based on the estimation of metformin, sitagliptin, pioglitazone, and glimepiride individually or in combination with other drugs [19-35]. The aim of the study was to develop a reversed-phase liquid chromatography method and validate the same according to the ICH guidelines [18]. The possible degradation products of metformin, sitagliptin, pioglitazone, and glimepiride were generated by stress degradation, and the developed method was used successfully to separate and identify the degradation products from the analyte. This method can be applied for the determination of stability of the APIs during pre-formulation and formulation studies for the development of FDC in pharmaceutical laboratories.

MATERIALS AND METHODS

Instrumentation

Analytes were scanned between 200 and 400 nm using UV-visible spectrophotometer (Shimadzu, model UV-1700). Experiments were carried out using Shimadzu prominence Modular HPLC system with LC 20AT solvent delivery unit, CBM 20A system controller, SIL 20A auto-sampler, CTO 20A column oven, and SPD 20 A UV detector. Samples were injected at the flow rate of 1.0 mL/min. The pH of the solutions was measured with the pH meter (Mettler Toledo, S20K). Refluxing of the drugs in specific degradation conditions was carried out using a Rotavapor (R-300, Buchi). A Shimadzu ATX-124 analytical balance was used for weighing.

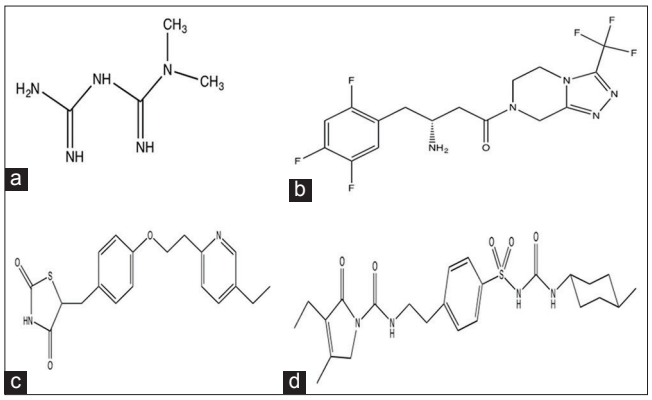

Fig. 1: (a) Metformin, (b) sitagliptin, (c) pioglitazone, (d) glimepiride

Reagents and chemicals

The metformin, sitagliptin, pioglitazone, and glimepiride reference materials were purchased from Mesochem Technology Inc., Beijing, China. Methanol and water were used of HPLC grade and purchased from Fisher Scientific, India. Potassium dihydrogen phosphate buffer was purchased from Sigma-Aldrich Company, India.

Selection of wavelength

Standard solution of metformin, sitagliptin, pioglitazone, and glimepiride, 10 µg/mL each, was scanned between 200 and 400 nm using a UV-visible spectrophotometer. Wavelength was selected from the overlay spectra of the above solutions.

Chromatographic separation

Analytes were separated on Agilent XDB-C18, 150 mm × 4.6 mm, 5 µm column using an isocratic elution mode having mobile phase composition of 20 mM potassium dihydrogen phosphate buffer (pH 4.0):acetonitrile (65:35% v/v). The detection was carried out at the wavelength of 225 nm. 20-µL fixed-loop was used for the injection of the samples with the flow rate of 1.0 mL/min.

Preparation of standard solutions

50 mg of metformin, 4 mg of sitagliptin, 2 mg of pioglitazone, and 1.5 mg of glimepiride were separately weighed and dissolved in methanol to obtain 500 µg/mL of metformin, 40 µg/mL sitagliptin, 20 µg/mL pioglitazone, and 15 µg/mL of glimepiride individual standard stock solutions. Working standard solution of mixture of metformin (50 µg/mL), sitagliptin (4 µg/mL), pioglitazone (2 µg/mL), and glimepiride (1.5 µg/mL) was prepared from the stock solution.

Method validation

System suitability test [18]

System suitability test is an integral part of the chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Linearity

The linearity was assessed by the analysis of combined standard solution in a range of 25–100 µg/ml for metformin, 2.5–10 µg/ml sitagliptin, 1–4 µg/ml pioglitazone, and 0.75–3 µg/ml glimepiride.

Precision

Results for inter-and intra-day precision were expressed as percentage relative standard deviation (%RSD) or coefficient of variance.

