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Method Development and Validation of RP-HPLC Method for Simultaneous Analysis of Three Component Tablet Formulation containing Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide

Narsimha rao Doredla[1]*, Chandana Mannepalli[2]

[1] Department of Pharmaceutical Analysis Sarada College of Pharmaceutical Sciences, Kondakavuru, Narasaraopet, Guntur (D.t),Andhra Pradesh, India.
Corres.: simham64@gmail.com . Phone No: +91-9985361467

[2]Department of Pharmaceutical Analysis, A.M Reddy Memorial College of Pharmacy, Petlurivaripalem, Narasaraopet, Andhra Pradesh, India.
Corres.: chandanamannepalli@gmail.com, Phone No: +91-7893539891

Abstract: A simple, rapid, and precise reversed-phase high-performance liquid chromatographic method for simultaneous analysis of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide in a tablet dosage form has been developed and validated. Chromatography was performed on a 25 cm × 4.6 mm i.d., 5-µm particle, C18 column with 60:40 (*v*/*v*) acetonitrile—0.5Mm potassium dihydrogen phosphate buffer (pH adjusted to 3.0 ± 0.1 with 5% orthophosphoric acid) as mobile phase at a flow rate of 1.2 mL min−1. UV detection was performed at 230 nm. Total run time was 10 min; metformin hydrochloride, pioglitazone hydrochloride, and glibenclamide were eluted with retention times of 1.75, 2.22, and 6.483 min, respectively. The method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the concentration ranges 200–1000 µg mL−1 for metformin hydrochloride, pioglitazone hydrochloride and glibenclamide. Limits of detection were 6.3, 15.4, and 8.2 ng mL−1 and limits of quantification were 19.09, 46.66, and 24.84 ng mL−1 for metformin hydrochloride, pioglitazone hydrochloride, and glibenclamide, respectively. The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of the three drugs in tablets. The validated method was successfully used for quantitative analysis of TriGlycomet tablets.

Key Words: RP-HPLC, validation, Metformin hydrochloride, Pioglitazone hydrochloride, Glibenclamide

Introduction

Metformin chemically N, Ndimethyl imidodicarbonimidic diamide hydrochloride is used as antidiabitic drug from the biguanide class used in the management of type 2 diabetes. Major action of metformin lay in increasing glucose transport across the cell membrane in skeletal muscle[(1)] Pioglitazone hydrochloride (PIO) is chemically [(±)-5-[[4-[2-(5-ethyl-2- pyridinyl) ethoxy] phenyl] methyl] -2, 4-] thiazolidinedione monohydrochloride. It is a potent agonist for peroxisome proliferator activated receptor-gamma (PPARγ), activation of which modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism. Glibenclamide is 1- [4- [2-(chloro-2-methoxybenzamido) ethyl]-benzene sulphonyl] 3cyclohexylurea,5-chloro-N-[2-[4[[[(cyclohexyl(amino)carbonyl]-amino]sulphonyl] phenyl] ethyl]-2-methoxy benzamide or 1-[[p-[-2-(5-chloro-oanisamido) ethyl]phenyl]-sulphonyl-3-cyclohexylurea,a sulphonyl urea derivative is a second generation oral hypoglycaemic agent which is more potent than those of first group[(2)] and is used to assist in the control of mild to moderately severe type II. diabetes mellitus (adult, maturity-onset) that does not require insulin, but that can be adequately controlled by diet alone. It is drug of choice for initiating treatment in noninsulin-dependent diabetes when diet and weight control fails. It stimulates the secretion and enhances the utilization of insulin by appropriate tissues[3]. The chemical structures of Metformin HCl Pioglitazone HCl and Glibenclamide are shown in fig. 1.

