High-performance Liquid Chromatographic Analysis of Pioglitazone, Gliquidone, Rosuvastatin and Simvastatin in Formulations and Human Serum

Arayne, M. Saeed*[,a] Sultana, Najma[a] Mirza, Agha Zeeshan[b] Shamshad, Hina[c]

[a] *United Biotech (Pvt) Ltd, Gulistan Jauhar, Karachi 75290, Pakistan*
[b] *Department of Chemistry, University of Karachi, Karachi 75270, Pakistan*
[c] *Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan*

Co-administration of HMG-CoA reductase with antidiabetic drugs is most common since antidiabetic drugs are mostly prescribed for long term therapy. In the present paper, we describe the simultaneous determination of antidiabetic (pioglitazone hydrochloride and gliquidone) in presence of statins (rosvastatin and simvastatin) in formulations and in human serum using RP-HPLC technique. The serum samples were subjected to protein precipitation with acetonitrile prior to an HPLC analysis. At a flow rate of 1 mL•min^{-1} isocratic elution was employed, using mobile phase consisting of methanol/water (90∶10, *V*∶*V*), pH 3.50 with phosphoric acid and absorbance was recorded at 235 nm. The assay was reproducible, linear (concentration range of 5—50 μg•mL^{-1}) and accurate. The LOD and LOQ values were 1.32, 0.28, 0.05 and 0.57 μg•mL^{-1} and 4.39, 0.93, 0.16 and 1.90 μg•mL^{-1} for pioglitazone hydrochloride, gliquidone, rosvastatin and simvastatin, respectively. There were no interfering peaks due to the excipients present in the pharmaceutical tablet and serum. Thus, the proposed method is simple and suitable for the analysis of active ingredient in tablet form and human serum.

Keywords analytical methods, liquid chromatography, pioglitazone hydrochloride, gliquidone, rosuvastatin, simvastatin, human serum

Introduction

Type 2 diabetes and heart failure commonly occur together and this combination is associated with poor outcomes. Approximately 10% of patients with type 2 diabetes have heart failure 2 to 4 times the rate in people without diabetes[1,2] and presence of heart failure also appears to be an independent risk factor for developing diabetes.[3] Type 2 diabetes is commonly associated with many comorbid conditions that are also risk factors for heart failure, including coronary artery disease, hypertension, renal dysfunction and obesity.[4,5] Furthermore, several of the metabolic and functional disturbances associated with diabetes, such as hyperglycemia, increased free fatty acids and insulin resistance may contribute to heart failure risk.[2,6]

Thiazolidinediones are peroxisome proliferator activated receptor subtype gamma (PPAR-γ) agonists that target insulin resistance directly. They are effective glucose lowering agents that have many potential cardiovascular benefits.[7,8] Pioglitazone (Figure 1) decreases insulin resistance via its action at the PPARγ.[7] It reduced the risk of the primary endpoint and the risk of secondary 'hard' (disease-related) macrovascular events in a high-risk patient population with type 2 diabetes.[9] In clinical trials, pioglitazone in monotherapy does not appear to impart an increase in the incidence of heart failure compared with metformin or sulfonylurea.[10-13] Numerous HPLC methods have been applied for the determination of pioglitazone and its metabolites in pharmaceutical dosage forms and biological fluids.[14-20]

Gliquidone (Figure 1) belongs to the class of sulfonylurea derivatives.[21] It improves glycemic control and lowers blood sugar level. Because of its short term effect, gliquidone is suitable for combined treatment.[22] Several methods have been described in the literature to determine gliquidone in dosage forms and biological fluids.[21,23-25]

For primary and secondary prevention of cardiovascular disease, statins are increasingly used. Simvastatin is potent cholesterol lowering agent that also has FDA indications for lowering triglycerides (Figure 1).[26] Rosuvastatin (Figure 1), a synthetic cholesterol lowering drug is commonly used for the treatment of dyslipidemia. It is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase[27-29] and used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and apolipo-

* E-mail: zee_amm@hotmail.com
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protein B in blood and reduce the risk of cardiovascular events in hyperlipidemic and normocholesterolemic patients.[30,31] Different HPLC methods with UV detection have been applied for the determination of simvastatin[32-35] and rosuvastatin[36-38] in dosage forms and in biological fluids.

