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A RP-HPLC method development and validation for simultaneous estimation of metformin HCl and rosiglitazone in bulk and tablet dosage form

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ABSTRACT

A rapid reverse phase high performance liquid chromatography method has been developed and validated for the determination of Metformin HCl and Rosiglitazone maleate in combined tablet dosage form. Isocratic chromatography was performed on a Symmetry C18 column (150X4.6mm, 5µm, XTerra) with a mobile phase consist of 70:30v/v Methanol : Phosphate buffer (pH 4 with ortho -phosphoric acid) with the flow rate of 0.5ml/min and the detection was monitored out by UV detector at 239 nm. The total run time was less than 10 min. The retention time for Metformin & Rosiglitazone maleate was found to be 3.333 and 5.694 min respectively. Various chromatographic parameters including Linearity, precision, accuracy, system suitability, LOD, LOQ and robustness have been evaluated. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Metformin & Rosiglitazone maleate in tablets.

Key words: Metformin HCl, Rosiglitazone malate, validation.

INTRODUCTION

Pharmaceutical products formulated with more than one drug, i.e. combination products, are intended to satisfy previously unmet needs of the patients by combining the therapeutic effects of two or more drugs in a single product. These combination products can be challenging to the analytical chemist who are involved in the development and validation of analytical methods. Simultaneous estimation of drug combination is done by separation using chromatographic methods like HPLC,HPTLC, and GC as these methods are accurate and precise having good reproducibility.

Metformin hydrochloride is chemically N, Ndimethylimidodicarbonimidicdiamide hydrochloride (1, 1-dimethylbiguanide hydrochloride) which acts by decreasingintestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity.It is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese peoples and those with normal kidney function.It is official in all Pharmacopoeia (I.P, B.P, EP, USP)[1-4]. Allthe pharmacopoeias describe HPLC method for estimation of MET. Aliterature survey revealed spectrophotometry, HPLC, LC-MS/MS and LC-electrospray tandem mass spectrometry methods forsimultaneous estimation of MET in pharmaceutical formulation[5-11].

Rosiglitazone (ROSI) isused as an anti-diabetic drug. Chemically it is 5-[4-(2-[methyl(pyridin-2-yl)amino]ethoxy)benzyl]-thiazolidine-2,4-dione. It acts by activation of theintracellular receptor class of the peroxisomeactivated receptors (PPARS). Its action is dependent on the presence of insulin. It isofficial in IP. Literature survey indicated difference spectrophotometry, HPLC, LC-MS/MS andLC–electrospray tandem mass spectrometry methods for ROSE in pharmaceutical formulation with otherdrugs[12-17].

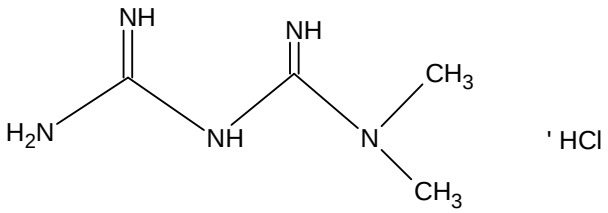


Figure 1: Metformin HCl

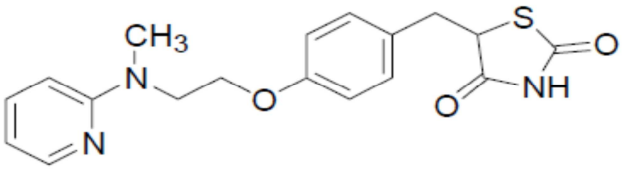


Figure 2: Rosiglitazone

Pharmaceutical validations among these methods undergo the world'Validation' means 'Assessment' of validity or action of providing effectiveness and validation as per ICH guidelines [18-20].

From the literature it was found that only few analytical methods reported for the simultaneous estimation of Metformin HCl and Rosiglitazone malate by gradient RP-HPLC in human plasma and LC/MS in Human plasma and this analysis method were considered to be tedious and time consuming processes. Hence the current study was an attempt to make a simple isocratic method for the simultaneous estimation of the Metformin HCl and Rosiglitazone malate by RP-HPLC.