Several assay techniques have been described for quantitative determination of metformin, pioglitazone and glibenclamide in individual and in combination. The UV Spectroscopy determination [(4-5)], UV and HPLC determination [(6)], HPLC determination [(7-15)], HPTLC determination[(16-19)]

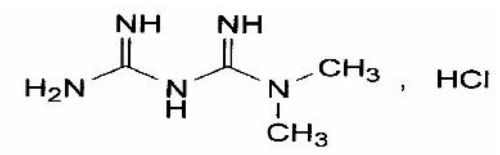


Fig1 (a) chemical structure of Metformin

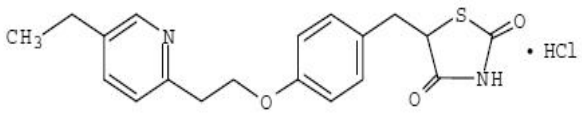


Fig1 (b) chemical structure of Pioglitazone

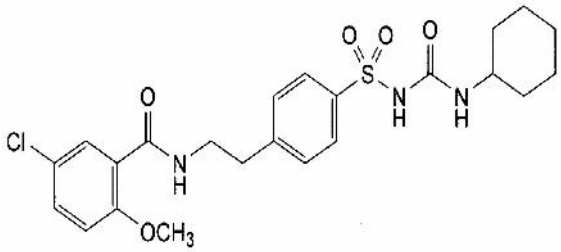


Fig1(c) chemical structure of Glibenclamide

Experimental

Materials and methods:

Glibenclamide (GLB) and Metformin HCl (MET) were supplied by Aribindo pharmaceuticals, India as gift sample and used as such. Methanol used was spectro grade from Qualigen fine chemicals Ltd, India. Water used was HPLC grade, Potassium Dihydrogen Phosphate - AR grade**,** Ortho Phosphoric Acid - AR grade**,** Acetonitrile - HPLC grade.

Instrumentation:

Quantitative HPLC was performed on an low pressure gradient LC–2010CHT SHIMADZU High-Pressure Liquid Chromatographic instrument for the analysis. The instrument is provided with solvent delivery module with PDA detector SHIMADZU phenomix, ODS Reverse phase column (250 X 4.6mm). An auto injector and window based CLASS VP software was used for its automatic operation, recording and analysis. A Sartorius electronic balance was used for weighing the materials**.**

Preliminary solubility studies of drugs

Solubility of three drugs was determined at 28±1 C. A small quantity of standard drugs were dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, and P^H 4, 7, 9 buffer solutions. By the solubility studies we determined that all the three drugs were dissolved in methanol.

Selection of mobile phase

Pure drug of Metformin (MET), Pioglitazone (PIO) and Glibenclamide (GLB) of mixed standard stock solution (100µg/mL of MET, 100µg/mL of PIO and 100µg/mL of GLB) were taken and 10µL sample was injected in to RP - HPLC system and run in different solvent systems. Different mobile phases systems like acetonirile: potassium dihydrogen phosphate buffer (KH_2PO_4), acetonitrile: acetic acid, acetonirile: water and

methanol: water: acetonitrile were tried in order to determine the best conditions for the effective separation of Metformin (MET), Pioglitazone (PIO) and Glibenclamide (GLB) The mobile phase consisting of acetonitrile and 0.5Mm potassium dihydrogen phosphate buffer (KH_2PO_4) pH is adjusted to 3±0.1 in the ratio of (60:40% *v/v*) was selected as it gave high resolution for MET, PIO and GLB with minimal tailing.

Preparation of mobile phase

The mobile phase consisting of acetonitrile: 0.5Mm potassium dihydrogen phosphate buffer (KH_2PO_4) pH is adjusted to 3±0.1 in a ratio of (60:40% *v/v*) was prepared and ultrasonicated for 20 minutes. The mobile phase was then filtered through a 0.45 µ membrane filter.

Buffer preparation:

8g of potassium dihydrogen was weighed and dissolved in 100ml of water and volume was made up to 1000mL with water. Adjust the pH to 3.0 ±0.1 using dilute Ortho phosphoric acid. The buffer was filtered through 0.45 µ filters to remove all fine particles and gases.

Selection of analytical wavelength

By appropriate dilutions of the standard stock solutions with methanol, various concentrations of MET,PIO and GLB were prepared separately and their overlain spectra was obtained using the double beam UV visible spectrophotometer 1700 in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. From the overlain spectra, it was observed MET; PIO and GLB exhibited strong absorbance at about 230 nm which was selected as the analytical wavelength for further analysis.

