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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Metformin HCL, Pioglitazone and Gliclazide in Bulk and Formulation.

Jawale Anup R*, Rane Sachin S, Chaudhari Rajesh Y, and Patil Vijay R.

T.V.E.S.'s Hon. Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur- 425503, Dist.-Jalgoan, (M.S.), India.

ABSTRACT

A new simple, rapid, precise, and specific assay method was development and validation of RP-HPLC stability indicating method for the simultaneous estimation of metformin HCL, pioglitazone and gliclazide in bulk and formulation. The analysts were separated by using RP HPLC on a RP- vertex C18 column (5 µm, 4.6mm, 250 mm). The mobile phase was methanol: acetonitrile: water at 1.0 mL/min satisfactorily resolved the binary mixture. The UV detector was operated at 228 nm for the determination of the drugs. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 20-100 µg/ml for metformin HCL, 4-12 µg/ml gliclazide and for 3-15 µg/ml pioglitazone with a R^2 0.9997, 0.9983 and 0.9989 values respectively, in this mixture. The optimized method proved to be specific, robust and accurate for the quality control in bulk drug and pharmaceutical formulations.

Keywords: Metformin Hcl, Pioglitazone and Gliclazide, Validation, RP-HPLC, Simultaneous Estimation

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*Corresponding author

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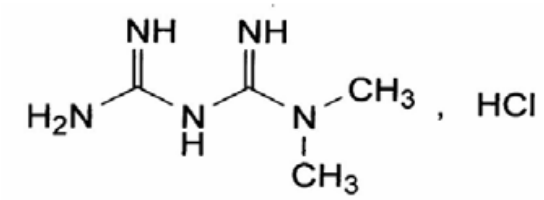
INTRODUCTION

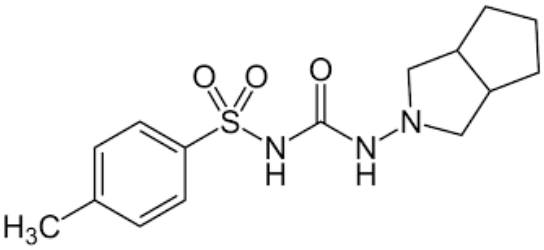
Metformin HCL (MET) is chemically N,N-dimethylbiguanide hydrochloride, gliclazide (GLZ) is N-(hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4 methylbenzenesulfonamide and pioglitazone is 5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione. MET is a compound that It acts by suppressing excessive hepatic glucose production and improving glucose clearance, its predominant effect is to decrease fasting plasma glucose and decreasing the intestinal absorption of glucose [1-3]. Gliclazide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). based on the pharmacological efficacy, gliclazide is considered a second-generation sulfonylurea which presents a higher potency and a shorter half-life [2,3] gliclazide belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release.[3,4] Pioglitazone is other antidiabetic medications to manage type 2 diabetes mellitus. It is administered as a racemic mixture, though there is no pharmacologic difference between the enantiomers and they appear to interconvert *in vivo* with little consequence. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α [3-6].

There often inadequate to use monotherapy, but combination therapy can simplify dosing regimens, improve compliance, decrease side effects and reduce cost. Analytical methods reported for quantitative determination of MET, GLZ and PIO individually in pharmaceutical formulations or biological fluids are high-performance liquid chromatographic HPLC [7-11] and methods reported for quantitative determination of MET individually in pharmaceutical formulations or biological fluids are HPLC and some of the reported for quantitative determination method in combination of MET, GLZ and PIO. Literature survey revealed that very few methods are reported for determination of MET, GLZ and PIO in pharmaceutical formulations, therefore it was thought worthwhile to develop simple, precise and robust analytical method for the same.

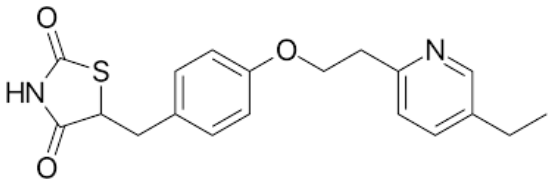
Figure 1: Chemical structures of Metformin HCL (MET), Gliclazide (GLZ) and Pioglitazone (PIO).