METERIALS AND METHODS

List of chemicals:
Metformin and Rosiglitazone were obtained as gift sample from Dr.Reddy's laboratories, Potassium dihydrogen phosphate, ortho-phosphoric acid, Methanol (HPLC grade) from Merck Ltd., Mumbai and water (HPLC grade) Loba chemicals.

Instrument Used: Waters HPLC, Alliance, with Empower 2 software, UV-3000 Labinida, Sonicator SE60US Enertech.

Chromatographic conditions:
The column used for separation was a symmetry C18 column (150X4.6mm, 5µm, XTerra). The mobile phase was prepared by mixing Methanol and phosphate buffer (pH 4 adjusted with ortho -phosphoric acid) in the ration of 70:30(v/v). The mobile phase was filtered using 0.45µm filter and degassed by ultra-sonic vibrations prior to use. The flow rate was 0.5 ml/min, column was maintained at ambient temperature, and detection wavelength was selected as 239nm.

Method development:
The method development was started with determination of absorbance maxima, at 239 nm as selected wavelength. The trails carried out to obtain an optimized method showing improved peak shape, plate count, and asymmetry. The chromatographic separation was achieved by the column used for separation was a symmetry C18 column (150X4.6mm, 5µm, XTerra). The mobile phase was prepared by mixing Methanol and phosphate buffer (pH 4 adjusted with ortho -phosphoric acid) in the ration of 70:30(v/v) was an optimized method with the retention time (3.33 and 5.694), USP Plate count was (2973 and 2117) and USP Tailing factors (1.7 and 1.4).

Preparation of solutions:
Buffer Preparation**:** 700mg of KH_2PO_4 dissolved in 1000ml Beaker. Dilute and make up with HPLC water. pH is adjusted to 4.0 ± 0.01 with orthophosphoric acid and filter it.

Preparation of Mobile Phase:Mix phosphate buffer 300 ml (30%) and 700 ml of Methanol (70%) degas in Sonicator for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation Of Standard Stock Solution: An accurately weighed quantity of 10 mg of Metformin and 10 mg of Rosiglitazone working standard are transferred separately into 10 ml clean dry volumetric flasks and add about 7 ml of diluent and sonicate to dissolve completely then diluted to required volume with same solvent

Assay for marketed formulation: Twenty tablets were weighed and ground to a fine powder. An amount of power equivalent to 500 mg of Metformin and 2 mg of Rosiglitazone were weighed accurately and transferred into a 100 ml volumetric flask and add about 70ml of diluent and sonicate for 30 min. and make the volume up to the mark with the same solvent, then the solution was filtered through 0.45 µm membrane filter. Pipette 1ml of above filtrate into a 100 ml volumetric flask and dilute up to the mark with diluent. The chromatogram is shown in figure 3-7.

Method validation
The method validation was done as per the ICH guidelines, and accordingly the parameters evaluated were Specificity, precision, accuracy, linearity, ruggedness, robustness and system suitability studies. For all the parameters %RSD were calculated.

Specificity: Specificity of an analytical method is its ability to measure accurately and specifically the concentration of analyte without interference from other API, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of sample and standard were compared.

Linearity and Range: The linearity of an analytical procedure is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within given range, was studied by analyzing five analyte concentrations of drug ranging from 05-25µg/ml for metformin and 10 to 50µg/ml rosiglitazone. The calibration curve is shown in Figure 8.

Accuracy: Accuracy refers to the closeness of a measured value to a standard or known value. The percentage recovery was studied for 50%, 100% and 150%, each level was injected three times dated are shown in table 1.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of same homogenous sample under prescribed conditions. This experiment was conducted to prove the repeatability of the assay results obtained by quantification methodology. System precision, method precision and intermediate precision was performed.

