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# A MODIFIED REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GLIMEPRIDE AND PIOGLITAZONE IN BULK AND DOSAGE FORMS

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## ABSTRACT

A modified reverse phase liquid chromatographic method was developed and validated for the simultaneous estimation of glimepride and Pioglitazone in bulk and pharmaceutical dosage forms. The developed and validated method was simple, precise, accurate and economical reverse phase liquid chromatography method. Estimation of drugs in this combination was done with a $C_{18}$ column (Zorbax 100-5 column, 250mm x 4.6mm) passing mobile phase of composition phosphate buffer pH 7: Acetonitrile in ratio 45:55 v/v. The flow rate was 1 ml/min and the column effluents were identified at 230 nm. The retention time of Pioglitazone and Glimepiride were 6.7 min and 8.3 min respectively. The method was found to be linear over a range of 10-50µg/ml for Pioglitazone and 3-15µg/ml for Glimepiride. The validated method proved as reproducible one with a %RSD value of less than 2 and having the selectivity and accuracy within the specified limits. Assay of marketed dosage forms was determined and found to be with 98% and 99% for Pioglitazone and Glimepiride respectively. The method was validated according to the guidelines of International Council for Harmonization (ICH) and was successfully utilized in the simultaneous estimation of the commercial dosage forms. This liquid chromatographic method can be applied for the qualitative and quantitative determination of selected drugs.

**KEYWORDS:** Pioglitazone, Glimepiride, RP-HPLC, Method development and validation.

## INTRODUCTION

Pioglitazone is chemically designated as *RS*)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2,4-dione is a hypoglycemic drug with a pKa value of 14.64. Its molecular formula is $C_{19}H_{20}N_2O_3S$ and it has a molecular weight of 165.52gm/mole. It is soluble in methanol, slightly soluble in acetone and acetonitrile. It is official drug in Martindale,[1] Merck index[2] and Indian Pharmacopeia 2007 and 2014.[3]

Glimepiride is an oral hypoglycemic drug which is chemically designated as1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl-3-(trans-4-methyl cyclohexyl) urea. The molecular formula is $C_{24}H_{34}N_4O_5S$ and molecular weight is 490.62 gm/mol. It is a white to off-white crystalline powder. That is soluble in methanol and dimethyl formamide. It is official drug in Martindale,[1] Merck index,[2] and Indian Pharmacopeia 2007 and 2014.[3]

After extensive literature survey it was proved that very few methods were reported for the estimation of Pioglitazone and Glimepiride individually or in combination by RP-HPLC [4-22] but few methods had been reported for simultaneous estimation of these two drugs. So we attempted to develop an accurate, rapid, precise, stable, sensitive and economically viable liquid chromatographic method for the simultaneous determination of selected drugs in the present research.

## MATERIALS AND METHODS

### Equipment used

The chromatographic separation was performed on Agilent 1120 compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase $C_{18}$ [Zorbax 100-5 column, 250mm x 4.6mm] was used. Elico SL-210 double beam UV visible spectrophotometer and Axis AGN204-PO electronic balances were used for spectrophotometric determinations and weighing purposes respectively.

### Reagents and chemicals

Pharmaceutical grade pure Pioglitazone and Glimepiride gift samples were procured from Mylan Laboratories, Hyderabad. Marketed formulation Tablets with dose of 50 mg of Pioglitazone and 15 mg of Glimepiride were procured from local market (Pioglar by Ranbaxy Pharma). HPLC grade Acetonitrile and Water were

procured from Merck specialties private limited, Mumbai.

**Chromatographic conditions**

Zorbax-$C_{18}$ column 5µm [250mm x 4.6mm] was used for the chromatographic separation at a detection wave length of 230 nm. Mobile phase with a composition of Phosphate buffer pH 7 and Acetonitrile in a ratio of 45:55 v/v was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 1 ml/min and the injection volume was 20µl.

**Preparation of Mobile phase**

Phosphate buffer pH 7 was prepared by dissolve 0.504 gm of disodium hydrogen phosphate and 0.301gm of Potassium dihydrogen phosphate of HPLC grade water and adjusts the pH to 7.0 with sufficient water was added to produce100 ml filtered through 0.45µ membrane filter and sonicated for 15 minutes.

**Preparation of Standard solutions**

25mg each of Pioglitazone and Glimepiride were accurately weighed and transferred into two 25ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (Pioglitazone) and B (Glimepiride) of concentration 1000 µg/ml of each drug. From the primary stock solutions 0.8ml and 0.5 ml were pipette out from A and B respectively, transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 80µg/ml and 50 µg/ml of Pioglitazone and Glimepiride respectively and this solution is (working stock solution A).