Metformin HCL



Gliclazide



Pioglitazone

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MATERIAL AND METHODS

Chemicals and reagents

Metformin HCl, Gliclazide and Pioglitazone were obtained as gift sample from Swiss Garnier Biotech Pvt. Ltd. Himachal Pradesh. Commercial pharmaceutical preparation Glycinorm Total-60 was procured from local pharmacy. Acetonitrile, methanol and water used were of HPLC grade (Qualigens Fine Chemicals, Mumbai, India). Ortho-phosphoric acid was AR grade (Qualigens Fine Chemicals, Mumbai, India). A 0.2 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Apparatus

The chromatographic system (Systronics Corporation, India) consisted of LC-138 at prominence solvent delivery module, a manual rheodyne injector with a 20 µL fixed loop and a UV-visible detector. The separation was performed on a Kromstar™ RP-Vertex C18 column (5 µm, 4.6mm* 250 mm) at an ambient temperature. Chromatographic data were recorded and processed using Clarify 2.0 Software A Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase. Shimadzu UV 1800 double beam UV visible spectrophotometer and Sansui-vibra DJ-150S-S electronic balance were used for Spectrophotometric and weighing purposes respectively.

Chromatography Conditions

Chromatographic separations of active substances were obtained by using Kromstar™ RP-Vertex C18 column (5 µm, 4.6mm* 250 mm). Mobile phase acetonitrile: methanol: water (10:30:60 v/v) (PH 5.0 was adjusted with sodium acetate buffer) was prepared, filtered through a 0.2 µm nylon filter and degassed for 5 min in an ultrasonicator. The mobile phase was pumped through the column at flow rate of 1.0 mL/min. Analyses were carried out at ambient temperature with detection at 228 nm. The injection volume was 20µL and each analysis required around 14 min.

Standard Solutions

Stock standard solutions of MET 1mg/mL, GLZ 1mg/mL and PIO 1mg/mL were prepared by dissolving 50 mg MET, 50mg GLZ and 50 mg PIO standard in 50 mL methanol. Working standard solutions of MET 100 µg/mL, GLZ 12µg/mL and PIO 3 µg/mL were prepared by diluting suitable aliquots of corresponding stock solutions with mobile phase.

Sample Solution

Twenty Glycinorm Total–60 tablets containing 500 mg of MET, 60mg GLZ and 15 mg PIO were weighed and ground to fine powder. A quantity of power equivalent to 500 mg of MET, 60mg GLZ and 15 mg PIO was transferred into 100 mL volumetric flask containing methanol (30 mL), sonicated for 15 min and the volume was made up to the mark and filtered through 0.45µm nylon membrane filter. This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The response of solution was measured at 228 nm and quantification of MET, GLZ and PIO was done by using present HPLC method.

Selection of Detection wavelength

All the three concentrations of given samples are scanned separately. Overly spectra clearly denote optimum wavelength at 228nm with all selected analyte with possible maximum absorbance as shown in figure 2.

Validation of Proposed Method [12,13]

Calibration curve (linearity)

Accurately measured aliquots of working standard solutions equivalent to 20-100 µgm/mL MET, 4-12 µgm/mL GLZ and 3-15 µg/mL PIO were transferred to series of 10 mL volumetric flasks and the

contents of the flasks were diluted to volume with mobile phase. A 20 µL aliquot of each solution was injected in triplicate into the liquid chromatography. The conditions including the flow rate of mobile phase at 1.0 mL/min, detection at 228 nm and run time program for 12 min, were adjusted. A calibration curve for each drug was obtained by plotting area under the peak versus concentration. The graphs of area vs concentration were recorded for all the drugs and are shown in (Fig. 4, 5 and 6).

Accuracy (% recovery)

Recovery studies were carried out by adding a known amount of pure drugs MET, GLZ and PIO to a pre analyzed sample solution. These studies were carried out by spiking 80%, 100% and 120% respective drug.

