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Method development and validation for simultaneous estimation of Pioglitazone and Glimepiride in tablet dosage form by RP-HPLC and UV- Spectrophotometric method.

[*1]Vinod Kumar K, [1]Sudhakar M, [2]Padmanabha Reddy Y, [2]Swapna A, [2]Rajani Sekhar V.
[1]Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, Andhra Pradesh, India. [2]Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, India.

Abstract
The present work deals with the development of a reliable method for the simultaneous estimation of Pioglitazone and Glimepiride in a combined tablet dosage form by using RP – HPLC method and UV-Spectrophotometric method. Both methods were validated and compared for sensitivity and linearity. The RP-HPLC method utilized Gliburide (Glibenclamide) as an internal standard and the mobile phase composition was Methanol and water which gave a retention time of 4.34 min and 5.19 min for Glimepiride and Pioglitazone respectively. The linearity range was between 1 -15µg/ ml and 3 - 45µg/ml for glimepiride and pioglitazone respectively. The accuracy of the method was found to be in the range of 98 – 102 %. The precision studies were carried out on three concentrations in three replicates and the % RSD was found to be less than 2%. The method proved specificity for both drugs. The UV spectrophotometric method utilized simultaneous equation for the estimation of the drugs. The linearity range was between 3- 24 µg/ml for glimepiride and 4 – 32 µg/ml for pioglitazone. The recovery was in the range of 103 – 110 %. The precision studies were carried out using three concentrations in three replicates and the %RSD was found to be less than 2 %. Both these methods have been successively applied to pharmaceutical dosage formulation and were validated according to ICH guidelines.

Key Words
Pioglitazone, Glimepiride, Simultaneous estimation, RP- HPLC.

Introduction
Pioglitazone, a thiazolidinedione derivative and Glimepiride, a sulfonyl urea are Anti- Diabetic agents. Pioglitazone acts by binding to PPARγ, which activates insulin-responsive genes that regulate carbohydrate and lipid metabolism[1]. It reduces the insulin resistance in liver and peripheral tissues and increases the expense of insulin dependent glucose where as Glimepiride acts as secret gouge and induces the secretion of insulin from pancreatic β- cells and also reduces the hepatic clearance of the insulin hormone[1]. Both drugs together produce a hypoglycemic effect. The present method involves the simultaneous estimation of both the drugs in a combined dosage form. Thorough survey of the literature has revealed that many methods have been developed for the estimation of the selected drugs individually. Many methods were also developed for the simultaneous estimation if these drugs which utilized the mobile phase compositions majorly consisting of acetonitrile and phosphate or ammonium acetate buffers[2-9]. UV-spectrophotometric methods also have been developed for the estimation of these drugs using Derivative Spectrophotometric method[10-11]. The present work utilizes methanol and water as mobile phase which is economic than the methods reported so far. The present work also involves development of UV- spectrophotometric method using simultaneous equation. This work concentrates on developing a specific, accurate, reliable, validated and cost effective method for the simultaneous estimation of the selected drugs.

***Corresponding Author:**
vinodpharma@gmail.com

Materials and Methods
Apparatus
Agilent LC 1120 equipped with manual rheodyne injector of injector volume 20 µl and variable wavelength detector. Agilent TC – C18 (2) column of dimensions 250 × 4.6 mm, 5 µ was used.
Systronics 2202 UV – Visible Double Beam Spectrophotometer equipped with photo diode detector was used. It was operated in single and multi-wavelength modes.

Axis LCGC electronic balance was used for weighing of the materials.

Chemicals

Pioglitazone and Glimepiride were procured from Hetero Labs. Methanol and Water o HPLC grade were purchased from Merck. Ultipore N_{66} nylon filters of 0.45 µm and 0.22µm were purchased from Pall Life Sciences.

Method development for RP–HPLC

Preparation of Standard Solution

The Standard Solutions of Pioglitazone and Glimepiride were prepared by dissolving 10 mg of each separately in small quantity of methanol and were sonicated for 15 minutes. Then the volume was made up with methanol. From this stock solution, a solution of concentration 100µg/ml was prepared for each drug.

Chromatographic conditions

The mobile phase consisted of methanol, water in the ratio of 72:28 contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 10min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0ml/min and the injection volume was 20µl.The column temperature was maintained at 23 ± 1^0C. The eluents were monitored at 230nm. The optimized chromatographic conditions were summarized in Table No. 1.

Selection of internal standard

The internal standard was selected on the basis of purity, polarity, solubility and absorption characteristics. Ramipril, Metformin and Gliburide (Glibenclamide) were tried and chromatographed with standard drugs. The elution times were found to be 2.5, 8.2, 3.9 minutes respectively. Of all the internal standards tried, Gliburide exhibited well resolved and symmetrical peak. For the present study, Gliburide was therefore, selected as internal standard. The standard chromatogram was shown in figure 1.

