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A Validated RP-HPLC Method for Simultaneous Estimation of Antidiabetic Drugs Pioglitazone HCl and Glimepiride

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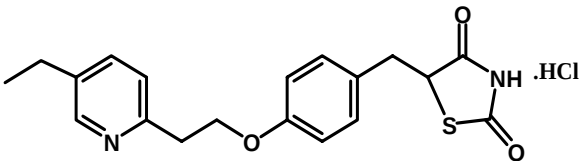
Abstract

A simple, fast and economic reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous and quantitative analyses of pioglitazone HCl and glimepiride in pharmaceutical dosage forms. The method was developed using the mobile phase comprising of potassium dihydrogen phosphate buffer (KH_2PO_4) at pH 3.4 and acetonitrile in the ratio of 40:60 (v/v) over C-18 bonded silica column (250 x 4.6 mm, 5 um, Phenomenex Inc.) at ambient temperature. The flow rate was at 0.8 min/min and the eluent was monitored by UV detection at 235 nm. The recoveries were found to be >97% for pioglitazone and >99% for glimepiride, demonstrative of accuracy of the protocol. Inter-day and intra-day precision of the new method were less than the maximum allowable limit (RSD% ≤ 2.0) according to ICH, USP and FDA guidelines. The method showed linear response with correlation coefficient (r^2) values of 0.9991 for pioglitazone and 0.9999 for glimepiride. Therefore, the method was found to be accurate, reproducible, sensitive and less time consuming and can be successfully applied for the assay of pioglitazone and glimepiride in combined formulations.

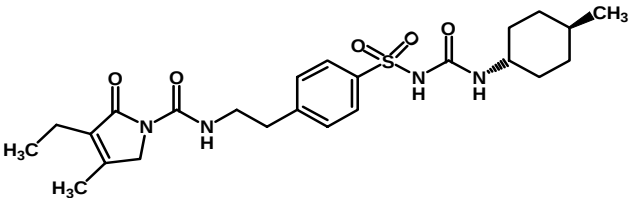
Key words: Antidiabetic, pioglitazone, glimepiride, reversed phase, HPLC, method validation

Introduction

Pioglitazone is an oral anti-hyperglycemic drug of the thiazolidinedione class. Chemically it is (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione (Figure 1A). It acts by decreasing insulin resistance. It is used in the treatment of type-II diabetes mellitus (Gillies and Dunn, 2000; Smith, 2001; Belfort *et al.*, 2006; DeFronzo *et al.*, 2011). Glimepiride is also an oral antihperglycemic drug belongs to sulfonylurea group. It is 3-ethyl-4-methyl-*N*-(4-[*N*-((1*r*,4*r*)-4-methylcyclohexy-lcarbamoyl) sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide (Figure 1B) and is effective at low doses in patients with non-insulin-dependent diabetes mellitus (Langtry and Balfour, 1998; Rosenkranz *et al.*, 1996; Müller *et al.*, 1995).



A



B

Figure 1. Structures of pioglitazone hydrochloride (**A**) and glimepride (**B**).

Drug requires some absolute necessities like quality, potency etc. to exhibit its best activities. It is obvious that a little change in the formulation or variations in the manufacturing process or use of low quality materials can affect the product stability and efficacy. Use of low quality or adulterated drug introduces more toxins in the body. It can be harmful to the patient to a high risk level. Quality and efficacy assessments and maintenance of proper dosage schedule are strongly emphasized to ensure their safety and efficacy. Therefore, these drugs required a

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simple, rapid and accurate method for simultaneous and routine analysis.

The literature survey reveals several analytical methods for quantitative estimation of pioglitazone hydrochloride and glimepride in pharmaceutical formulations and in body fluids. These methods include high performance liquid chromatography [HPLC] for pioglitazone hydrochloride, glimepride and for both in other combinations (Sane *et al.*, 2004; Mamdouh *et al.*, 2012; Kalyankar *et al.*, 2010; Ramesh *et al.*, 2010; Lakshmi *et al.*, 2009; Karthik *et al.*, 2008; Navaneethan *et al.*, 2011). The present work was aimed to develop a simple, rapid and accurate RP-HPLC method for the simultaneous quantification of pioglitazone hydrochloride and glimepride in bulk form or in their pharmaceutical formulations and to validate the method according to ICH and FDA guidelines with respect to the parameters of accuracy, precision, linearity and specificity (Sultan *et al.*, 2012; Sultan, *et al.*, 2011(a, b); FDA, 2012; ICH (Q2A and Q2B); USP, 2009).