Method precision (repeatability)

The precision of the developed method was assessed in terms of repeatability, intraday and inter-day precision by analyzing six replicate standard samples. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and on 3 days for inter-day precision.

Intermediate precision (reproducibility)

The intra-day and inter-day precisions of the proposed method were determined by estimating the corresponding responses 5 times on the same day and on 5 different days for present method. The results are reported in terms of relative standard deviation (RSD).

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

LOD and LOQ of the drug were calculated using the equations according to International Conference on Harmonization (ICH) guidelines.

Robustness

Robustness of the method was determined by making slight changes in chromatographic conditions. Effect of % of water (59, 60 and 61%) in mobile phase on the retention time and slight changes in flow rate were applied as variable parameters. Flow rate varied at three levels (0.9, 1, 1.1). One factor at the time was changed to estimate the effect. Thus standard solution at varied pH (pH 4.9, 5 and 5.1) three pH levels was performed.

Specificity

Specificity is the ability of the analytical method to measure analyte response in presence of interferences including degradation products and related substances. Specificity was checked by determining MET, GLZ and PIO in laboratory prepared binary mixture and in binary mixture containing different degradation products.

System suitability Test (SST)

In the system suitability test tertiary solution of 100 µg/ml of MET, 12 µg/ml GLZ and 3 µg/ml of PIO (n=6) was prepared and injected. Then the system suitability parameters like retention time, theoretical plates, tailing factor and resolution were calculated from the chromatogram.

Analysis of MET, GLZ and PIO in Combined Tablet Dosage Form

Tablets containing MET (500 mg), GLZ (60 mg) and PIO (15 mg) of the brand Glycinorm Total-60 from Glenmark Pharma. Ltd. India, were purchased from the local market. The responses of the tablet dosage form were measured at 228 nm for quantification of MET, GLZ and PIO by using LC method above. The amounts of MET, GLZ and PIO present in sample solutions were determined by adjusting the responses into the regression equations for MET, GLZ and PIO.

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RESULTS AND DISCUSSION

The absorption spectra of MET, GLZ and PIO greatly overlap; so conventional determination of these compounds in mixture is not possible. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for MET, GLZ and PIO were obtained with a mobile phase consisting of Acetonitrile: methanol: water (10:30:60 v/v), pH 5 adjusted using Ortho-phosphoric acid buffer. Quantification of the drugs was performed at 228 nm. Resolution of the components with clear baseline separation was obtained.

Validation of the Proposed Method

Linearity

Linear correlation was obtained between peak areas and concentrations of MET, GLZ and PIO in range of 20–100 µg/mL, 4- 12 µg/mL and 3-15 µg/mL respectively. The linearity of calibration curves was found to be acceptable over the concentration ranges of for MET, GLZ and PIO with a R^2 0.9997, 0.9983 and 0.9989 values respectively (Table- 1, Fig- 4, 5 and 6). The results show that good correlation existed between the peak area and concentration of the analysts.

Accuracy

The recovery experiments were performed by the standard addition method. The recoveries obtained were 99.80, 99.92 and 99.87% for MET, GLZ and PIO, respectively (Table 2). The high values indicate that the method was accurate. The recovery studies showed that the results were within acceptable limits, above 99.5% and below 100.5%.

Method precision

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 0.110, 0.058 and 0.061 i.e. % RSD below 2.0 indicating that the method is reproducible. The results are shown in (Table No.2)

Intermediate precision

The intra-day RSD values for MET, GLZ and PIO were 0.7674, 1.1703 and 1.2222 %, respectively. The inter-day RSD values for MET, GLZ and PIO were 0.5296, 1.7377 and 0.5154 % respectively. The % RSD (< 2%) values indicate that the method was sufficiently precise (Table 2).

LOD and LOQ

LOD values for MET, GLZ and PIO were found to be 2.240049 µg/mL, 0.499942 µg/mL and 0.586203 µg/mL, respectively. LOQ values for MET, GLZ and PIO were found to be 6.788027 µg/mL, 1.514974 µg/mL and 1.776373 µg/mL respectively (Table 2). These data showed that the method was sensitive enough for the determination of MET, GLZ and PIO.