Preparation of Calibration curve

The separate standard calibration curves were plotted for each component. Different volumes of stock solutions were accurately transferred in to 25 ml volumetric flasks and diluted to mark to yield concentration range 1-15µg/ml for Glimepiride and 3-45µg/ml for Pioglitazone. 9 solutions of each were prepared and the final volume was made up to the mark. The calibration line was obtained by plotting the peak area against concentration of drug.

System suitability

The system suitability tests were carried out and the results were tabulated in Table No. 2.

Analysis of Glimy-P tablets

Ten tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets of 2mg Glimepiride and 15mg Pioglitazone was extracted with methanol in a 100ml volumetric flask, and 50ml of methanol was added to the same. The flask was sonicated for 10min and volume was made up to the mark with methanol. The above solution was filtered using whatman 1 filter. 1ml was transferred into a 10ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 2 µg/ml of Glimepiride and15 µg/ml of Pioglitazone. The solution was sonicated for 10min and injected under above chromatographic conditions and peak area was measured. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated and shown in Table No. 3 and Figure 2.

Method development for UV - Spectrophotometer

Preparation of Standard Solution

The standard solutions were prepared by dissolving 100 mg of each drug in 100 ml of methanol separately. The stock solution was suitably diluted with methanol to get a concentration of 10 µg/ ml of Pioglitazone and Glimepiride.

Preparation of Test sample

20tablets (GLIMY-P GLI 2mg & PIO 15mg) were accurately weighed and powdered and weighed powder equivalent to 100mg of GLI and PIO and dissolved in 100ml methanol(100%) in 100ml volumetric flask and filtered. 10ml from the above solution was pipette out and diluted to 100ml with methanol.10ml from the above solution was pipette out and dilute with 100ml methanol.

Procedure

The standard solutions of 10µg/ml GLI and PIO were scanned in the wave length range of 200nm-400nm for simultaneous equation method. The maximum wavelength of two drugs was found to be 225.6 nm for GLI and 267.2 nm. The concentration was estimated by using simultaneous equation. The absorptivities (A1%, 1cm) of both the drugs at the wavelengths were determined. The absorbance and absorptivities values at the particular wavelength were substituted in the following to obtain the

concentration of x and y drugs. The results were represented in Table No. 4 and 5.

$$Cx = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$Cy = \frac{A_1\, ax_2 - A_2\, ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Validation[12-13]

Both methods were validated for the following characteristics: linearity, accuracy, precision, specificity, limit of detection, limit of quantitation, robustness, and ruggedness.

Specificity

Specificity of the HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities, degradants or excipients. A volume of 20 µl of working placebo sample solution was injected and the chromatogram was recorded. No peaks were found at retention time of 4.34 and 5.14 min. Hence, the proposed method was specific for Pioglitazone and Glimepiride.

Linearity

The linearity of calibration curves in pure solution was checked over the concentration range of 1- 15 µg/ml for Glimepiride and 3 -45 µg/ml for Pioglitazone through HPLC method. In UV method, the linearity was determined over a concentration range of 1 -30 µg/ml for Glimepiride and 2 -26 µg/ml for Pioglitazone. The data was represented in Table No.6.

Precision

The intra – day and inter – day precision of the HPLC method was tested by computing the %RSD for three replicates over three concentration ranges of Glimepiride and Pioglitazone. The precision of the UV method was tested by measuring the absorbance at wavelength of maximum absorbance of both drugs in three replicates. The results were shown in Table No. 9.

Accuracy

Accuracy was determined by percentage recovery studies. The reference standard of the drug was spiked at 80%, 100%, 120% level to the formulation and recovery studies were carried out in three replicates using HPLC and UV methods. The percentage recovery and % relative standard deviation were calculated and the results were shown in Table No.7 and Table No. 8 respectively.

Limit of Detection and Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by examining the signal to noise ratio in HPLC method and the LOD and LOQ of UV method were determined by visual evaluation. The results were tabulated in Table No. 10.

Robustness

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the pump flow rate (± 0.1%) and organic solvent content (± 2%). None of the alterations caused a significant change in peak area RSD, USP tailing factor and theoretical plates. Although the changes in the retention time were significant, yet quantitation was possible. The results were tabulated in Table No. 11.

Conclusion

The validated HPLC and UV methods employed here proved to be simple, rapid, precise, accurate and cost effective. The specificity experiment showed that there was no interference from the excipients. The low LOD and LOQ values proved the method to be sensitive. The proposed method can be applied for routine analysis for the estimation of bulk drugs and pharmaceutical dosage forms.