Repeatability

A standard solution containing 50 µg/ml of metformin, 5 µg/ml of sitagliptin, 2 µg/ml of pioglitazone, and1.5 µg/ml of glimepiride was injected 6 times, areas of peaks were measured, and %RSD was calculated to determine the repeatability of the method.

Intra- and inter-day precision

A standard solution containing 20, 50, and 75 µg/ml of metformin; 2.5, 5, and 7.5 µg/ml of sitagliptin; 1, 2, and 3 µg/ml of pioglitazone, and 0.75, 1.5, and 2.25 µg/ml of glimepiride was analyzed 6 times on the same day for the determination of intraday precision and on 3 different days for the determination of interday precision, and %RSD was calculated.

Accuracy

Accuracy was calculated at three different levels in terms of percentage recovery by spiking known amount of standard solution (80%, 100%, and 120%) to the solution of a synthetic laboratory mixture of metformin, sitagliptin, pioglitazone, and glimepiride.

Specificity and selectivity

The specificity of the method was established through the study of resolution factors of the drug peak from the nearest resolving peak and also among all other peaks.

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

The LOD and LOQ were estimated at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

Robustness

Robustness of the method was investigated by varying the chromatographic conditions, such as changing the flow rate by ±10%, i.e., 0.8 ml/min and 1.2 ml/min; changing the ratio of mobile phase was with ±2, i.e. 20 mM potassium dihydrogen phosphate buffer (pH 4.0):acetonitrile (63:37% v/v and 67:33% v/v); and changing the pH of the buffer in the mobile phase with ±0.2%, i.e., 3.7 and 3.3. Robustness of the developed method was indicated by the overall %RSD between the data, at each variable condition.

Analysis of synthetic laboratory mixture

Synthetic laboratory mixture of 50 mg of metformin, 4 mg of sitagliptin, 2 mg of pioglitazone, and 1.5 mg of glimepiride was weighed individually and spiked with 1 mg hydroxypropyl cellulose (E463) and 1 mg microcrystalline cellulose (E460(i)) as the excipients into a 100 ml volumetric flask. The analytes were extracted with 5 ml methanol by sonication in the ultrasonicator bath, and then, the solution was filtered through Whatman filter Paper No. 42. The final concentration of a mixture of 50 µg/ml of metformin, 5 µg/ml of sitagliptin, 2 µg/ml of pioglitazone, and 1.5 µg/ml of glimepiride was made with mobile phase. Samples were analyzed using the developed assay. The areas of resulting peaks were measured at 225 nm.

Stress degradation studies

Acid hydrolysis

Forced degradation in acidic condition was performed by adding 1 ml of standard solutions of metformin (500 µg/ml), sitagliptin (40 µg/ml), pioglitazone (20 µg/ml), and glimepiride (15 µg/ml) each individually to 6 ml methanol:water (1:1). To start the reaction, pH 3.0 was adjusted using 0.1 M hydrochloric acid. The mixture was incubated at 45°C for 2 h. The solution was then allowed to reach at room temperature, neutralized to pH 7.0 by the addition of 0.1 M sodium hydroxide, and diluted to 10 ml with the mobile phase so as to get a final concentration of 50 µg/ml for metformin,

4 µg/ml for sitagliptin, 2 µg/ml for pioglitazone, and 1.5 µg/ml for glimepiride.

Alkaline hydrolysis

Alkali-induced, forced degradation was performed by adding 1 ml of standard solutions of metformin (500 µg/ml), sitagliptin (40 µg/ml), pioglitazone (20 µg/ml), and glimepiride (15 µg/ml) each, respectively, to 6 ml methanol:water (1:1). Adjusting the pH 12.0 using 0.1 M sodium hydroxide started alkali hydrolysis. The mixture was incubated at 45°C for 2 h (n=3). The solution was then allowed to reach at room temperature, neutralized to pH 7.0 by the addition of 0.1 M hydrochloric acid, and diluted to 10 ml with the mobile phase to get a final concentration of 50 µg/ml for metformin, 4 µg/ml for sitagliptin, 2 µg/ml for pioglitazone, and 1.5 µg/ml for glimepiride.