Specificity

Specificity is the ability of the analytical method to measure analyte response in presence of interferences including degradation products and related substances. Specificity was checked by determining MET, GLZ and PIO in laboratory prepared binary mixture and in binary mixture containing different degradation products (Table 2 and 3).

Robustness

The method was found to be robust with no significant changes on test result upon change of analytical conditions like different flow rate, amount methanol in mobile phase and pH of mobile phase with the standard deviation was found to be Bellow 1 and % RSD is less than 2 for all results. It was found that under small deliberate changes of chromatographic factors, there was no considerable change in under study parameters (Table 4).

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System Suitability Test

A sample solution of 100 µg/ml of MET, 12 µg/ml of GLZ and 3 µg/ml of PIO (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results (Table 4) obtained from system suitability tests are in agreement with the official requirements.

Table 1: Regression analysis of the calibration curves for MET, GLZ and PIO in the proposed HPLC Method

Parameter	Metformin HCl	Gliclazide	Pioglitazone
Linearity Range (µg/mL)	20-100	4-12	3-15
Detection Wavelength (nm)	228		
Slope ± SD	69.506	27.845	116.45
Intercept ± SD	219.83	18.422	0.26
Correlation coefficient	0.9997	0.9983	0.9989

SD- Standard deviation

Table 2: Summary of the validation parameters for the proposed HPLC method

Parameter	MET	GLZ	PIO
LOD	2.240049	0.499942	0.586203
LOQ	6.788027	1.514974	1.776373
Accuracy	99.80	99.92	99.87
Repeatability (%RSD, n = 5)	0.110	0.058	0.061
Precision (%RSD)			
Inter-day, n = 5	99.49 (0.5296)	98.16 (1.7377)	98.66 (0.5154)
Intra-day, n = 3	99.16 (0.7674)	98.91 (1.1703)	98.33 (1.2222)

LOD = Limit of detection.
LOQ = Limit of quantification
RSD = Relative standard deviation.

Table 3: Assay results for the combined dosage form using the proposed HPLC method

Formulation	MET	GLZ	PIO
Glycinorm Total 60	99.32 ±0.25849	99.52 ±0.33515	99.78 ±0.05594

SD =Standard deviation, 5 determinations

Table 4: System suitability test parameters for MET, GLZ and PIO for the proposed HPLC method

System Suitability Parameters	MET	GLZ	PIO
Retention Time (tR)	2.82	3.40	3.85
Capacity Factor (k)	1.827	2.408	2.859
Theoretical Plate Number (N)	3034.43	4409.60	5080.05
Asymmetry	1.546	1.295	1.205
Resolution Factor (R)	0	2.836	2.146