System precision: 20µl of standard solution was injected for six times and measured the peak area for all six injections in HPLC. The % RSD for the area of six replicate injections was calculated.

Method precision: 20µl of sample solution was injected for six times and the peak area of the resulting chromatogram was used for the calculation of standard deviation and relative standard deviation shown in Table 2.

Intermediate precision: The ruggedness of an analytical method is the degree of reproducibility of test results obtained by same samples under different conditions. The standard solutions was injected two times for two days, calculated the mean and %RSD table 3.

LOD and LOQ: The Detection and quantification limits for the metformin and Rosiglitazone was performed and calculated using S/N ratio method.

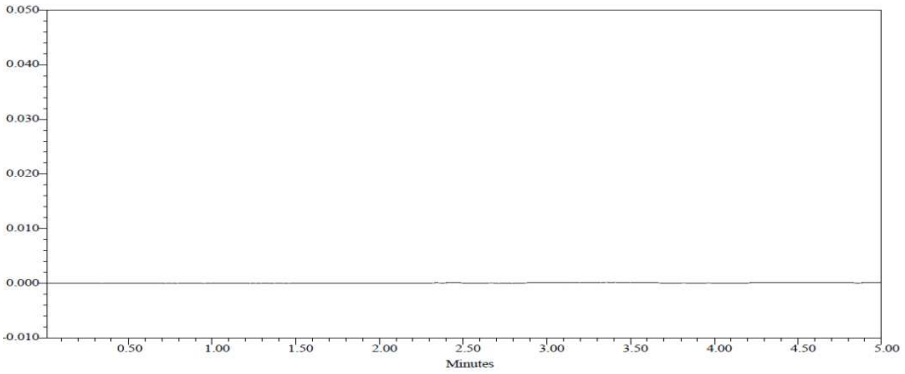
Robustness of an analytical method is measure of its capacity to remain unaffected small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.Robustnessmeasures the lack

ofinternal influences on the test results. As part of the Robustness, deliberate change in the Flow rate and Mobile Phase composition was made to evaluate the impact on the method.

Change in flow rate: The flow rate was varied at 0.4 ml/min to 0.6ml/min. Standard solution 15ppm of Metformin& 30ppm of Rosiglitazone was prepared and analysed using the varied flow rates along with method flow rate.

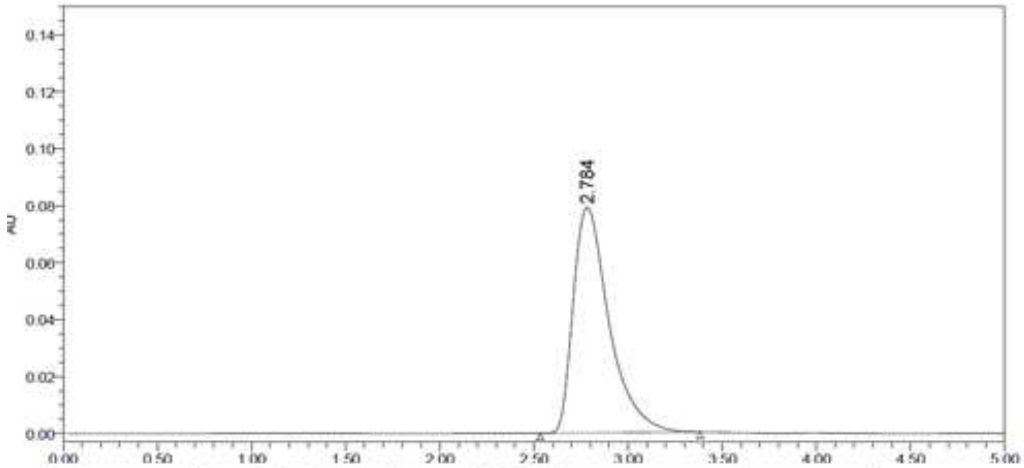
Change in Organic composition: The Organic composition in the Mobile phase was varied from 60% to 80%. Standard solution 15 µg/ml of Metformin &30µg/ml of Rosiglitazone was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method Table 4 & 5.