Materials and Methods

Drugs and Materials: Working standard of pioglitazone HCl (potency 99.95%) and glimepiride (potency 99.99%) were kind gift of Drug International Ltd., Dhaka, Bangladesh. For the estimation of pioglitazone and glimepiride formulated as tablets, samples produced by renowned pharmaceutical industries of Bangladesh were collected from the market. HPLC grade acetonitrile and methanol were procured from Active Fine Chemicals Ltd., Dhaka Bangladesh.

Instrumentation

HPLC system: High Performance Liquid Chromatography system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data was recorded with LC-solutions software.

Column: An analytical reversed phase C-18 (ODS) column [(4.6 mm x 250 mm; 5 µm), Phenomenex, Inc] was used to analyze the samples.

Preparation of mobile phase: To prepare buffer solution of pH 3.4, sodium dihydrogen phosphate (NaH_2PO_4) (195.5 mg) was taken in a 1000 mL volumetric flask. About 500 ml of double distilled water was added into the flask, dissolved the salt and finally water was added up to the mark. Then pH was adjusted to 3.4 by adding dilute phosphoric acid, sonicated for 10 minutes and then filtered through a 0.22 µm millipore filter. HPLC grade actonitrile was also filtered and degassed before use into the HPLC system.

Preparation of standard solution: Standard solution of the pure drug was prepared by dissolving 49.062 mg of glimepiride (MW of glimepiride = 490.617, and potency = 99.99%) powder equivalent to 100 µmole glimepiride in a 100 ml volumetric flask using mixture of methanol/acetronitrile (50:50) and 39.290 mg of poiglitazone HCl (MW of poiglitazone HCl = 392.90, and potency = 99.95%) powder equivalent to 100 µmole poiglitazone in100 ml volumetric flask using methanol as mobile phase. The final concentrations of both solutions were obtained 1 µmole/ml. Appropriate from these solution were further diluted to get standards of varying concentrations (0.5, 0.25, 0.125, 0.0625 and 0.03125 µmole/mL).

Preparation of test samples: Ten tablets were weighed, made into fine powder in a mortar with pestle and average weight was taken. Accurately weighed powder equivalent to average weight of each tablet (5 µmole of pioglitazone and 0.5339 µmole of glimepiride) were taken in a 50 ml volumetric flask and 10 ml of HPLC-grade methanol/acetronitrile (50:50) mixture was added and sonicated to mix uniformly. The final volume was adjusted with mobile phase to get the concentration of 0.1 µmole/ml for pioglitazone and 0.107µmole/ml for glimepiride. Then both solutions were further diluted to get the concentrations of 0.05µmole/ml for pioglitazone and 0.00535µmole/ml for glimepiride, which were further diluted to get the concentrations of 0.025µmole/mL for pioglitazone and 0.00265µmole/ml for glimepiride.

Chromatographic conditions: All analyses were done at ambient temperature under isocratic conditions. The mobile phase contained potasium dihydrogen phosphate buffer (KH_2PO_4) at pH 3.4 and acetonitrile in the ratio of 40:60 (v/v) at the flow rate 0.8 ml/min. The injection volume was kept at 20 µL for all analyses. Before analysis, every standard and sample was filtered through 0.45 µm filter tips. The column eluate was monitored at 235 nm.

Method Validation

Specificity: The specificity of the HPLC method was evaluated to ensure that there was no interference from the excipients present in the pharmaceutical formulation. The specificity was studied by injecting the excipients, standard solution and pharmaceutical preparation of pioglitazone and glimepiride.