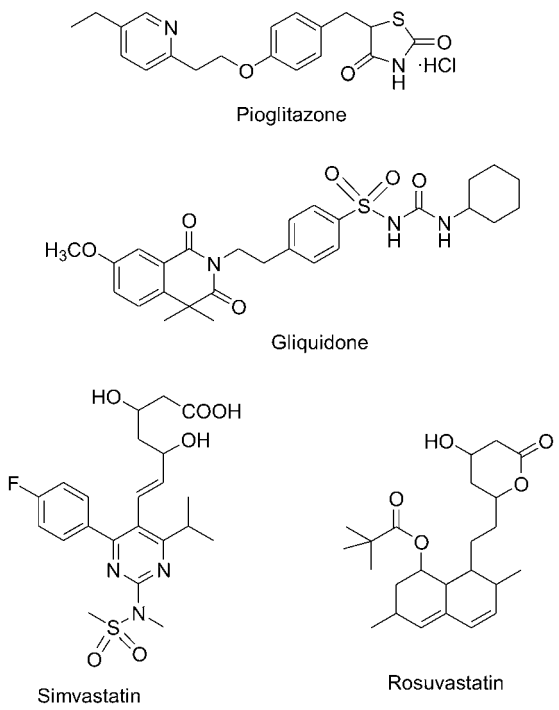

Figure 1 Structures of pioglitazone, gliquidone, simvastatin and rosuvastatin.

Treatment of dyslipidemia in diabetes is thus important, with focus on lowering LDL cholesterol. All these drugs belong to different classes that can be co-administrated in number of cases. Combination of thiazolidinediones and fibrates may lower serum HDL cholesterol in some patients.[39,40] It is also reported that statin treatment, significantly decreased pancreatic β-cell function in patients with type 2 diabetes.[41] Furthermore, cotreatment with statin and thiazolidinediones significantly increased plasma adiponectin concentrations in patients with type 2 diabetes.[42]

Due to the drug combination on these formulations there has been a need for creating reliable quantitative method to determine these drugs in commercial samples and in human serum. The aim of our work was to develop a new method for the simultaneous determination of pioglitazone hydrochloride, gliquidone, rosuvastatin and simvastatin. Numerous workers have reported simultaneous determination of same classes of drugs by HPLC but the method of all above co-administrated drugs is not reported simultaneously by HPLC. The proposed method was successfully applied to the determination of these drugs in commercial tablets, human serum and for drug-drug interaction studies. The established method was validated with respect to specificity, linearity, precision, accuracy and ruggedness.

Experimental

Instrumentation

Shimadzu HPLC system, equipped with LC-10 AT VP pump, SPD-10 AV VP UV-vis detector utilizing Purospher® STAR RP-18 encapped (5 μm, 25 cm×0.46 cm) column. The chromatographic and integrated data were recorded using Shimadzu model CBM-102 Communication Bus Module. Shimadzu Class-GC 10 software (version 2) was used for data acquisition and mathematical calculations.

Chemicals and reagents

Pharmaceutical grade of gliquidone reference standard was kindly gifted by Pharmatec (Private) Limited Karachi. Pioglitazone hydrochloride and simvastatin were kindly supplied by Ali Gohar Pharmaceutical (Private) Limited Karachi and rosuvastatin was kindly gifted by Pharmevo (Private) Limited. Glurenor® (30 mg) tablets, Poze® (45 mg), X-plended (20 mg) and Limitrol® (10 mg) tablets were purchased from pharmacy. HPLC grade methanol was purchased from Merck Germany. Other chemicals used were of analytical grade.

Chromatographic conditions

All analyses were done at ambient temperature (24±2) ℃ under isocratic conditions. Phosphate buffer-water-methanol solution was studied with a view to select a suitable mobile phase. A good compromise of resolution, peak width and run time was the suitability criterion. The trial runs indicated that the phosphate buffer-water-methanol mixture was more appropriate. The mobile phase consisted of a mixture of methanol and water (90∶10, V∶V), pH adjusted to 3.50 with phosphoric acid. The flow rate was 1 mL•min^{-1} and absorbance was recorded at 235 nm. The retention times were obtained as 2.57 min for pioglitazone, 3.39 min for rosuvastatin, 5.55 min for gliquidone and 8.95 min for simvastatin (Figure 2).