RESULTS AND DISCUSSION



	Baseline Noise (mV)
1	0.048

Figure 3: Blank Chromatogram



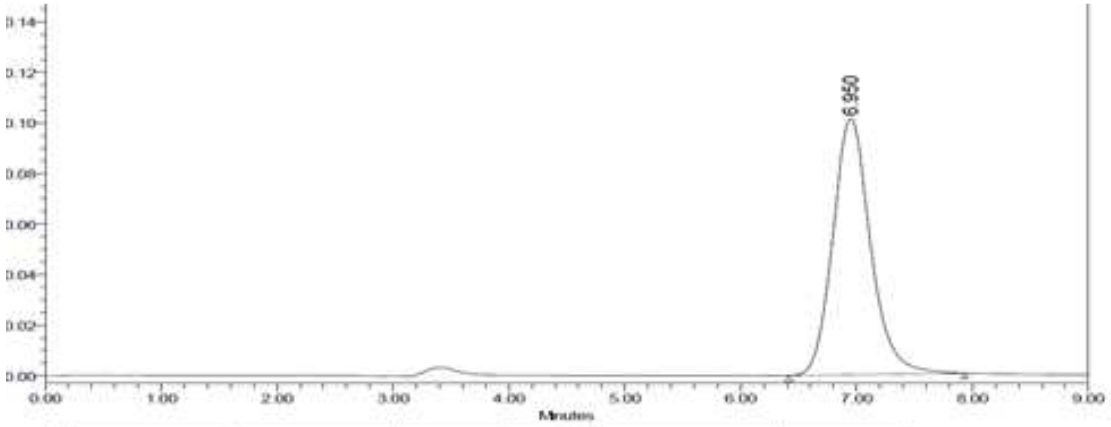
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	2.784	1047731	79327	1119.2	1.6

Figure 4: Chromatogram of Metformin

After several trails with various solvents, mobile phase system composed of Methanol and Phosphate buffer in the proportion of 70:30 respectively was chosen for the simultaneous estimation of Metformin and Rosiglitazone in combined dosage form by RP-HPLC. This mobile phase composition offered maximum resolution for the drug at the detection wavelength of 239nm. Mobile phase with the flow rate of 0.5 ml/min gave optimum separation with

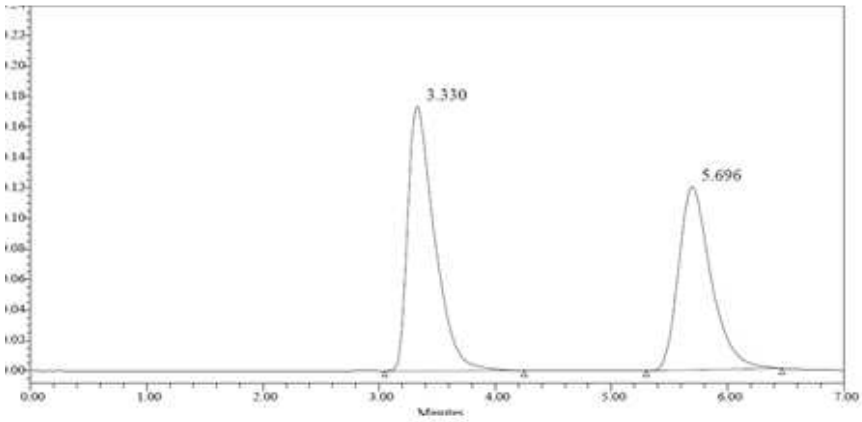
good resolution between the peaks. A reverse phase C_{18} column was used as stationary phase. The retention time of Metformin and Rosiglitazone were found to be 3.330 and 5.696 minutes, respectively. The total time of analysis was less than 10 minutes.

The simultaneous estimation of the metformin and rosiglitazone is performed with the optimized method. The percentage purity for Metformin and Rosiglitazone were found to be 99.2 and 98.8, respectively.



