

Simultaneous Determination of Gliquidone, Pioglitazone Hydrochloride, and Verapamil in Formulation and Human Serum by RP-HPLC

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Abstract

In the present study, a reverse-phase high performance liquid chromatography method was developed, validated and applied for the simultaneous determination of gliquidone, pioglitazone hydrochloride and verapamil in tablets and human serum. Chromatographic separation was achieved on a C18 column (5 μm , 25 \times 0.46 cm) with a mobile phase consisting of methanol–water–acetonitrile (80:10:10 v/v/v) with a flow rate of 0.7 mL/min and pH adjusted to 3.50 with phosphoric acid at 230 nm. Glibenclamide was used as internal standard. The experimentally derived limit of detection and limit of quantitation were determined to be 0.24, 0.93, 0.40, and 0.80, 3.11, 1.36 $\mu\text{g}/\text{mL}$ for gliquidone, pioglitazone, and verapamil, respectively. There were no interfering peaks due to the excipients present in the pharmaceutical tablets. Thus, the proposed method is simple and suitable for the simultaneous analysis of active ingredients in dosage forms and human serum.

Introduction

Diabetes mellitus is a global health problem of steadily increasing proportions with approximately 95% of patients being affected by the type 2 form of the disease (1).

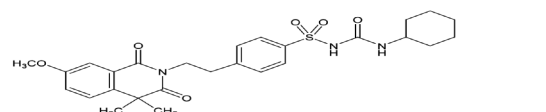
The sulfonylurea constitutes long-established group of drugs with a proven track record in the treatment of type 2 diabetes. Gliquidone (Figure 1), a sulfonylurea used for the treatment of non-insulin dependent diabetes mellitus (NIDDM), is a safe and also effective treatment option for new-onset diabetes mellitus (NODM). It has shown similar efficacy in comparison to rosiglitazone (2). Literature survey revealed that quantification of gliquidone has been achieved by UV spectrophotometry (3), atmospheric pressure chemical ionization liquid chromatographic-mass spectrometric (APCI-LC-MS), LC-MS (4), and high performance (HP) LC (5–7).

Pioglitazone (Figure 1) is a thiazolidinedione compound used in the treatment of type 2 diabetes and in most countries

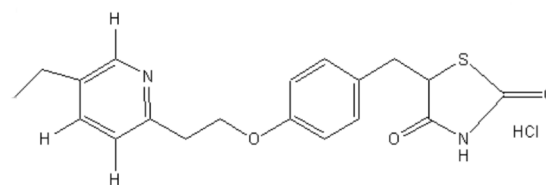
approved either as monotherapy or in combination with sulfonylureas or metformin (8). It had a positive effect on important components of the lipid profile in a dose-dependent manner and it is effective glucose lowering agent that has many potential cardiovascular benefits (9,10).

Numerous HPLC methods have been applied for the determination of pioglitazone and its metabolites in pharmaceutical dosage forms and biological fluids (11–17).

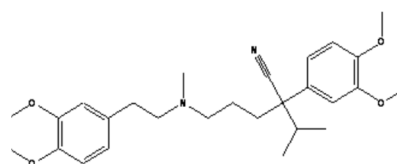
Calcium channel blockers block the entry of calcium into the muscle cells of the heart and are primarily used to reduce systemic vascular resistance and treat hypertension. Calcium channel blockers may be associated with increased risks of cardiovascular events, cancer and gastrointestinal haemorrhage (18). A calcium channel blocker verapamil (Figure 1), reduce cardio vascular disease (CVD) events in people with diabetes and



Gliquidone



Pioglitazone Hydrochloride



Verapamil

Figure 1. Chemical structures of gliquidone, pioglitazone hydrochloride, and verapamil.

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hypertension (19,20) and use of verapamil, significantly reduced the risk of developing diabetes (21). Several investigations have been determined verapamil through HPLC (22–27).

Management of type 2 diabetes mellitus usually involves combined pharmacological therapy to obtain adequate glucose control and treatment of concurrent pathologies. In addition, triple therapy with the addition of a thiazolidinedione to metformin-sulfonylurea combination has been recently evaluated and allows glucose targets to be reached before insulin therapy is considered. Some antihypertensive agents may favor hypoglycemic episodes when co-prescribed with sulfonylurea (28) and diabetic patients often take anti-hypertensive medications (29) and co administered with antidiabetic drugs. Due to combination of these drugs, there is a need for the simultaneous determination of gliquidone and pioglitazone hydrochloride with verapamil by HPLC.

The present study deals with the simultaneous determination of antidiabetic gliquidone and pioglitazone with verapamil. Many of reported methods require expensive instrument and laborious extraction procedures. No method was reported for the simultaneous determination of these co-administered drugs. The proposed method was successfully applied to the determination of these drugs in tablets and human serum. The established method was validated with respect to linearity, limit of detection and quantification, precision, accuracy, and specificity.