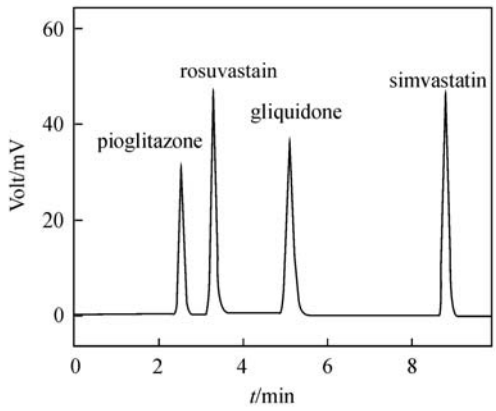

Figure 2 Representative chromatogram of pioglitazone, rosuvastatin, gliquidone and simvastatin.

Preparation of standard and sample solutions

Accurately weighed amounts, equivalent to 10 mg of standards of gliquidone, pioglitazone hydrochloride, rosuvastatin and simvastatin were transferred to 100 mL volumetric flask, separately and volumes were completed with mobile phase. The resulting solutions of 100 μg•mL^{-1} were sonicated for 25 min and filtered through membrane filter. Aliquots of each solution were accordingly diluted with mobile phase in order to obtain solutions with final concentration of 5—50 μg•mL^{-1}.

Procedure for formulations

20 tablets of each drug were individually weighed and triturated and an amount of powder equivalent to 10 mg of each drug was transferred to 100 mL volumetric flask. The content of the flask was shaken for about 60 min for maximum solubility of drugs and volumes were completed with mobile phase. The resulting solutions were filtered through Wattmann filter paper to separate out the insoluble excipients and further dilutions were carried out to obtain desire concentration. Final solutions were filtered through a 0.45 μm Millipore filter (USA) before injection into the system.

Serum drug analysis

The reported procedure was used for serum analysis.[43,44] Multiple blood samples (10 mL) of 10 healthy volunteers were collected and centrifuged at 3000 r/min for 10 min. Plasma was separated and deproteinated by acetonitrile and the supernatant obtained was filtered through a 0.45 micron pore size membrane filter. Different volumes of stock were taken, further diluted appropriately and spiked in plasma to prepare the calibration standards. These were stored at −20 ℃ and 20 μL volume of each sample was injected.

Method development

Calibration curves

6 different concentration levels (5, 10, 15, 20, 25 and 50 μg•mL^{-1}) were obtained of each standard solution, conveniently diluted with mobile phase. Each solution was injected in the chromatographic system (n=3) and mean values of peak areas were plotted against concentrations.

Assessment of linearity and recovery

The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation and intercept values (Table 1). The linearity of each standard curve was assessed by plotting the peak area ratio of drugs versus concentration. The recovery of analytes in human serum was expressed as ratio of mean peak area obtained from extraction before spiked to extraction after spiked multiplied by 100.

Selectivity and specificity

The selectivity and specificity of proposed method was evaluated through possible interference due to excipients presented in the pharmaceutical formulations (Figure 2). For that, placebo of each tablet sample was prepared by mixing respective excipients. The method specificity was assessed by comparing the chromatograms obtained from the drug and the most commonly used excipients mixture with those obtained from blank (excipients solution in methanol without drug).

Table 1 Regression statistics and LOD and LOQ

Drug	Regression equation	r^2	LOD/(μg•mL^{-1})	LOQ/(μg•mL^{-1})
Pioglitazone	y=12907x+41612	0.9961	1.32	4.39
Gliquidone	y=30579x+24762	0.9988	0.28	0.93
Rosuvastatin	y=29122x+4146	0.9989	0.05	0.16
Simvastatin	y=29780x+105685	0.9976	0.57	1.90

Limit of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the coefficient of variation (CV) and the slope (S) of the calibration curves.[45] The LOD and LOQ of pioglitazone, gliquidone, rosuvastatin and simvastatin were 1.32, 4.39; 0.28, 0.93; 0.05, 0.16 and 0.57, 1.90 μg•mL^{-1}, respectively.

Accuracy

To evaluate the accuracy of the proposed method, recovery tests were carried out with all samples at three different concentrations, *i.e.* 8, 10, 12 μg•mL^{-1} (n=3) in synthetic samples using placebo mixtures and were performed by adding known amounts of standard solutions to sample followed by analysis using proposed method. The percentage of recovery was calculated as indicated by Association of Official Analytical Chemists International (Table 2).[46]

Table 2 Accuracy and precision

Analyte	Spiked concentration/(μg•mL^{-1})	Precision RSD/%	Accuracy
Pioglitazone	8	1.94	100.84
	10	0.86	100.76
	12	1.44	102.77
Gliquidone	8	1.50	98.33
	10	1.05	100.60
	12	1.38	98.01
Rosuvastatin	8	1.14	101.41
	10	1.18	99.55
	12	1.92	102.37
Simvastatin	8	1.57	100.76
	10	1.79	98.69
	12	1.18	101.21

Precision

The precision of the method is expressed as relative standard deviation (RSD) amongst responses in each case. All solutions were prepared fresh and responses were determined after replicate injection of sample solutions (Table 2).