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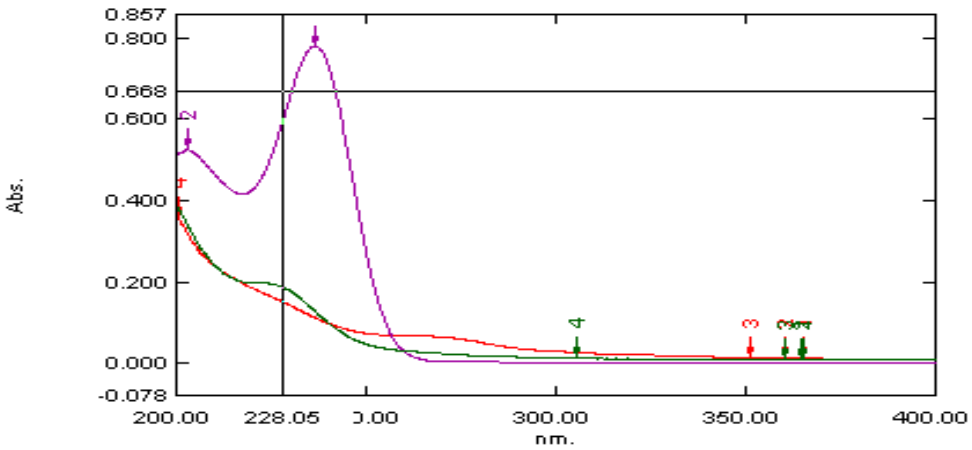
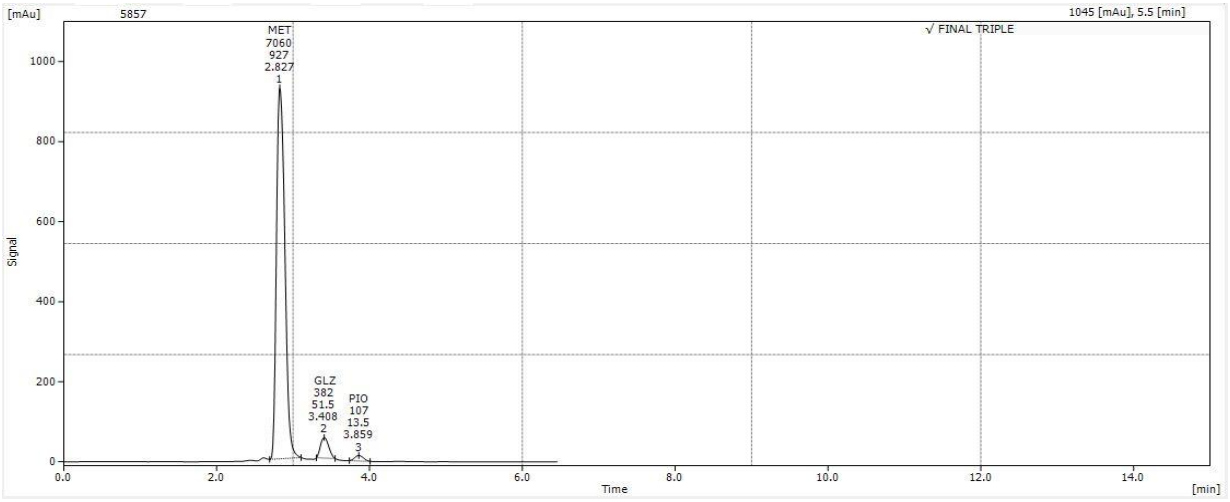


Figure 2: Overlay Spectra of Samples.

Figure 3: Typical liquid chromatogram obtained for a 20 µL injection of a tablet of MET, GLZ and PIO