Experimental

Materials

Pharmatec (Private) Limited Karachi was kindly gifted gliquidone reference standard. Pioglitazone hydrochloride and glibenclamide were obtained from Hilton Pharma Ltd. Karachi. Glurenor (gliquidone 30 mg) of Pharmatec (Pvt.) Ltd. Karachi, Poze (pioglitazone hydrochloride 45 mg) tablets of AGP (Pvt.) Ltd. and verapamil (Calan 40 mg) from Wilson's Pharma (Pvt.) Ltd. were purchased from the local pharmacy. Methanol used was of HPLC grade.

Equipments

A Shimadzu HPLC (Japan) system equipped with LC-10 AT VP pump, a Rheodyne manual injector fitted with a 20 μ L loop, a Purospher STAR RP-18 end capped (5 μ m, 25 \times 0.46 cm) column, and an SPD-10 A VP UV-vis detector (Japan) were utilized. Chromatographic system was integrated via Shimadzu model CBM-102 to PIV computer. Shimadzu CLASS-GC software (version 2) was used for data acquisition and mathematical calculations.

Solution preparations

Separate stock solutions of gliquidone, pioglitazone, verapamil, and glibenclamide were prepared separately in a 100 mL volumetric flask by dissolving 10 mg of each drug and volume was made up by 100 mL of methanol so that final concentration was 100 μ g/mL. Five milliliters (20 μ g/mL) of internal standard was added in a series of 25 mL volumetric flasks and different aliquots of gliquidone, pioglitazone, and verapamil standard

solution were added to attain concentration range of 5, 10, 15, 20, and 50 μ g/mL using the same solvent as diluent.

Assay in formulations

Twenty tablets of each drug were powdered and equivalent to 10 mg of gliquidone, pioglitazone, and verapamil were weighed and transferred separately into 100 mL calibrated flasks and make up with methanol. The content of the flask was shaken for ~ 60 min. This solution was filtered through Wattmann filter paper to separate out the insoluble excipients and further dilutions were carried out to obtain desired concentration. Final solutions were filtered through a 0.45 μ m Millipore filter (Billerica, MA) before injection in HPLC.

Serum drug analysis

The reported procedure used for serum analysis (30). In brief, multiple blood samples (10 mL) of ten healthy volunteers were collected and centrifuged at 3000 rpm for 10 min. The serum separated and deproteinated by acetonitrile and supernatant obtained was filtered through 0.45 micron pore size membrane filter. Serum thus obtained was mixed in ratio of 1:1 with drug solutions and then serially diluting it with blank serum to attain the desired concentrations. These were stored at -20°C and 10 μ L volume of each sample was injected and chromatographed.

Result and Discussion

Method development and validation

Method validation characteristics were tested in accordance with ICH guidelines (31). Linearity, method accuracy (% recovery of individual measurements) and method precision (%RSD) using samples in three replicates were tested. Chromatographic separation was carried with a mobile phase consisting of methanol–water–acetonitrile (80:10:10, v/v/v) with a flow rate of 0.7 mL/min and pH adjusted to 3.50 with phosphoric acid at 230 nm. Glibenclamide was used as internal standard.

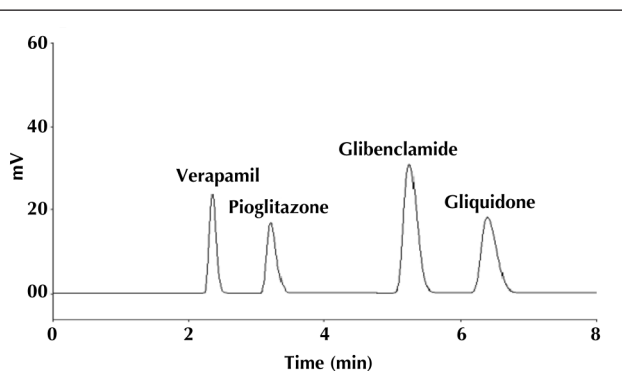
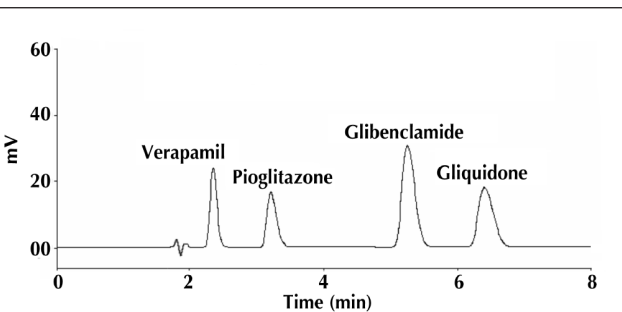
Linearity of the assay was demonstrated by running standards at different concentrations over the range 5–50 μ g/mL. Precision and accuracy were assessed using three spiked samples at each of three concentrations. Measured concentrations were determined by application of the appropriate standard curve obtained on each occasion. Precision was assessed in terms of %RSD, while accuracy was determined from % recovery calculations. Recovery of each drug from serum was assessed using spiked

Table I. Statistical Results of Linear Regression Analysis in the Determination of Gliquidone, Pioglitazone, and Verapamil

Statistical parameters	Gliquidone	Pioglitazone	Verapamil
Slope	25686	10724	19797
Intercept	46124	36656	35057
Linear r^2	0.9992	0.9963	0.9984
LOD (μ g/mL)	0.24	0.93	0.40
LOQ (μ g/mL)	0.80	3.11	1.36