Application in human serum

There was no significant difference between the amount of drug spiked in serum and the amount recovered. The method applied in our study involved the direct injection of the plasma samples after precipitation of protein with acetonitrile. The recovery values (Table 3) in human serum clearly indicated the applicability of the present method for the required purpose (Figure 3).

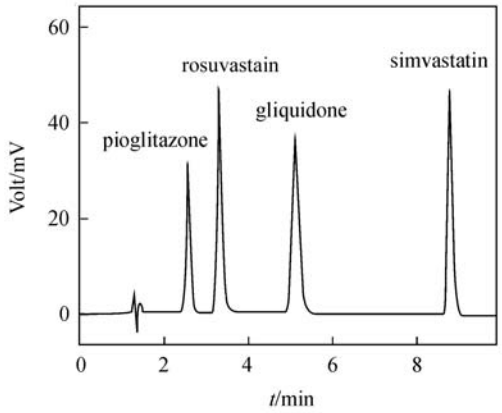

Figure 3 Representative chromatogram of pioglitazone, rosuvastatin, gliquidone and simvastatin in human serum.

Discussion

Several HPLC methods have been developed for determination of these co-administered in biological fluids; however, these methods used solid-phase extraction for sample preparation and different relatively expensive, laborious and complicated techniques. The method used in the present study provided a simple and reliable procedure for the determination of these drugs simultaneously in human serum. Although there is no structural similarity between these drugs yet they have similarity in the solubility behavior in the mobile phase. The proposed method is simple and does not involve laborious time consuming sample preparations. Calibration curves showed linearity over a concentration range from 5 to 50 μg•mL^{-1}. The correlation coefficients for pioglitazone, gliquidone, rosuvastatin and simvastatin obtained with linear regression of curve were 0.9961, 0.9988, 0.9989 and 0.9976, respectively. The LOD and LOQ of pioglitazone, gliquidone, rosuvastatin and simvastatin were 1.32, 4.39; 0.28, 0.93; 0.05, 0.16 and 0.57, 1.90 μg•mL^{-1}, respectively. The recovery values obtained confirmed the accuracy of the proposed method (Table 2). The results proved specificity of the proposed method. Table 1 shows the statistically treated linear regression data of pioglitazone, gliquidone, rosuvastatin and simvastatin. The chromatograms obtained for drugs spiked plasma samples are represented in Figure 2. No interfering peaks were observed in chromatogram which further confirmed the specificity of the method.

Conclusion

The proposed HPLC method enabled quantitative determination of pioglitazone, gliquidone, rosuvastatin and simvastatin in pharmaceutical formulations and serum. All calibration curves were found to be linear with correlation coefficients of above 0.996. Analytical results of samples were in accordance with those of standard solution in the same concentrations. The method will also be preferential for its use of low volume of serum and the simplicity of the sample preparation, the short-run time and the absence of matrix effect. The proposed HPLC method is fast, precise, accurate, sensitive and efficient and can be used for routine analysis in quality control laboratories.

Table 3 Recovery of pioglitazone, gliquidone, rosuvastatin and simvastatin in human serum

Concentration/ (μg•mL^{-1})	Gliquidone		Pioglitazone		Rosuvastatin		Simvastatin	
	Recovered/ (μg•mL^{-1})	RSD/%	Recovered/ (μg•mL^{-1})	RSD/%	Recovered/ (μg•mL^{-1})	RSD/%	Recovered/ (μg•mL^{-1})	RSD/%
5	4.96	1.21	5.04	0.84	5.01	0.79	4.94	0.47
10	10.02	1.34	9.81	1.77	10.15	0.49	9.97	0.68
15	15.08	1.05	14.96	1.35	14.88	0.62	15.29	1.52
20	20.31	0.52	19.68	0.28	19.64	1.22	20.31	0.69
25	25.41	0.47	25.22	0.61	24.82	1.55	25.48	1.74

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