References

1. Goodman Gilman, The Pharmacological Basis of Therapeutics, 11th Ed, 2006, 1703 -1706.
2. Ravi Sharma, Gagan Sharma, Darpan Chopra and Pavan Kumar Jain,Analytical method development and validation for the simultaneous estimation of Pioglitazone and Glimepiride in tablet dosage form by HPLC, IJPSR, 2(3), 637-642.
3. J. Ramesh, K.C. Arul prakasam, A.Suresh, A. Phani Kumar, M.Anudeep, Simultaneous estimation Pioglitazone and Glimepiride by RP-HPLC method, J. Global Trends in Pharm.sci, 1(1), 2010, 68-78.
4. B. Praveen Kumar Reddy, D. Boopathy, Bibin Mathew, and P.Perumal,Method development and validation of Simultaneous determination of Pioglitzone and Glimepiride in pharmaceutical dosage form, International Journal of ChemTech Research, 2(1), 2010, 50-53.

5. T.M. Kalyankar, M.R.Badgujar and R.B. Kakde, Simultaneous Determination of Pioglitazone and Glimepiride by RP-HPLC in pharmaceutical dosage form Journal Pharmacy Research, 3(12), 3078-3080.
6. A. Karthik, G.Subbramanian, C.Mallikarjuna Rao,Krishnamurthy Bhat,Simultaneous determination of Pioglitazone and Glimepiride in bulk drug and pharmaceutical dosage form by RP-HPLC, Pak.J.Pharm.Sci, 21(4),2008,421-425.
7. K.S. Lakshmi, T. Rajesh, Shrinivas Sharma,Determination of Pioglitazone and Glimepiride in pharmaceutical formulations and Rat plasma by RP-LC, International Journal of Pharm Tech Research, 1(3), 2009, 496-499.
8. H. Havaldar, L. Vairal, Simultaneous estimation of Glimepiride, Rosiglitazone and Pioglitazone in the pharmaceutical dosage form, E- Journal of Chemistry, 7(4), 2010, 1326-1333.
9. K.S. Lakshmi and T.Rajesh, Development and validation of RP-HPLC method for Simultaneous determination of Glipizide, Rosiglitazone, Pioglitazone, Glibenclamide and Glimepiride in Pharmaceutical dosage form, J.Iran. Chem. Soc., 8(1), 2011,31-37.
10. R.Patel Deepa, J.Patel Laxmanbhai, B.Patel Advaita, Simultaneous estimation of Glimepiride, Pioglitazone and Metformin by Derivative Spectrophotometry method, International Research Journal ofPharmacy, 2(3), 2011, 111-114.
11. K.S Lakshmi, T. Rajesh, S.Sharma, S.Lakshmi,Development and validation of UV derivative Spectrophotometric method for the determination of Metformin, Pioglitazone and Glimepiride in Pharmaceutical Formulations, DER Pharma chemica, 1(2), 20158- 66.
12. ICH – Q2B, Validation of Analytical Procedure : Geneva, Switzerland, 1996BS 7501 EN 45001. General Criteria for the operation of Testing Laboratories 1989.
13. ICH, Q2a, Validation of Analytical Procedure, Methodology, International Conference on Harmonization, Geneva, October 1994.

Table No. 1: Optimized chromatographic conditions.

S.No	Parameters	Glimepiride	Pioglitazone
1	Mobile Phase	Methanol : Water	Methanol : Water
2	Ratio	72:28	72:28
3	Detector	PDA	PDA
4	Detection Wavelength	230nm	230nm
5	Column (Stationary Phase)	C_{18} 250X4.5mm id, 5µm	C_{18} 250X4.5mm id, 5µm
6	Flow Rate	$1ml.min^{-1}$	$1ml.min^{-1}$
7	Column Temp.	23^oC	23^oC
8	Retention time	4.34	5.14
9	Volume of injection (µl)	20	20

Table No. 2 System Suitability parameters

Parameter	Glimepiride	Pioglitazone	Gliburide (internal standard)	Acceptance Criteria
Retention time (min)	4.34	5.14	3.9	--
Plate count	5276	5942	4391	>2000
Tailing factor	0.49	1.05	0.99	≤ 2
Asymmetry (10 %)	0.99	1.01	0.99	0.9 - 1.2
Capacity factor	0.73	1.68	2.8	1 -10
Resolution	--	3	2.6	>2
HETP	0.047	0.042	--	--

Table No. 3: Assay of the tablet dosage form by HPLC method.