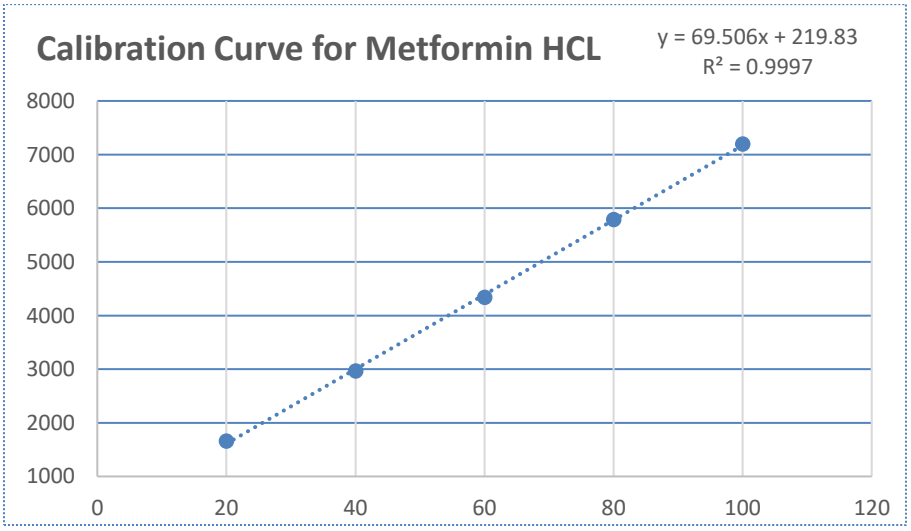


Figure 4: Calibration Curve for Metformin HCL

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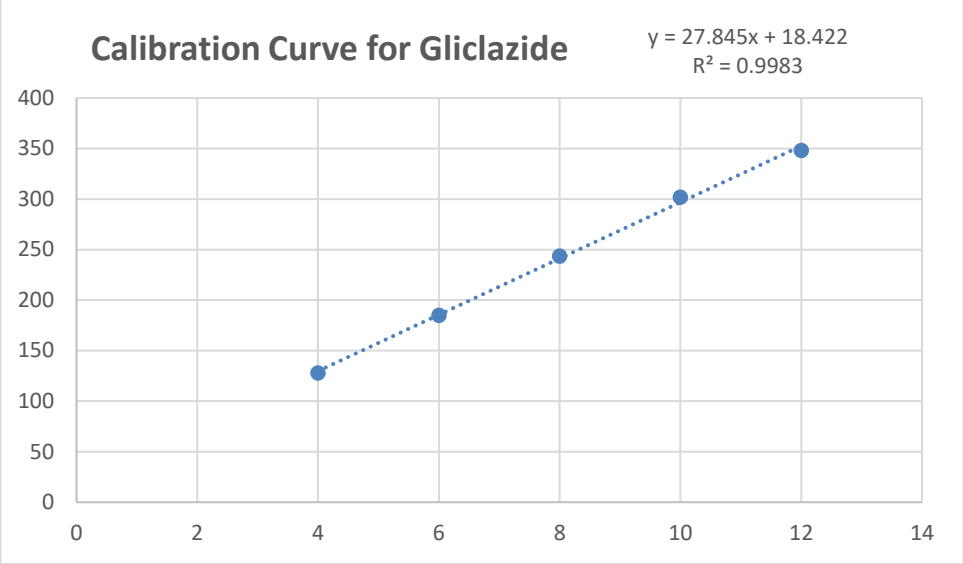

Figure 5: Calibration Curve for Gliclazide

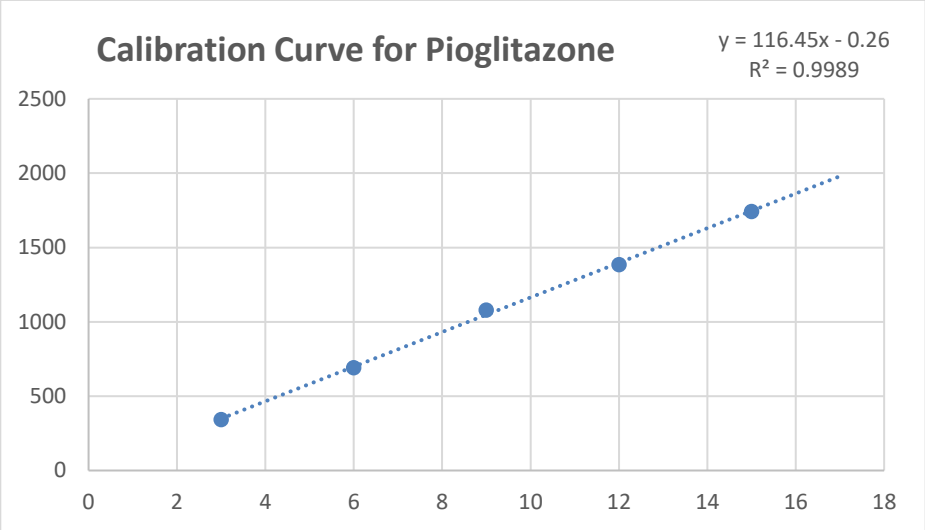

Figure 6: Calibration Curve for Pioglitazone

CONCLUSIONS

The proposed RPHPLC method presented in this paper has advantages of simplicity, precision and convenience for separation and quantitation of MET, GLZ and PIO in combination and can be used for the assay of their respective dosage form. Moreover, the proposed method is a stability indicating assay method that can determine MET, GLZ and PIO in presence of their degradation products. Thus, the proposed method can be used for the quality control of MET, GLZ and PIO in typical laboratories.

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