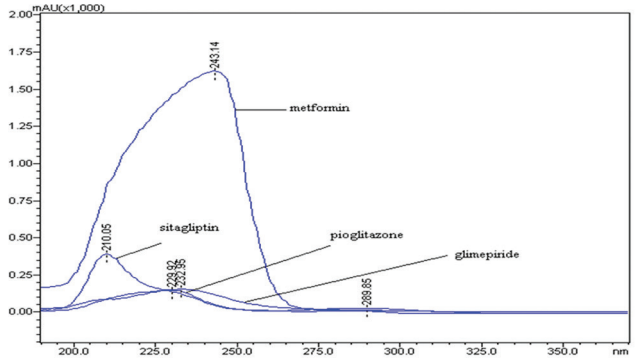

Fig. 2: Overlay ultraviolet spectrum of metformin, sitagliptin, pioglitazone, and glimepiride showing selection of wavelength detection

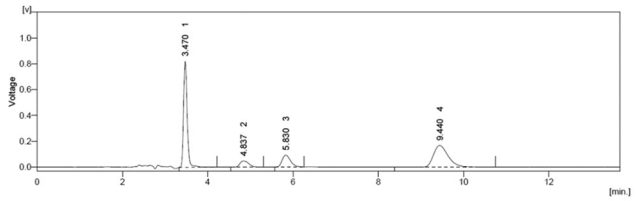

Fig. 3: Chromatogram of metformin (1), sitagliptin (2), pioglitazone (3), and glimepiride (4) in 20 mM potassium dihydrogen phosphate buffer (pH 4.0): acetonitrile (65:35 %v/v) with flow rate - 1.0 ml/min

Oxidative degradation

To evaluate the effect of oxidizing conditions, 1 ml of the standard solutions of metformin (500 µg/ml), sitagliptin (40 µg/ml), pioglitazone (20 µg/ml), and glimepiride (15 µg/ml) each separately was added to 2 ml of 3% hydrogen peroxide solution, and the mixture was refluxed at 45°C for 2 h. The solution was then allowed to reach room temperature and diluted to 10 ml with the mobile phase to get a final concentration of 50 µg/ml for metformin, 4 µg/ml for sitagliptin, 2 µg/ml for pioglitazone, and 1.5 µg/ml for glimepiride each individually.

Thermal degradation

To evaluate the effect of temperature, 1 ml of a standard solution of metformin (500 µg/ml), sitagliptin (40 µg/ml), pioglitazone (20 µg/ml), and glimepiride (15 µg/ml) was each incubated at 60°C for 2 h. The solutions were then allowed to reach room temperature and diluted to 10 ml with the mobile phase to get a final concentration of 50 µg/ml for metformin, 4 µg/ml for sitagliptin, 2 µg/ml for pioglitazone, and 1.5 µg/ml for glimepiride.

Photolytic degradation

To study the effect of UV light, 1 ml of each of the standard solutions of metformin (500 µg/ml), sitagliptin (40 µg/ml), pioglitazone (20 µg/ml), and glimepiride (1.5 µg/ml) was exposed to short and long wavelength UV light (254 nm and 366 nm, respectively) for 4 h. The volume was made up by the mobile phase to get final concentration equivalent to 50 µg/ml of metformin, 4 µg/ml of sitagliptin, 2 µg/ml pioglitazone, and 1.5 µg/ml of glimepiride.

Synthetic laboratory mixture was also treated with described acidic, alkaline, oxidative, thermal, and photolytic degradation conditions. 20 µL of the resulting solutions were injected into the HPLC system, and the chromatograms were recorded.

RESULTS AND DISCUSSION

Method development

As metformin, sitagliptin, pioglitazone, and glimepiride showed absorbance response at a wavelength of 225 nm, it was selected as a wavelength of detection. Fig. 2 represents the overlay UV spectrum.

Analytes were separated on Agilent XDB-C18, 150 × 4.6 mm, 5µm column using an isocratic elution mode having mobile phase composition of 20 mM potassium dihydrogen phosphate buffer (pH 4.0):acetonitrile (65:35% v/v). Analytes were detected at 225 nm. 20 µL fixed-loop injector was used for the injection of the samples with the flow rate of 1.0 mL/min. Retention times were 3.47 min, 4.83, 5.83, and 9.44 min for metformin, sitagliptin, pioglitazone, and glimepiride, respectively, as shown in Fig. 3.