Formulation	Labeled claim (mg)	Peak area mean ± SD (n=3)	Amount found(mg) Mean ± SD	Assay	% RSD
Glimepiride	2	3930971±1518	2.03±0.007	101.5	0.34
Pioglitazone	15	14168143±135232	15.1±0.14	100.6	0.954

Table No. 4: Absorbance data.

Name of the Drug	λ_1 225.6 nm	λ_2 267.2 nm
Glimepiride	0.013(ax_1)	0.0388(ax_2)
Pioglitazone	0.0499(ay_1)	0.0173(ay_2)
Tablet	0.780(A_1)	0.358(A_2)

Table No. 5: Assay of the tablet dosage form by UV – method.

Formulation	Labeled claim (mg)	Amount found(mg) Mean	Assay
Glimepiride	2	2.2	110
Pioglitazone	15	15.5	103.3

Table No. 6: Linearity data.

Parameter	HPLC		UV	
	Glimepiride	Pioglitazone	Glimepiride	Pioglitazone
Linearity (µg/ml)	1 -15	3 -45	3 – 24	4 – 32
Regression equation	y = 0.047x + 0.079	y = 0.034x + 0.058	y = 0.045x + 0.061	y = 0.024x + 0.016
Correlation coefficient	0.999	0.998	0.998	0.999
Intercept	0.079	0.058	0.061	0.016
Slope	0.047	0.034	0.045	0.024

Table No. 7: Accuracy of HPLC method.

Drug	Test (spiked)	% Recovery (n = 3)	% RSD
Glimepiride	80 %	98.3	1.41
	100 %	98.2	0.51
	120 %	102.3	0.84
Pioglitazone	80 %	99.2	1.12
	100 %	102.0	0.39
	120 %	100.3	1.20

Table No. 8: Accuracy of UV method.

Drug	Label claim mg/tablet	Amount added	Amount found	% recovery	%RSD
Glimepiride	2	2mg	2.2mg	103.0	0.128
Pioglitazone	15	15mg	15.5mg	110.0	0.269

Table No. 9: Precision.

Precision	HPLC		UV	
	Glimepiride	**Pioglitazone**	**Glimepiride**	**Pioglitazone**
Repeatability	0.66	0.52	0.66	0.55
Intermediate precision				
Day 1	0.52	0.51	0.69	0.62
Day 2	0.48	0.36	0.61	0.51
Day 3	0.44	0.18	0.68	0.52

Table No. 10: LOD and LOQ.

Parameter	HPLC		UV	
	Glimepiride	**Pioglitazone**	**Glimepiride**	**Pioglitazone**
LOD	700 ng/ml	760 ng/ml	0.54 µg/ml	0.37 µg/ml
LOQ	2.12 µg/ml	2.31 µg/ml	1.58 µg/ml	1.2 µg/ml

Table No. 11: Robustness.

S.No	Parameter	Modification	Retention time		Asymmetry	
			GLI	**PIO**	**GLI**	**PIO**
1	Flow rate	0.8ml/min	5.88	6.92	1.20	1.26
		0.9ml/min	5.13	6.06	1.10	1.21
		1.0ml/min	4.31	5.23	1.00	1.00
		1.1ml/min	4.10	4.88	1.13	1.23
		1.2ml/min	3.80	4.50	1.21	1.26
2	Mobile phase composition (Methanol : Water)	74:26	4.1	4.9	1.18	1.10
		72:28	4.3	5.1	1.09	1.08
		70:30	4.8	5.6	1.10	1.14

Table No. 12: Comparison of HPLC and UV methods.

Parameters	HPLC		UV	
	Glimepiride	**Pioglitazone**	**Glimepiride**	**Pioglitazone**
Linearity	1 -15 µg/ml	3 -45 µg/ml	5 – 30 µg/ml	4 -24 µg/ml
Precision (%RSD)	0.53	0.39	0.66	0.55
Accuracy	99.6 %	100.5 %	103 %	110 %
LOD	700 ng/ml	760 ng/ml	0.54 µg/ml	0.37 µg/ml
LOQ	2.12 µg/ml	2.31 µg/ml	1.58 µg/ml	1.2 µg/ml