**Preparation of Sample Solution**

Twenty tablets of Pioglitazone and Glimepiride were weighed and crushed. Tablet powder equivalent to 300 mg of Pioglitazone and 40 mg of Glimepiride was weighed accurately and transferred to a 25 ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. The volume was made up with the mobile phase and filtered with 0.45µmembrane filter and sonicated for 20 min. 0.8 ml of this solution was pipette out and transferred to a 10 ml volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 80µg/ml of Pioglitazone and 50 µg/ml of Glimepiride (working stock solution B).

**Optimization of RP-HPLC method**

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Pioglitazone and Glimepiride. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Phosphate buffer pH 7 and Acetonitrile (45:55 v/v) using Zorbax-$C_{18}$ column 5µm [250mm x 4.6mm].

**Validation of the RP-HPLC method**

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

**System suitability**

System suitability was carried out with five injections of solution of 100% concentration having 50µg/ml of Pioglitazone and 15 µg/ml of Glimepiride in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factors (T) were reported in table 1.

**Linearity**

For the determination of linearity, appropriate aliquots were pipette out from working stock solution A to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50µg/ml of Pioglitazone and 3-15µg/ml of Glimepiride. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Pioglitazone and Glimepiride were shown in figure 3 and figure 4 their corresponding linearity parameters were given in table 2.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were given in table 2.

**Precision**

The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (50µg/ml of Pioglitazone and 15µg/ml of Glimepiride) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were given in table 3.

**Accuracy**

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analyzed sample and contents were reanalyzed by the proposed method and the percent recovery was reported. The results were given in table 4.

**Specificity**

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients. The optimized chromatogram of Pioglitazone and Glimepiride without any interference was shown in figure 2.

**Robustness**

Robustness of the method was verified by altering the chromatographic conditions like wavelength detection, flow rate, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ±2 nm in the detection wave length and ±0.2 ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. %RSD was reported in the table 5

**Ruggedness**

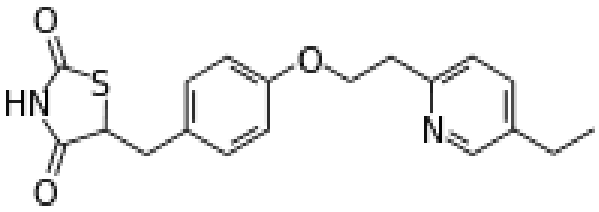
Ruggedness of the method was verified by altering method parameters like different instruments, analysts, laboratories, reagents, days etc. The solution containing 50 µg/ml of Pioglitazone and 15 µg/ml of Glimepiride was injected into HPLC three times under different parameters like different analysts. %RSD was reported in the table 6.

**Assay of Marketed Formulations**

20 µl of sample solution of concentration 50 µg/ml of Pioglitazone and 15µg/ml of Glimepiride was injected into chromatographic system and the peak responses were measured. The solution was injected three times in to the column. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples. A typical chromatogram for assay of marketed formulation was shown in figure 5 and the obtained values were reported in the table 7.

## RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, Phosphate buffer pH 7 and Acetonitrile in the ratio 45:55 v/v was selected as mobile phase because of better resolution and symmetric peaks. Pioglitazone and Glimepiride were found to show appreciable absorbance at 230nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Pioglitazone and Glimepiride at different Rts was shown in figure 2.