Selection of Flow rate

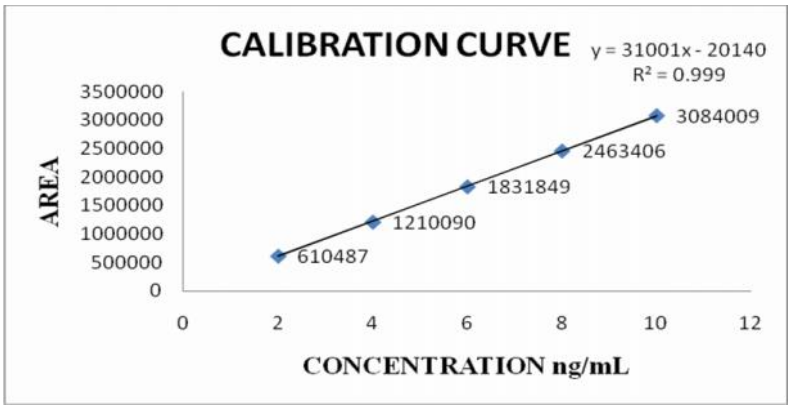
Pure drug of Metformin (MET), Pioglitazone (PIO) and Glibenclamide (GLB) was injected into the RP - HPLC system using mobile phase of acetonitrile and potassium dihydrogen phosphate buffer (KH_2PO_4) pH is adjusted to 3±0.1 in the ratio of (60:40% *v/v*) for this different flow rates of 0.5mL/min,0.8mL/min,1mL/min,1.2mL/min and1.5mL/min were tried. The best retention time and separation was obtained at 1.2mL/min so 1.2mL/min was used as flow rate.

Preparation of standard stock solutions

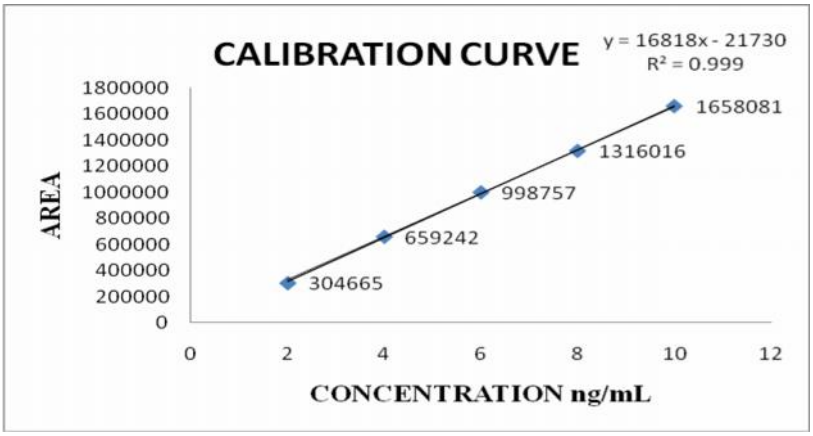
About 10 mg of MET, PIO and GLB was accurately weighed and transferred to 100 ml volumetric flasks respectively. It was dissolved in methanol and the solution was made up to volume with same solvent to obtain stock solutions of concentration 100µg/ml of MET, PIO and GLB.

Preparation of Standard Calibration curves

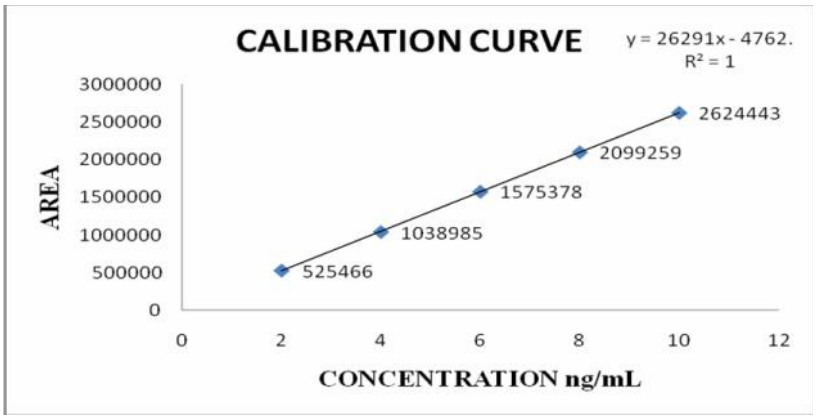
From the standard stock solutions of all three drugs, were filtered and sonicated and filled in vails and different injection volumes like 2µL,4µL,6µL,8µL and 10µL were injected in to HPLC to obtain the concentrations of 200ng-1000ng/mL. Chromatographed and the peak areas were measured. **Calibration plots of concentration against peak area were then constructed for MET, PIO, and GLB as shown in Fig:** From the calibration plots unknown assay samples were quantified by reference to these calibration plots.