Table 1: System suitability parameters for metformin, sitagliptin, pioglitazone, and glimepiride

System suitability parameters	Metformin	Sitagliptin	Pioglitazone	Glimepiride
Theoretical plates per column (N)	7657	4137	3672	2449
Symmetry factor/tailing factor	1.31	1.51	1.57	1.65
Resolution	4.97	2.63		7.32

Table 2: Results from regression analysis for metformin, sitagliptin, pioglitazone, and glimepiride

Description	Metformin	Sitagliptin	Pioglitazone	Glimepiride
Linearity and range	25–100 µg/ml	2–10 µg/ml	1–4 µg/ml	0.75–3 µg/ml
Regression coefficient	0.999	0.999	0.999	0.999
Slope (m)	93.23	264.67	330.61	2365.7
Intercept (c)	−80.72	−33.91	−2.34	−85.41

Method validation

The method was validated as per the ICH guidelines [17] with respect to parameters defining linearity, precision, accuracy, specificity, and robustness.

The number of theoretical plates, peak tailing, and resolution factor was determined to define system suitability parameters for metformin, sitagliptin, pioglitazone, and glimepiride. The results for system suitability data are listed in Table 1.

Linearity and range were assessed by the analysis of combined standard solution with the range of 25–100 µg/ml for metformin, 2.5–10 µg/ml sitagliptin, 1–4 µg/ml pioglitazone, and 0.75–3 µg/ml glimepiride. Standard calibration curve for metformin, sitagliptin, pioglitazone, and glimepiride is represented in Figs. 4-7, respectively. The data for regression analysis are listed in Table 2. A standard solution containing 50 µg/ml of metformin, 4 µg/ml of sitagliptin, 2 µg/ml of pioglitazone, and 1.5 µg/ml of glimepiride, respectively, was injected 6 times, and areas of peaks were measured to determine the repeatability of the method. %RSD. value for the determination of repeatability is represented in Table 3.

A standard solution containing 25, 50, and 75 µg/ml of metformin; 2.5, 5, and 7.5 µg/ml of sitagliptin; 1, 2, and 3 µg/ml of pioglitazone; and 0.75, 1.5, and 2.25 µg/ml of glimepiride was analyzed 3 times on the same day for the determination of intraday precision and on 3 different days for the determination of interday precision. %RSD values for intra- and inter-day precision are represented in Table 4. The accuracy of the method was confirmed by recovery study from the synthetic laboratory mixture at three levels of standard addition. The results are shown in Tables 5-8 for metformin, sitagliptin, pioglitazone, and glimepiride, respectively. Percentage recovery was in the range of 99.4–100.994% for metformin, 99.2–100.9 sitagliptin, and 99.3–100.4% for glimepiride. LOD was 2.21 µg/ml, 0.34 µg/ml, 0.10 µg/ml, and 0.12 µg/ml for metformin, sitagliptin, pioglitazone, and glimepiride, respectively, whereas LOQ was 6.7 µg/ml, 1.04 µg/ml, 0.31 µg/ml, and 0.37 µg/ml for metformin, sitagliptin, pioglitazone, and glimepiride, respectively. The method was robust with %RSD values <2% with the deliberate changes in the composition of mobile phase, changes in the pH, or change in the flow rate. Applicability of the proposed method was evaluated by analyzing a synthetic laboratory mixture, and the results are shown in Table 9. The assay results were >99.0% for metformin, sitagliptin, pioglitazone, and glimepiride each, respectively, in synthetic laboratory mixture.

Establishment of stability indicating a method for the assessment of degradation behavior

The stressed samples were assayed using developed reversed-phase HPLC (RP-HPLC) method. Following degradation, behavior was observed under different stress conditions for the HPLC studies on the combination of metformin, sitagliptin, pioglitazone, and glimepiride (Tables 10-13).

Significant degradation was observed in the presence of acidic, basic, neutral oxidative, and photolytic stress conditions for metformin, sitagliptin, pioglitazone, and glimepiride, respectively (n=5). Percentage degradation for the standard drug was 14%, 13%, 12%, 13%, and 13% for metformin; 14%, 12%, 12%, 12%, and 13% sitagliptin; 17%, 27%, 14%, 18%, and 19% for pioglitazone; and 18%, 20%, 12%, 18%, and 18% for glimepiride in the presence of acidic, basic, thermal, oxidative, and photolytic degradation, respectively. Percentage degradation in synthetic laboratory mixture was 16%, 13%, 12%, 11%, and 15% for the metformin; 17%, 13%, 12%, 13%, and 14% for sitagliptin; 17%, 27%, 19%, 17%, and 21% for pioglitazone; and 16%, 15%, 15%, 13%, and 14% for glimepiride in the presence of acidic, basic, thermal, oxidative, and photolytic degradation, respectively. The percentage degradation was calculated by the formula: % Degradation=(Average peak area of untreated stock solution - average peak area of stock solution under specific degradation condition)/(average peak area of untreated stock solution) ×100).