Accuracy: The accuracy of an analytical method expresses the nearest between the expected value and the value obtained. It is expressed by calculating the percent recovery (R %) of analyte recovered by assay of spiked samples. In this case, aliquot equivalent to 0.0125, 0.02 and 0.05 µmole/mL of standard solutions were analyzed.

Precision: Precision of the assay was investigated with respect to both repeatability and reproducibility. The precision of an analytical method is the degree of agreement among individuals test result where the method is applied repeatedly to multiple samplings. It was checked by intra- and inter-day repeatability of responses after replicate injections and expressed as RSD%. The calculation formula for RSD% = (Standard deviation of recovered conc. /Mean recovered conc.) x 100%. In the current method development and validation process, precision was determined by three replicate analyses of each of three concentrations levels of 0.0125, 0.025 and 0.05 µmole/mL of standard solutions using the proposed method.

Linearity: Five different concentration levels 0.5, 0.25, 0.125, 0.0625 and 0.03125 µmole/ml were prepared from standard solution of pioglitazone and glimepiride. Then 20 µl of each solution was injected thrice times into the HPLC using auto-sampler and the analyses were monitored at 235nm. The average peak areas were plotted against concentrations. The linearity of the proposed method was determined by using calibration curves to calculate coefficient of correlation, slope and intercept values by using the following equation, $y = mx + c$; where, y is the peak area, x is the concentration of drug, m is the slope and c is the intercept.

Robustness: The robustness of the method was studied by minor but deliberate changes in the method like mobile phase composition, pH, buffer, flow rate, detection wavelength, etc.

Results and Discussion

The reversed phase HPLC method has been developed and validated as per ICH, USP and FDA guide-lines for determination of pioglitazone HCL and glimepiride in pharmaceutical formulations by using the mobile phase comprising potasium dihydrogen phosphate buffer and acetonitrile in the ratio of 40: 60 (v/v) at ambient temperature at a flow rate of 0.8 ml/min with UV detection at 235nm. The retention time of pioglitazone and glimepiride was obtained at 4.5 ± 0.1 min and 10.0 ± 0.1 min, respectively (Figure 2).