1. HPLC Linearity graph for Metformin



2. Linearity graph for Pioglitazone



3. Linearity graph for Glibenclamide

Analysis of the marketed formulation

Twenty tablets (TriGlycomet*, manufactured by USV LIMITED, Mumbai) were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10 mg of powder was transferred to a 10 ml volumetric flask and dissolved in methanol. After the immediate dissolution, the volume was made up to the mark with same solvent. The solution was sonicated for about 5 minutes and then filtered through a 0.2µm membrane filter. The filtrate was taken in to the HPLC vail and injected in to HPLC under the optimized chromatographic conditions. Area of each peak was measured at selected wavelengths. The amount of each drug present in the sample was determined using the prepared standard calibration curves of MET, PIO, and GLB Fig. represents the chromatogram of MET, PIO, and GLB in Tablet formulation. The results of analysis of tablet formulation are reported in Table 1.

Table 1: Results from assay of the tablet formulation by HPLC

Drug	Label claim (mg per tablet,*n* = 6)	Amount found (mg)	Drug content (%)	S.D.	COV (%)	S.E.
MET	500	504.25	100.85	1.185	1.184	0.684
PIO	15	15.26	101.76	0.198	0.194	0.112
GLB	5	5.01	100.56	1.433	1.425	0.827

MET: Metformin, PIO: Pioglitazone, GLB: Glibenclamide, S.D: standard deviation; COV: coefficient of variance; S.E: standard error

Validation

Linearity

Linearity was determined separately for MET, PIO, and GLB by plotting peak area against concentration. From these calibration plots it was clear that response was a linear function of concentration over the ranges 200–1000 ng/mL for Metformin hydrochloride Pioglitazone hydrochloride and Glibenclamide as shown in graphs 1, 2, 3. The linear regression equations for MET, PIO, and GLB

MET y = 31002x − 20160 (r2 =0.999)

PIO y = 18818x − 18773 (r2 =0.999)

GLB y = 26291x− 4762 (r2 =1)

Where *y* is response (peak area) and *x* the concentration

Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentrations, 80, 100, and 120% of those expected, in accordance with ICH guidelines, by replicate analysis (n = 6). Standard drug solutions were added to a preanalyzed sample solution and percentage drug content was measured. The results from study of accuracy are reported in Table 2. From these results it was clear that the method enables very accurate quantitative estimation of MET, PIO, and GLB in tablet dosage form, because all the results were within acceptable limits, i.e. COV < 2.0% and S.D. < 1.0.

Precision

Precision was studied both intra-day and inter-day. Six replicate sample solutions were prepared from the stock solution. For study of intra-day precision the concentrations of the three drugs were measured three times on the same day at intervals of 1 h. In the inter-day study the drug concentrations were measured on three different days. The results are reported in Table 3.

LOD and LOQ

The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the blank and S is the slope of the calibration plot. The results are reported in Table 4.

Table 2: Results from Accuracy study by HPLC

Drug	Amount taken (mg mL−1)	Amount added		Recovery (%,±S.D.)	COV (%)
		%	mg mL−1		
MET	500	80	400	101.46 ± 0.43	0.423
		100	500	101.75 ± 0.33	0.324
		120	600	100.80 ± 0.72	0.714
PIO	15	80	12	102.67 ± 0.84	0.818
		100	15	100.49 ± 0.90	0.895
		120	18	101.19 ± 0.61	0.603
GLB	5	80	4	99.96 ± 0.73	0.730
		100	5	102.57 ± 0.63	0.614
		120	6	101.74 ± 0.81	0.796

MET: Metformin, PIO: Pioglitazone, GLB: Glibenclamide, S.D: standard deviation;
COV: coefficient of variance

Table 3: Results from determination of intra-day and Inter-day precision by HPLC

Drug	Intra-day precision (COV, %)	Inter-day precision (COV, %)		
		Day 1a	Day 2a	Day 3a
MET	0.393	0.573	0.437	0.625
PIO	0.239	0.369	0.613	0.873
GLB	0.439	0.781	0.469	0.581