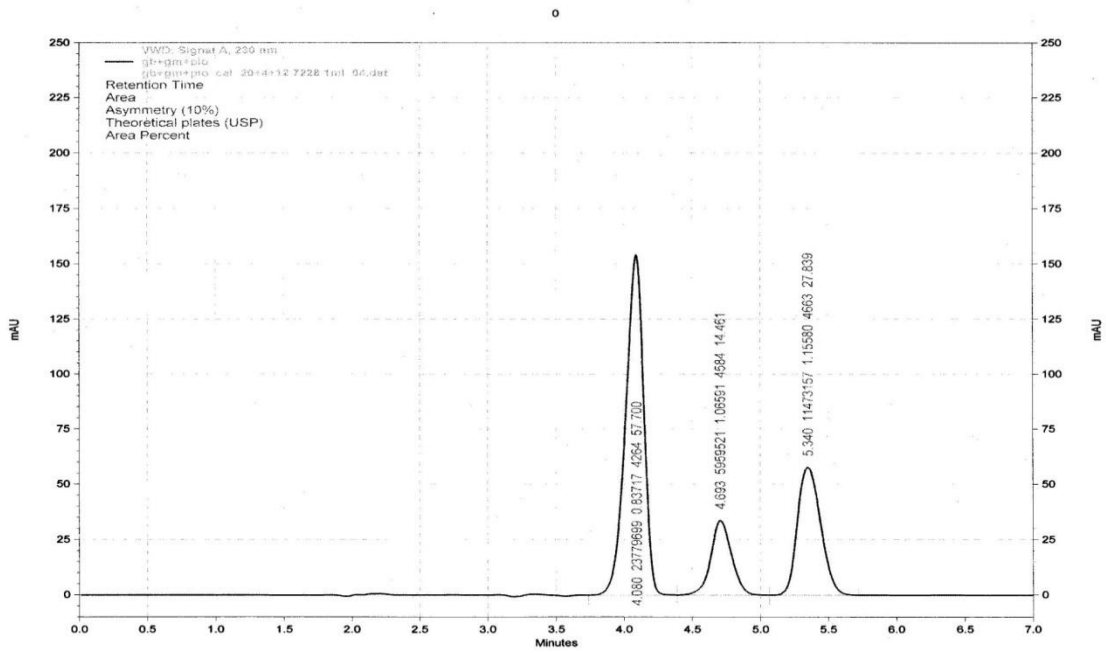

Figure 1: Typical Chromatogram of pioglitazone, Glimipride and IS.

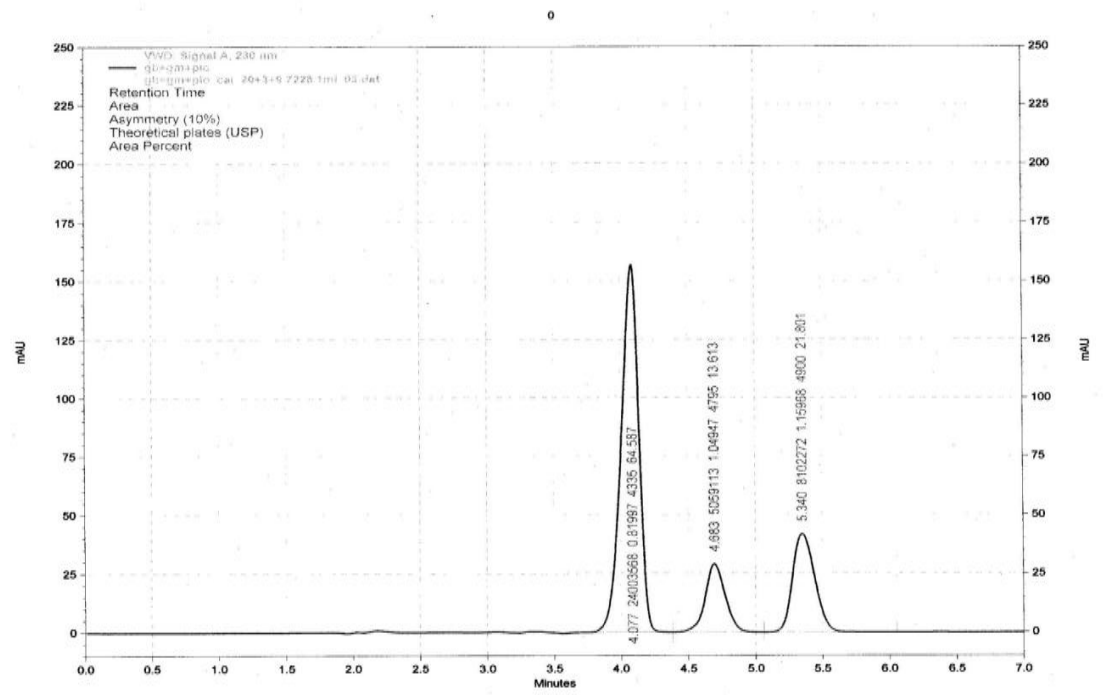

Figure 2: Chromatogram representing the assay of the tablet dosage form.

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