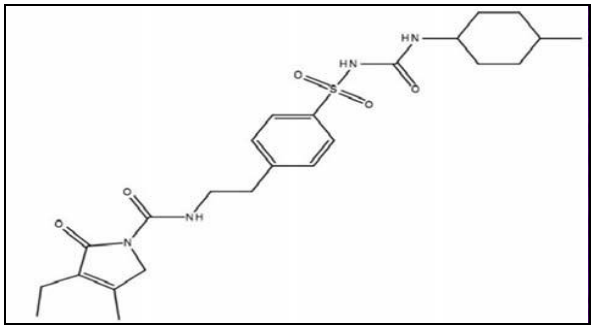




**Fig. 1: Chemical Structures of a) Pioglitazone and b) Glimepiride.**

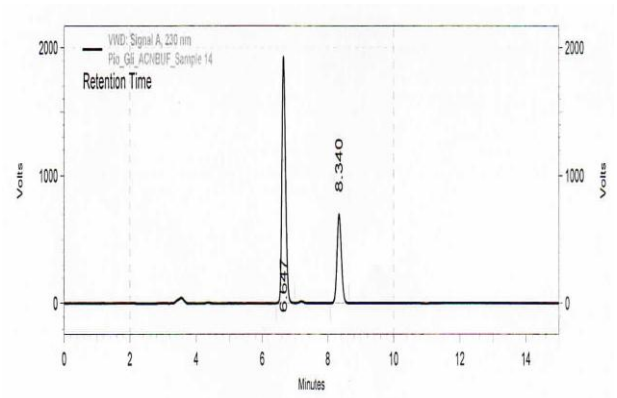



**Fig. 2: Optimized chromatogram of Pioglitazone and Glimepiride.**

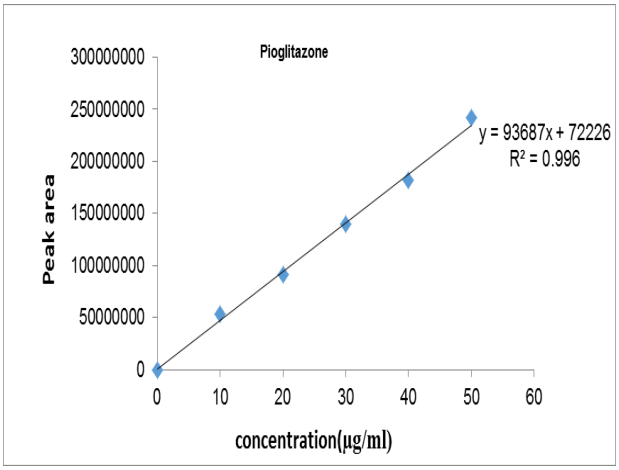



**Fig. 3: Calibration plot of Pioglitazone.**

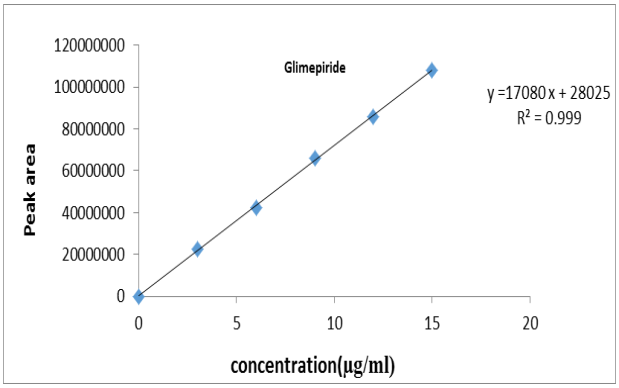



**Fig. 4: Calibration plot of Glimepiride.**

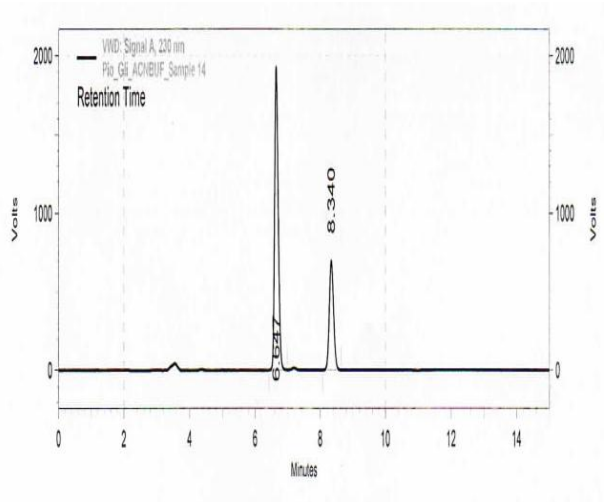



**Fig. 5: Assay chromatogram of Pioglitazone and Glimepiride in Tablet formulation.**

**Table 1: System Suitability Parameters (n=5).**

| Parameters | Pioglitazone | Glimepiride |
|---|---|---|
| Retention time (min) | 6.6 | 8.3 |
| Theoretical plates (N) | 8125 | 9145 |
| Tailing factor (T) | 1.2 | 1.3 |
| Resolution ($R_s$) | 1.657 | |

**∗n= No. of determinants**

**Table 2: Results for Linearity (n=3).**

| Parameter | Pioglitazone | Glimepiride |
|---|---|---|
| Linearity Range (µg/ml) | 10-50 | 3-15 |
| Regression Equation | y = 93687x + 72226 | y = 17080x + 28025 |
| Slope (m) | 93687 | 17080 |
| Intercept (c) | 72226 | 28025 |
| Regression Coefficient ($r^2$) | 0.9999 | 0.9998 |
| Limit of Detection (µg/ml) | 0.425 | 0.52 |
| Limit of Quantitation (µg/ml) | 1.40 | 1.716 |