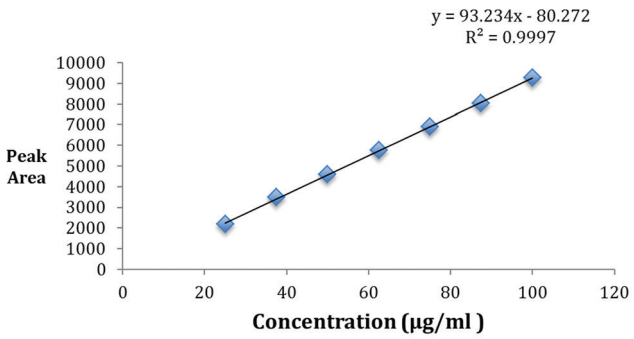

Fig. 4: Standard calibration curve of metformin (25–100 µg/ml)

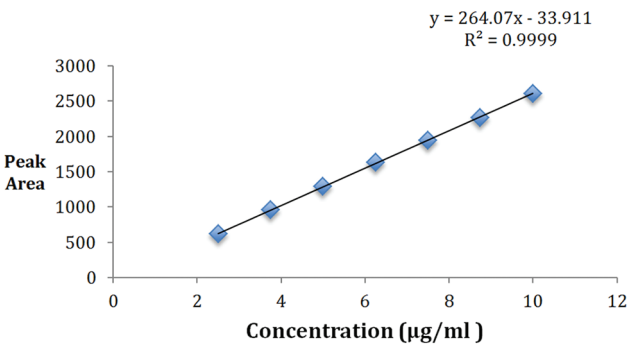

Fig. 5: Standard calibration curve of sitagliptin (2–10 µg/ml)

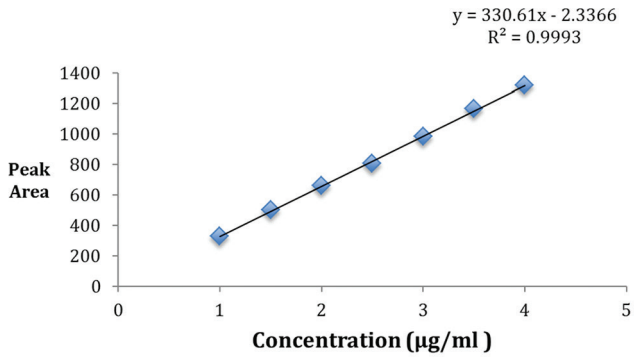

Fig. 6: Standard calibration curve of pioglitazone (1–4 µg/ml)

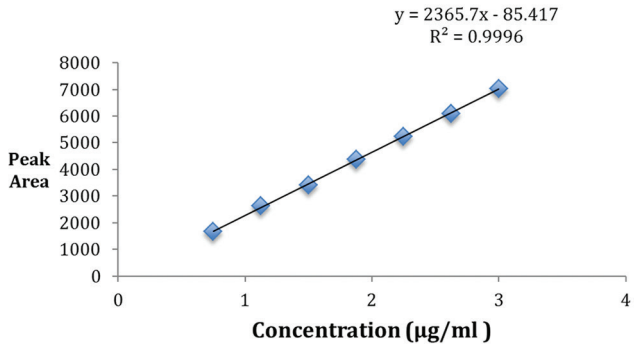

Fig. 7: Standard calibration curve of glimepiride (0.75–3 µg/ml)

Table 3: Repeatability data for metformin, sitagliptin, pioglitazone, and glimepiride