A Mean from six determinations COV: coefficient of variance;

Table 4: Results from determination of LOD and LOQ by HPLC

Drug	LOD (ng mL−1)	LOQ (ng mL−1)
MET	6	19.09
PIO	15.4	46.66
GLB	8.2	24.84

MET: Metformin, PIO: Pioglitazone,
GLB: Glibenclamide,
LOD: limit of detection; LOQ; limit of quantitation**;**

Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of all three drugs. It was observed that three sharp peaks for MET, PIO, and GLB were obtained at retention times 1.75, 2.22, and 6.483 min, respectively; these peaks were not obtained from placebo solution. The specificity of the method was assessed by comparing chromatograms obtained from drug standards with that obtained from tablet solutions. The retention times of the drug standards and the drugs from sample solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the tablets.

System-suitability study

Under the optimum chromatographic conditions, the retention times obtained for MET, PIO, and GLB were 1.362, 3.418, and 7.395 min, respectively Resolution (*RS*) between MET and PIO and between PIO and GLB was 2.05 and 3.94, respectively. Capacity factors, tailing factors, and number of theoretical plates are reported in Table 5.

Table 5: Results from system-suitability study by HPLC

Property (*n* = 6)	**MET**	**PIO**	**GLB**
*r*t	1.75	2.22	6.483
*T*f	1.37	1.22	1.10
k′	1.23	1.51	4.42
N	2304	7696	12012
RS	–	2.05	3.94

MET: Metformin, PIO: Pioglitazone, GLB: Glibenclamide, rt: retention time;
Tf: tailing factor; k′: capacity factor; N: number of theoretical plates; RS: resolution

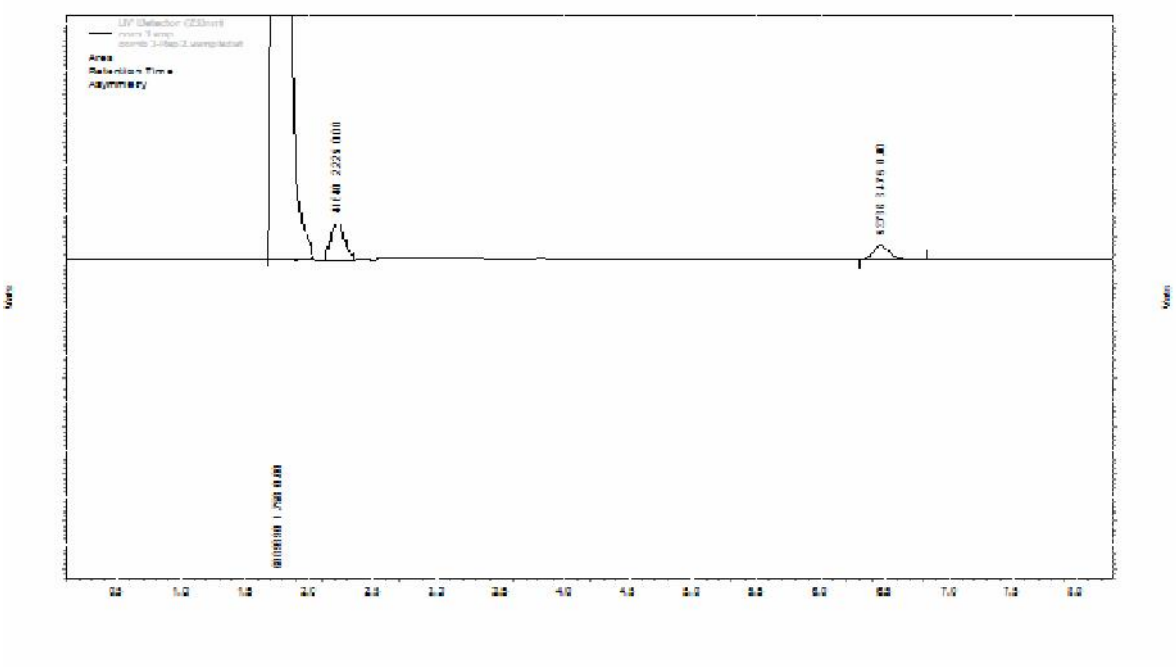


Figure-2: HPLC Chromatogram of Mixed Standard drugs of Metformin, Pioglitazone and Glibenclamide