**∗n= No. of determinants**

**Table 3: Results of Precision (n=6).**

| Drug | Intraday Precision (%RSD) | Interday Precision (%RSD) |
|---|---|---|
| Pioglitazone | 1.51 | 0.478 |
| Glimepiride | 1.22 | 0.85 |

**∗n= No. of determinants**

**Table 4: Results for Accuracy (n=3).**

| Recovery Level | *Pioglitazone* | | | | *Glimepiride* | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount Added (µg/ml) | | Amount Found (mg) | %Recovery | Amount Added (µg/ml) | | Amount Found (mg) | % Recovery (w/w) |
| | Std. | Test | | | Std | Test | | |
| 80% | 10 | 30 | 39 | 97.5 | 2 | 10 | 11.5 | 95.8 |
| 100% | 20 | 30 | 48.9 | 97.8 | 5 | 10 | 14.8 | 98.6 |
| 120% | 30 | 30 | 59.1 | 98.5 | 8 | 10 | 17.1 | 95 |

**∗n= No. of determinants**

**Table 5: Results for Robustness (n=3).**

| Parameters (n=3) | %RSD | |
|---|---|---|
| | Pioglitazone | Glimepiride |
| Detection wavelength at 228nm | 0.018 | 0.026 |
| Detection wavelength at 232nm | 0.03 | 0.02 |
| Flow rate 0.6ml/min | 0.2 | 0.06 |
| Flow rate 1ml/min | 0.032 | 0.036 |

**∗n= No. of determinants**

**Table 6: Results for Assay (n=3) of Marketed formulation.**

| Drug | Label claim (mg/tab) | Amount recovered | % Amount found in drug |
|---|---|---|---|
| Pioglitazone | 50 | 49 | 98% |
| Glimepiride | 15 | 14.9 | 99.3% |

**∗n= No. of determinants**

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Pioglitazone and Glimepiride at 6.7min and 8.3 min respectively without any interference. The parameters were given in table 1.

Concentration range of 10-50 µg/ml for Pioglitazone and 3-15 µg/ml of Glimepiride were found to be linear with correlation coefficients 0.999 and 0.999 for Pioglitazone and Glimepiride respectively. The results were given in table 2.

The limits of detection for Pioglitazone and Glimepiride were found to be 0.425 µg/ml and 0.52µg/ml respectively and the limit of Quantitation were 1.40µg/ml and 1.716µg/ml respectively. Values were represented in table 2.

The proposed method was found to be precise and reproducible with %RSD of 0.478 and 0.85 for Pioglitazone and Glimepiride respectively. %RSD was reported in table 3.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 97.5% to 98.5% and 95 to 98.6% for Pioglitazone and Glimepiride respectively. This indicates that the method was accurate. Values obtained were given in table 4. The method was found to be robust after changing the conditions like detection wavelength (± 1nm) and flow rate (± 0.2 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 5.

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination with a percentage purity of 98% for Pioglitazone and 99% for Glimepiride. The typical chromatogram for assay of marketed formulations was shown in figure.5 and Values obtained were given in table 6.

## CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Pioglitazone and Glimepiride from their formulations. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged and robust. So the established method can be employed in the routine analysis of the marketed formulations.

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## REFERENCES

1. Martindale (The complete drug reference), 34th edition, 2005; 632-655.
2. Merck Index 14th edition, 2006; 9151-3568.
3. Indian Pharmacopeia, 7th edition, 2014; 2-3: 1652-2834.
4. K. S. Lakshmi T. Rajesh. Development and Validation of RP-HPLC Method for Simultaneous Determination of Glipizide, Rosiglitazone, Pioglitazone, Glibenclamide and Glimepiride in pharmaceutical dosage forms and human plasma**.** Journal of the Iranian Chemical Society, 2011; 8(1): 31-37.
5. Sami El Deeb, Udo Schepers, Hermann Wätzig. Fast HPLC Method for the Determination of Glimepiride, Glibenclamide, and Related Substances using Monolithic Column and Flow Program. Journal of Separation Science, 2006; 29(11): 1571-1577.
6. P. K. Sahoo, R. Sharma, and S. C. Chaturvedi. Simultaneous Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride by RPHPLC Method from Combined Tablet Dosage Form. Indian Journal of Pharmaceutical Sciences, 2008; 70(3): 383–386.
7. Karthik Arumugam, Subramanian Ganesan, Chamallapudi Mallikharjuna Rao, Nayanabhirama Udupa. Simultaneous Determination of Pioglitazone and Glimepiride in Bulk Drug and Pharmaceutical Dosage Form by RP-HPLC Method. Pakistan Journal of Pharmaceutical Sciences, 2008; 21(4): 421-425.
8. Gadapa Nirupa, and Upendra M. Tripathi. RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and its Pharmaceutical Dosage Forms. Journal of Chemistry, 2013; 1-8.