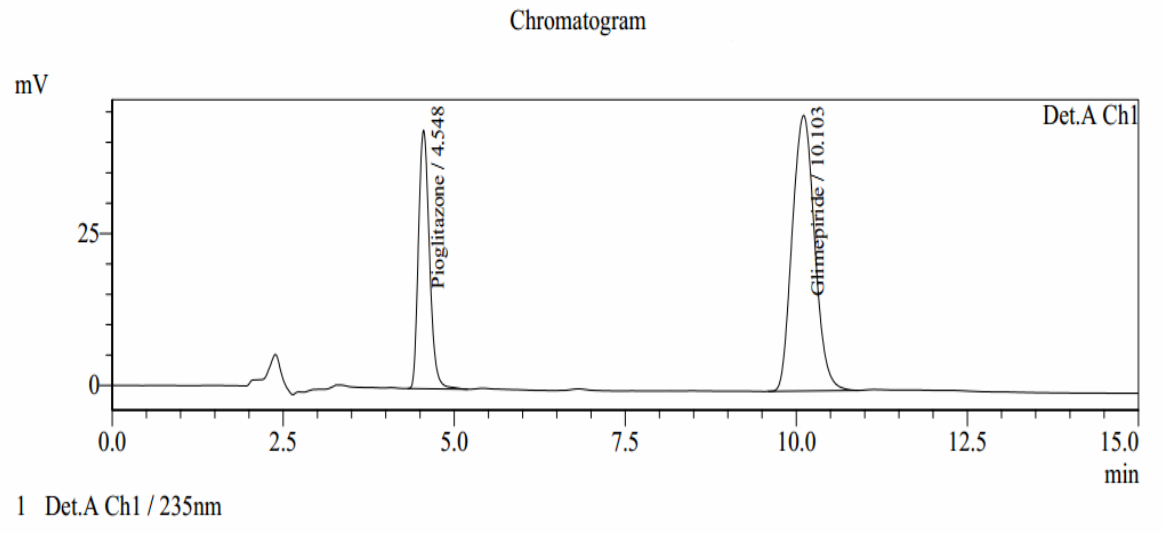


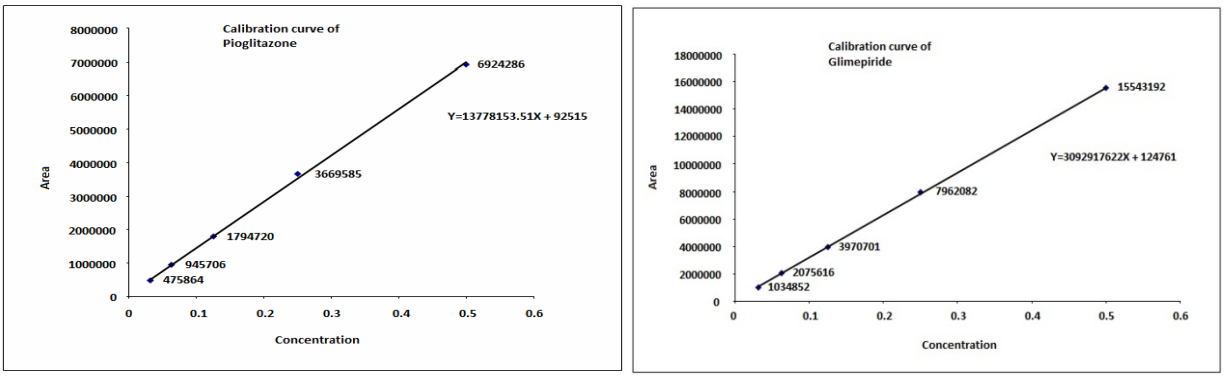
Figure 2. HPLC chromatogram of standard pioglitazone and glimepiride.

The specificity of the method was monitored by analyzing the placebo (containing all the ingredients of the formulation except the analyte), standard solution and market preparation containing pioglitazone and glimepiride. No peak was detected close to the retention time of pioglitazone and glimepiride at the wavelength of detection and hence proved high degree of specificity of the method.

When peak areas (y) were plotted against concentration levels of 0.5, 0.25, 0.125, 0.0625 and 0.03125 µmole/ml of standard solutions of pioglitazone and glimepiride, good correlation coefficients were obtained. The correlation coefficients (r^2) were obtained as 0.9991 for pioglitazone and 0.9999 for glimepiride which were within the acceptable range of guidelines and showed good linearity of the newly developed method. The slope (m) and intercept (c) of the calibration curves were found as 13778153.51 and 92515 for pioglitazone; and 30929176.22 and 124761 for glimipiride. (Table 1, Figure 3)

Table 1. Linearity of the method.

Drug	Injected conc. (µmole/mL)	Mean area (n=3)	Intercept (c)	Slope (m)	Correlation coefficient(r^2)
Pioglitazone	0.03125	475864	92515	13778153.51	0.9991
	0.0625	945706			
	0.125	1794720			
	0.25	3669585			
	0.5	6924286			
Glimepiride	0.03125	1034852	124761	30929176.22	0.9999
	0.0625	2075616			
	0.125	3970701			
	0.25	7962082			
	05	15543192			



A **B**

Figure 3. Linearity of curve for pioglitazone (A) and glimepiride (B).

The accuracy was evaluated at three different concentrations with spikes which were conducted in successive analysis (n=3) using the proposed method and the value was expressed as percentage of recovery (R %) between the mean concentrations found and added concentration for both of these drugs. The average percentage of recovery was found to be 99.87%, 98.82%, and 99.94% for 0.05, 0.025 and 0.0125 µmole/mL, respectively (Table 2).