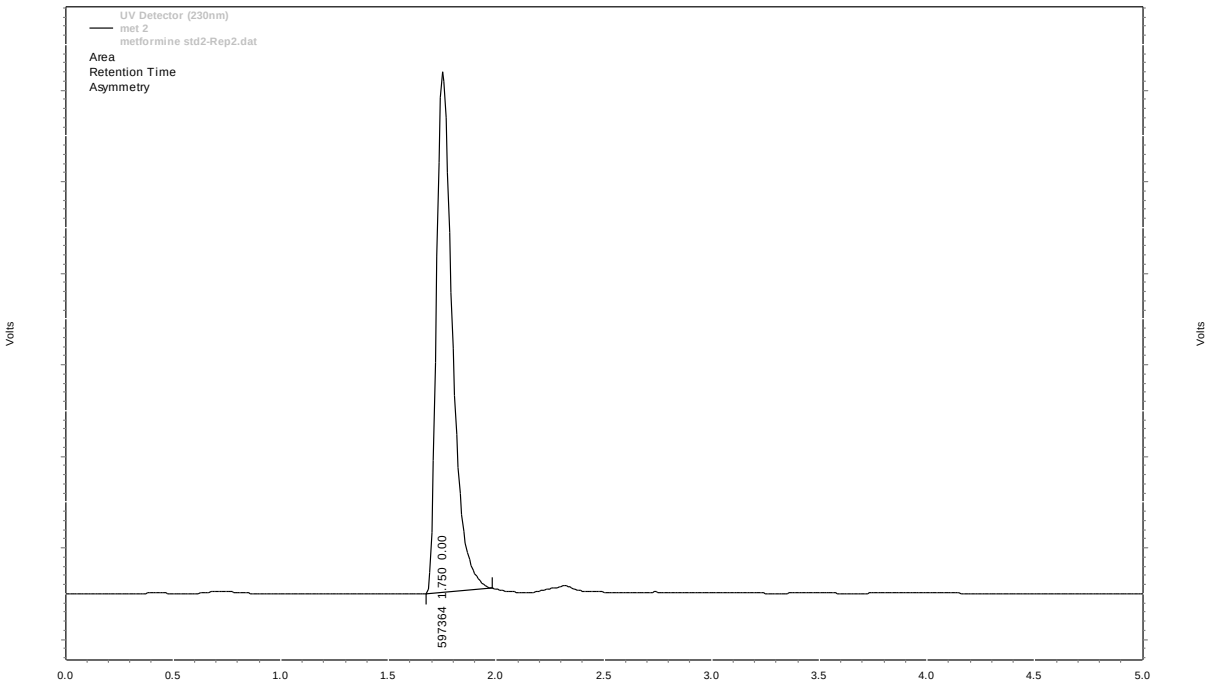


Figure-3: HPLC Chromatogram of Metformin Standard

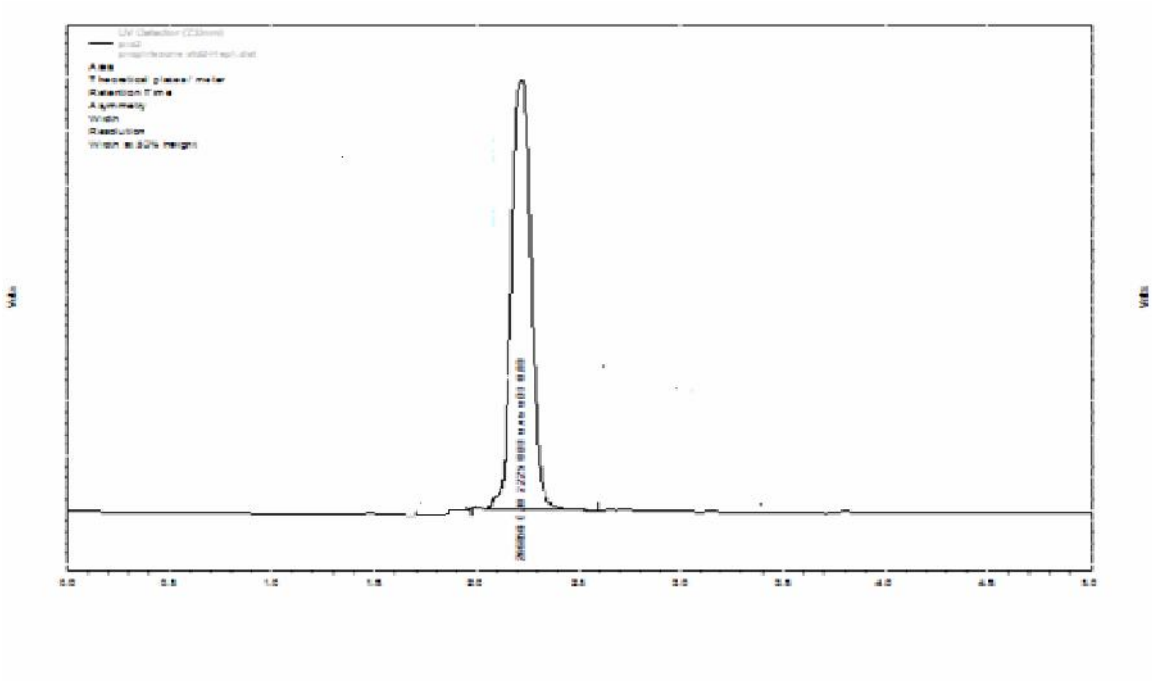


Figure-4: HPLC chromatogram of Pioglitazone Standard