9. D. Adukondalu, P. S. Malathy, J. V. Rao, and Y. M. Rao, Development and Validation of HPLC Method for Detection of Pioglitazone hydrochloride in Dosage Forms. International Journal of Pharmacy and Biological Sciences, 2011; 1(4): 474–478.
10. D. B. Pathare, A. S. Jadhav, and M. S. Shingare, RP-LC Determination of the cis-isomer of Glimepiride in a Bulk Drug Substance. Chromatographia, 2007; 66(7-8): 639–641.
11. N. Satheeshkumar , S. Shantikumar, R. Srinivas. Pioglitazone: A review of analytical methods**.** Journal of Pharmaceutical Analysis, 2014; 4(5): 295–302.
12. Deepti Jain**,** Surendra Jain**,** Deepak Jain**,** Maulik Amin. Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by RP-HPLC in Tablet Formulation Journal of Chromatographic Science, 2008; 46(6): 501-504.
13. G. Navaneethan, K. Karunakaran and K. P. Elango. Simultaneous Estimation of Pioglitazone, Glimepiride and Glimepiride Impurities in Combination Drug Product by a Validated Stability-Indicating RP-HPLC Method. Journal of Chilean of Chemical Society, 2011; 56(3): 815-818.
14. M Suchitra, D Sunitha, C Parthiban, B Siddartha and C Madhavi. Method Development and Validation of Metforminm, Glimepiride and Pioglitazone in Tablet Dosage Forms by RP-HPLC. International Research Journal of Pharmacy, 2013; 4(8): 250-254.
15. Rani G Shobha, Lohitha M, Preethi P Jaya, Madhavi R, Sunisitha B and Mounika D. Glimepiride Review of Analytical Methods. Asian Journal of Pharmaceutical Analysis, 2014; 4(4): 178-182.
16. Khohinur Hossain , Asma Rahman , Md. Zakir Sultan , Farhana Islam , Md. Akteruzzaman , Md. Abdus Salam and Mohammad A. Rashid. A Validated RP-HPLC Method for Simultaneous Estimation of Antidiabetic Drugs Pioglitazone HCl and Glimepiride. Bangladesh Pharmaceutical Journal, 2013; 16(1): 69-75.
17. M. S. V. Sakuntala, S. V. U. M. Prasad, S. Sri Devi, S. Kishore Yadav and K. Srinivas Reddy. A RP-HPLC Method Development and Validation for the Simultaneous Estimation of Glimepiride and Pioglitazone HCl in Tablet Dosage Forms. Journal of Chemical and Pharmaceutical Research, 2012; 4(1): 154-159.
18. Ravi Sharma, Gagan Sharma and Darpan chopra, Analytical Method Development and Validation for the Simultaneous Estimation of Glimepiride and Pioglitazone in Tablet Dosage Form by RP-HPLC. International Journal of Pharmaceutical Sciences and Research, 2012; 2(3): 637-641.
19. Kishore L, Kaur N. Estimation of Pioglitazone and Glimepiride in its Pharmaceutical Dosage Form by Spectrophotometric Methods. Der Pharmacia Lettre, 2011; 4: 276-284.
20. Freddy HH, Dharmendra LV. Simultaneous Estimation of Glimepiride, Rosiglitazone and Pioglitazone Hydrochloride in the Pharmaceutical Dosage Form. E-Journal of Chemistry, 2010; 7(4): 1326-33.
21. Praveen Kumar Reddy B, Boopathy D, Bibin Mathew, Prakash M, Perumal P. Method Development and Validation of Simultaneous Determination of Pioglitazone and Glimepiride in Pharmaceutical Dosage Form by RP-HPLC. International Journal of Chem Tech Research, 2010; 2(1): 50-53.
22. Kalyankar. T.M, Badguja. M.R, Mitkare S.S and Kakde R.B. HPTLC Method for Simultaneous Analysis of Pioglitazone HCL and Glimepiride in Pharmaceutical Preparations. Journal of Pharmacy Research, 2010; 3(12): 3118-3120.