Metformin			Sitagliptin			Pioglitazone			Glimepiride		
Concentration (µg/ml)	Peak area	%RSD	Concentration (µg/ml)	Peak area	%RSD	Concentration (µg/ml)	Peak area	%RSD	Concentration (µg/ml)	Peak area	%RSD
10	4965.27	1.05	5	1229.64	0.62	2	651.80	0.62	1.5	3907.47	0.91
	4888.48			1228.36			651.13			3869.07	
	4979.95			1233.26			653.71			3918.92	
	5035.73			1246.84			660.90			3962.10	
	4984.42			1234.37			654.32			3893.33	
	5024.40			1244.22			659.54			3953.97	

%RSD: Percentage relative standard deviation

Table 4: Intra- and inter-day precision for metformin, sitagliptin, pioglitazone, and glimepiride

Metformin		Sitagliptin		Pioglitazone		Glimepiride	
Concentration (µg/ml)	Mean±SD (n=6)	Concentration (µg/ml)	Mean±SD (n=6)	Concentration (µg/ml)	Mean±SD (n=6)	Concentration (µg/ml)	Mean±S.D (n=6)
Intraday precision							
25	2472.31±7.57	2.5	622.99±1.92	1	327.81±3.92	0.75	1970.41±16.21
50	4971.43±37.39	5	1231.71±8.56	2	656.60±3.71	1.5	3936.09±22.23
75	7545.32±47.21	7.5	1852.50±32.91	3	989.04±3.77	2.25	5930.89±35.75
Interday precision							
25	2478.76±22.67	2.5	622.97±8.52	1	330.41±3.02	0.75	1971.97±26.27
50	4925.49±52.36	5	1218.69±14.60	2	645.12±9.24	1.5	3859.72±68.98
75	7444.71±132.51	7.5	1846.43±28.15	3	981.28±10.88	2.25	5853.19±96.37

SD: Standard deviation

Table 5: Accuracy in terms of percentage recovery for metformin

Concentration Level (%)	Sample amount (µg/ml)	Amount of standard added (µg/ml)	Metformin		
			Amount recovered (µg/ml)	% recovery	% Mean recovery±SD
80%	25	20	20.24	101.21	100.70±0.93
	25	20	19.93	99.62	
	25	20	20.26	101.29	
100%	25	25	25.19	100.74	99.67±0.92
	25	25	24.78	99.12	
	25	25	24.79	99.17	
120%	25	30	29.57	98.57	98.84±0.66
	25	30	29.88	99.60	
	25	30	29.51	98.36	

SD: Standard deviation

Table 6: Accuracy in terms of % recovery for sitagliptin

Concentration Level (%)	Sample amount (µg/ml)	Amount of standard added (µg/ml)	Sitagliptin		
			Amount recovered (µg/ml)	% recovery	% Mean recovery±SD
80%	2.5	2	2.02	101.21	100.06±1.15
	2.5	2	2.00	100.07	
	2.5	2	1.98	98.91	
100%	2.5	2.5	2.52	100.70	100.29±1.57
	2.5	2.5	2.46	98.48	
	2.5	2.5	2.54	101.51	
120%	2.5	3	2.99	99.81	99.24±0.95
	2.5	3	2.94	98.14	
	2.5	3	2.99	99.78	

SD: Standard deviation

Table 7: Accuracy in terms of % recovery for pioglitazone

Concentration Level (%)	Sample amount (µg/ml)	Amount of standard added (µg/ml)	Pioglitazone		
			Amount recovered (µg/ml)	% Recovery	% Mean recovery±SD
80%	1	0.8	0.79	99.08	99.41±0.63
	1	0.8	0.80	100.14	
	1	0.8	0.79	99.00	
100%	1	1	1.01	100.81	101.14±0.42
	1	1	1.01	101.01	
	1	1	1.02	101.61	
120%	1	1.2	1.20	99.90	98.51±1.26
	1	1.2	1.18	98.23	
	1	1.2	1.17	97.43	

SD: Standard deviation

Table 8: Accuracy in terms of % recovery for glimepiride

Concentration Level (%)	Sample amount (µg/ml)	Amount of standard added (µg/ml)	Glimepiride		
			Amount recovered (µg/ml)	% recovery	% Mean recovery±SD
80%	0.75	0.6	0.60	100.26	99.82±0.69
	0.75	0.6	0.60	100.17	
	0.75	0.6	0.59	99.02	
100%	0.75	0.75	0.76	100.80	100.67±1.01
	0.75	0.75	0.75	99.61	
	0.75	0.75	0.76	101.62	
120%	0.75	0.9	0.90	99.90	98.51±1.2
	0.75	0.9	0.88	98.22	
	0.75	0.9	0.88	97.41	