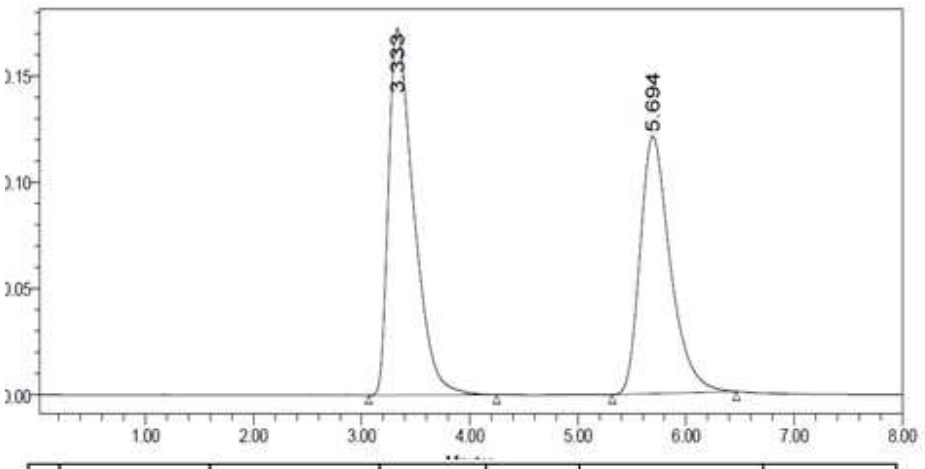
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rosiglitazone	6.950	2291945	101431	2216.5	1.2

Figure 5: Chromatogram of Rosiglitazone



	Name	Retention Time (min)	Area (µV*sec)	USP Plate Count	USP Tailing
1	Metformin	3.330	2775962	2973.4	1.7
2	Rosiglitazone	5.696	2339523	2017.2	1.4

Figure 6: Chromatogram of Mixed Standards



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	3.33	2755683	160535	2741.9	1.7
2	Rosiglitazone	5.694	2329967	71732	2216.3	1.3

Figure 7: Chromatogram for Marketed formulations

The specificity of the method where there is no interference of other substances in the retention time of the analytical peak. The system suitability of the developed method where theoretical plates (2973.4 and 2117.2), the tailing factor was (1.7 and 1.4) and resolution factor were within the acceptance criteria.

From the calibration curve constructed by plotting concentration vs. peak area, it was found that there exists a linear relationship in the concentration range of 5 to 25µg/ml for Metformin with 0.999 as the value of correlation coefficient and for Rosiglitazone the linearity in the range of 10 to 50µg/ml with 0.9998 as the value of correlation coefficient