Table II. Inter- and Intra-day Accuracy and Precision

Analyte	Spiked conc. (µg/mL)	Inter day Precision % RSD	Intra day Accuracy %	Precision % RSD	Accuracy %
Gliquidone	8	0.38	99.7	1.23	100.53
	10	0.86	99.3	1.02	100.22
	12	0.58	100.4	0.99	99.91
Pioglitazone	8	0.86	99.53	0.49	99.43
	10	1.08	99.78	1.29	98.34
	12	1.04	101.0	1.00	101.92
Verapamil	8	1.13	100.6	0.96	100.82
	10	1.53	97.56	0.82	100.32
	12	0.75	101.2	0.87	98.12

**Figure 2.** Representative chromatogram of verapamil, pioglitazone, glibenclamide, and gliquidone.**Figure 3.** Representative chromatogram of verapamil, pioglitazone, glibenclamide, and gliquidone in serum.**Table III. Recovery of Gliquidone, Pioglitazone, and Verapamil in Human Serum**

Conc. µg/mL	Gliquidone		Pioglitazone		Verapamil	
	Recovered Conc. µg/mL	% RSD	Recovered Conc. µg/mL	% RSD	Recovered Conc. µg/mL	% RSD
5	4.92	1.14	5.07	0.65	4.98	0.33
10	9.84	1.64	9.92	0.81	10.16	1.25
15	15.14	0.38	15.14	1.28	14.86	1.06
20	19.84	1.22	19.95	0.36	19.74	1.37
25	24.88	1.4	25.14	1.35	25.25	1.08

drug-free serum at five different concentrations, and was estimated by comparing the mean peak ratios of the extracted spiked serum to the mean peak area ratios of equivalent aqueous standard solutions.

Calibration curves

Different concentration levels (5, 10, 15, 20, 25, and 50 µg/mL) were obtained for each standard solution, diluted with methanol. Each solution was injected in the chromatographic system. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation and intercept values.

Linearity, limit of detection, and quantitation

Under the above described experimental conditions, linear correlation between the peak area and applied concentration was found in the concentration range 5–100 µg/mL, as confirmed by the correlation coefficients of 0.9992, 0.9963, and 0.9984 for gliquidone, pioglitazone, and verapamil, respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following equation.

$$\text{LOD} = \frac{3.3\sigma}{S} \quad \text{LOQ} = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the response, S is the slope of the calibration curve. The LOD and LOQ were determined to be 0.24, 0.93, and 0.40, and 0.80, 3.11, and 1.36 µg/mL for gliquidone, pioglitazone, and verapamil, respectively (Table I).

Precision

Precision data on the intra- and inter-day variation for all the drugs were performed using three concentration levels (8, 10, and 12 µg/mL) in three replicates on two different days and are summarized in Table II. Both inter- and intra-day RSD were less than 2%, indicating a sufficient precision.

Accuracy

Recoveries from pharmaceutical dosage forms after spiking with 80%, 100%, and 120% of additional standards and %RSD for all analyzed concentrations (Table II), confirming the accuracy of the method.

Specificity

The specificity of the method was tested by adding a known quantity of standard drug solutions to the tablet excipients placebo solutions. A 1:1 blend of drug and placebo was prepared and drug was then extracted from this blend using methanol. It is clear from the chromatograms of drugs alone and with excipients (i.e., microcrystalline cellulose, croscarmellose sodium, magnesium stearate, lactose, and hydroxypropylmethylcellulose) spiked in drugs (Figure 2), that no significant interference was found in the migration time of drug peaks during analysis. Peaks of all drugs were identified by comparison of their retention times and verapamil, pioglitazone, glibenclamide, and gliquidone were eluted at 2.40, 3.38, 5.20, and 6.56 min, respectively.

Application in human serum

Recovery of drugs from serum was assessed using spiked drug-free serum at different concentrations and was estimated by comparing the mean peak ratios of the extracted spiked serum to the mean peak area ratios of equivalent standard solutions. No significant difference between the amount of drug spiked in serum and the amount recovered was observed after spiking the analyte in the serum sample. The recovery values (Table III) in human serum clearly indicate the applicability of the present method for the required purpose (Figure 3). This method involved the direct injection of the serum samples after precipitation of protein with acetonitrile.

Conclusion

Using the described chromatographic conditions, gliquidone, pioglitazone, and verapamil and the internal standard, glibenclamide, were well separated. For all compounds, sharp and symmetrical peaks were obtained with good baseline resolution and minimal tailing, thus facilitating the accurate measurement of the peak area. No interfering peaks were found in the chromatogram due to tablet excipients. The proposed HPLC methods are simple, rapid, accurate, precise, sensitive, and easy to apply for determination of these drugs in pharmaceuticals and human serum, as can be seen from validation data. The proposed method does not involve laborious time consuming sample preparation. The method reported here provides high recovery of the investigated drugs. The validation data demonstrate good precision and accuracy, which proves the reliability of the proposed method. Finally, this method could be implemented for monitoring gliquidone, pioglitazone, and verapamil in clinical samples.

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