SD: Standard deviation

CONCLUSION

The overall demand for the development of FDC for antidiabetic drugs has been growing in the pharmaceutical market. This increases the need for the development of cost effective and high throughput assays. Proposed reversed-phase HPLC method was able to successfully separate and quantify metformin, sitagliptin, pioglitazone, and glimepiride simultaneously in the presence of their

Table 9: Analysis of synthetic mixture by developed method

Synthetic laboratory mixture	Analyte			
Weight (mg) of synthetic laboratory mixture	**Metformin (500 mg)**	**Sitagliptin (50 mg)**	**Pioglitazone (20mg)**	**Glimepiride(15 mg)**
Assay mean±SD (n=6)	99.61±0.45	99.24±0.97	99.53±0.69	100.82±0.93

SD: Standard deviation

Table 10: Percentage degradation of metformin with retention time of the degradation products

S. No.	Conditions	Retention time of metformin/ degradation products (min)	% Degradation of metformin (n=5)	% Degradation of metformin in synthetic mixture (n=5)
1	Untreated stock solution (10 µg/ml)	3.78	-	-
2	Acid hydrolysis	4.51, 7.96	14.11	16.93
3	Alkali hydrolysis	2.53, 3.35, 5.12	13.63	13.02
4	Oxidative degradation	3.03, 5.35	12.36	12.09
5	Thermal degradation	2.64, 4.66	13.82	11.49
6	Photolytic degradation	2.44, 4.31	13.57	15.42

Table 11: Percentage degradation of sitagliptin with retention time of the degradation products

S. No.	Conditions	Retention time of sitagliptin/ degradation products (min)	% Degradation of sitagliptin (n=5)	% Degradation of sitagliptin in synthetic mixture (n=5)
1	Untreated stock solution (10µg/ml)	5.06	-	-
2	Acid hydrolysis	4.12,7.29	14.31	17.08
3	Alkali hydrolysis	2.83, 5.00	12.22	13.08
4	Oxidative degradation	2.90, 5.14	12.73	12.39
5	Thermal degradation	2.90, 5.12	12.55	13.59
6	Photolytic degradation	2.82, 4.97	13.86	14.03

Table 12: Percentage degradation of pioglitazone with retention time of the degradation products

S. No.	Conditions	Retention time of pioglitazone/ degradation products (min)	% Degradation of simvastatin (n=5)	% Degradation of pioglitazone in synthetic mixture (n=5)
1	Untreated stock solution (10 µg/ml)	8.57	-	-
2	Acid hydrolysis	4.85, 7.01	17.35	17.75
3	Alkali hydrolysis	4.86, 7.54	27.45	27.38
4	Oxidative degradation	4.87, 8.52	14.27	19.65
5	Thermal degradation	4.33, 4.84	18.30	21.77
6	Photolytic degradation	4.84, 5.41	19.56	19.17

Table 13: Percentage degradation of glimepiride with retention time of the degradation products

S. No.	Conditions	Retention time of glimepiride/ degradation products (min)	% Degradation of simvastatin (n=5)	% Degradation of glimepiride in synthetic mixture (n=5)
1	Untreated stock solution (10 µg/ml)	8.57	-	-
2	Acid hydrolysis	10.43	15.27	15.92
3	Alkali hydrolysis	7.64	15.60	15.52
4	Oxidative degradation	9.64, 11,66	12.50	15.54
5	Thermal degradation	10.43, 11.66	18.43	18.40
6	Photolytic degradation	9.99	18.92	19.07

degradation products. This implies the stability indicating nature and specificity of the method. The developed validated stability indicating RP-HPLC method is simple, precise, accurate, robust, and reproducible resolving all the degradation products from the analytes of interest. The method validated as per the ICH guidelines and can be successfully used for the quantitative determination of metformin, sitagliptin, pioglitazone, and glimepiride and the stability of the analytes during the pre-formulation studies in pharmaceutical laboratories.

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AUTHORS' CONTRIBUTIONS

Nidhi Kotecha has developed, validated, and performed the computations. Dr. Jayvadan Patel supervised and encouraged to investigate the findings of this work. Both the authors discussed the results and contributed to the final manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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