The precision of proposed method was checked by intra-day and inter-day repeatability of responses after replicate injections of standard solution. The precision of pioglitazone was determined by triplicate injection of 0.05, 0.025 and 0.0125 µmole/ml each day for two

different days where the mean concentrations were found as 0.050703, 0.024927 and 0.01261 µmole/mL for day-1 associated with RSD% of 1.0%, 0.36% and 0.28%, respectively; and 0.049895, 0.025225 and 0.01248 µmole/mL for day-2 associated with RSD% of 0.043%, 0.028% and 0.23%, respectively (Table 3 and 4). To determine the precision of glimepiride, the mean concentrations were found as 0.049347, 0.02442 and 0.012533 µmole/ml for day-1 associated with RSD% of 0.51%, 0.78% and 0.51%, respectively; and 0.04947, 0.2451 and 0.012535 µmole/ml for day-2 associated with RSD% of 0.36%, 0.29% and 0.51%, respectively for same concentrations. The RSD% for intra-day and inter-day assays of pioglitazone and glimepiride in the same laboratory did not exceed more than 2% (Table 3 and 4).

All experimental results were in the range of the acceptable precision and accuracy, which indicate that the newly developed method is sensitive enough and accurate for determination of pioglitazone and glimepiride. Therefore, the method was applied for quantitative and simultaneous analysis of combined pharmaceutical preparation of pioglitazone and glimepiride formulated by local manufacturer. The quantity of active drugs was determined for three marketed preparations (sample-1, sample-2 and sample-3) for each drug and the potency of pioglitazone was found to be 99.94%, 101.37% and 99.79%, respectively; where as 100.05%, 99.73% and 100.14% for glimepiride, respectively (Table 5).

Table 2. Accuracy of the developed method.

Drug	Standard + Spike (µmole/ml)	Injected conc. (µmole/ml)	Mean recovery (%)
Pioglitazone	0.05 + 0.0	0.05	99.885
	0.025 + 0.0	0.025	98.820
	0.0125 + 0.0	0.0125	99.940
Glimepiride	0.00535 + 0.5	0.50535	99.925
	0.0002675 + 0.25	0.250267	99.418
	0.00001338 + 0.125	0.1250138	100.01

Table 3. The intraday precision of the developed method.

Drug	Injected conc. (µmole/ml)	Intra-day					
		Day-1			Day-2		
		Mean recovered conc. (n =3) (µmole/ml)	SD	RSD%	Mean recovered conc. (n= 3) (µmole/ml)	SD	RSD%
Pioglitazone	0.05	0.050703	0.00051	1.0	0.049895	0.000021	0.043
	0.025	0.024927	0.00009	0.36	0.025225	0.000007	0.028
	0.0125	0.01261	0.00004	0.28	0.01248	0.000028	0.23
Glimepiride	0.05	0.049347	0.00025	0.51	0.04947	0.000184	0.36
	0.025	0.02442	0.00019	0.78	0.02451	0.000071	0.29
	0.0125	0.012533	0.00006	0.51	0.012535	0.000064	0.51

Table 4. The inter-day precision of the developed method.

Drug	Injected conc. (µmole/ml)	Inter-day		
		Mean recovered conc. (n =3) (µmole/ml)	SD	RSD%
Pioglitazone	0.05	0.050299	0.0005716	1.14
	0.025	0.025076	0.000211	0.84
	0.0125	0.012545	0.000092	0.73
Glimepiride	0.05	0.049408	0.000087	0.18
	0.025	0.024465	0.000064	0.26
	0.0125	0.012534	0.0000012	0.01

Table 5. Sample analysis.

Drug	Sample Code	Injected conc. (µmole/mL)	Mean recovered (µmole/mL)	Average % Recovery = (Mean recovered conc./ injected conc.) ×100
Pioglitazone	Sample-1	0.1	0.09994	99.94
	Sample-2	0.05	0.05068	101.37
	Sample-3	0.025	0.02494	99.79
Glimepiride	Sample-1	0.0107	0.010705	100.05
	Sample-2	0.00535	0.005336	99.73
	Sample-3	0.00265	0.002654	100.14

CONCLUSION

To attain the objective, a rapid and sensitive reversed phase high performance liquid chromatographic method was developed and validated according to the guidelines of FDA, ICH and USP. Since there were good separation and resolution of the chromatographic peaks, the proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination and quantification of pioglitazone and glimepiride. The sample recoveries were in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of pioglitazone and glimepiride in combined dosage forms.

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