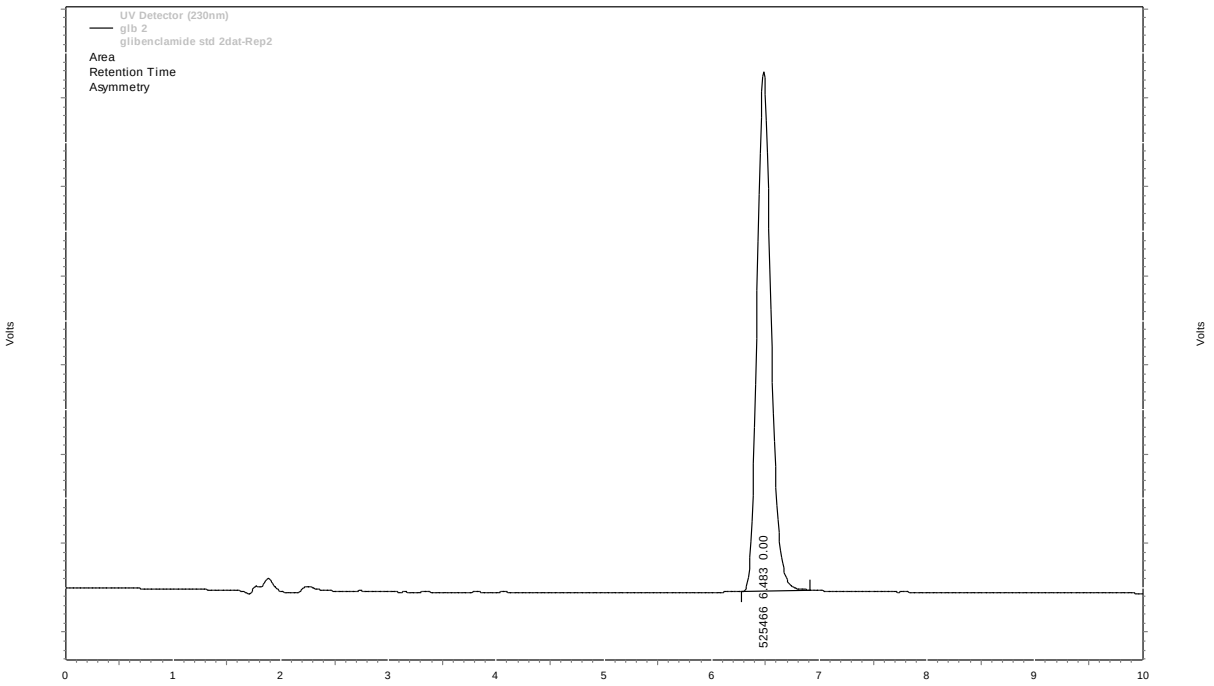


Figure-5: HPLC chromatogram of Glibenclamide Standard

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