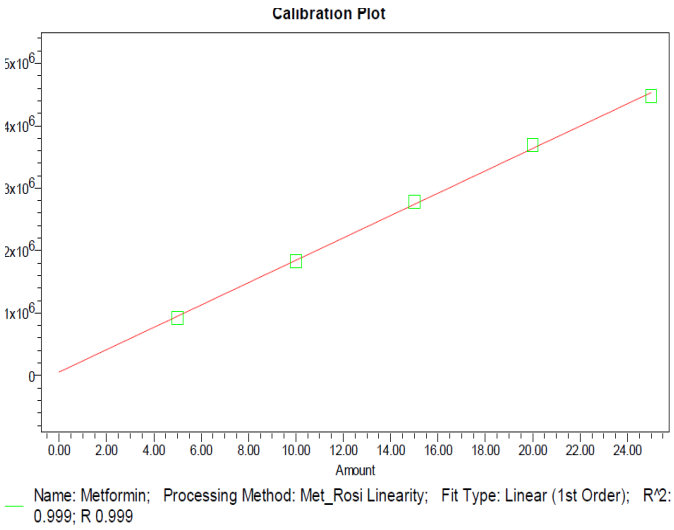


Figure 8: Linier curve for Metformin

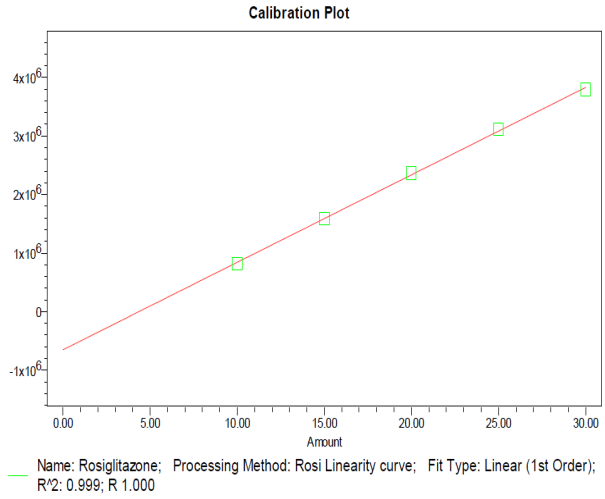


Figure 9: Linier curve for Rosiglitazone

The accuracy of the method was studied by performing recovery studies at 50%, 100% and 150% level. The standard drug at the concentration level of 50%, 100% and 150% were added to the sample and the analysis was carried out as per the assay method. The results were expressed in terms of percentage recovery. The values were

found to be 101.8 and 101.9 at 50% level, 100.4 and 100.5 at 100% level and 98.4 and 98.9 at 150%level for Metformin and Rosiglitazone, respectively.

Table 1: Accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean% Recovery
50%	1517158	2.68	2.73	101.9%	100.4%
100%	2791036	5.01	5.02	100.5%	
150%	4068986	7.4	7.32	98.9%	

Table 2: Accuracy results for Rosiglitazone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean% Recovery
50%	1305233	5.48	5.57	101.8%	100.2%
100%	2348433	10.0	10.0	100.4%	
150%	3408990	14.8	14.6	98.4%	

For precision studies, the sample solution was prepared at working concentration and analysis was carried at replicate. The percentage relative standard deviation was calculated for the peak areas of each drug and it was found to be 0.35 for Metformin and 0.63 for Rosiglitazone.

Table 3: Method Precision

Injection	Area of Metformin	Area of Rosiglitazone
1	2825475	2362906
2	2821743	2365959
3	2836183	2371390
4	2847213	2391369
5	2833801	2395639
Mean	2832883	2377453
Standard Deviation	9950.7	15040.6
% RSD	0.35	0.63

For Intermediate precision, the sample solution at working concentration was analyzed in replicate as per the assay method. The percentage relative standard deviation for the assay values was found to be 0.21 and 0.21 for Metformin and Rosiglitazone, respectively.

Table 4: Intermediate Precision

INJECTION	Area of Metformin	Area of Rosiglitazone
1	2818573	2377526
2	2815798	2372829
3	2820018	2376520
4	2828646	2384601
5	2827993	2384077
Mean	2822206	2379111
Standard Deviation	5788.3	5086.4
% RSD	0.21	0.21

Limit of measurements such as limit of detection and limit of quantification were found to be 2.93µg and 9.95µg for Metformin and for Rosiglitazone 3.06µg and 10.9µg, respectively.

For robustnessthe sample solution was prepared and run at changed flow rates of 0.4ml/min & 0.6ml/min as per the assay method. The variation in flow rate affected the method significantly. The method is robust only in less flow condition.

Table 5: Robustness studies for change in low rate

S.No	Flow Rate (ml/min)	Metformin		Rosiglitazone	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.4	2995.9	1.7	2893	1.5
2	0.5	2955	1.7	2356	1.4
3	0.6	2117.9	1.7	2855	1.2

Similarly by changing the mobile phase composition by changing the organic ratio by 10% the assay carried out. The variation in 10% Organic composition in the mobile phase affected the method significantly.

Table 6: Robustness studies for change in mobile Phase

S.No	Change in Organic Composition in the Mobile Phase	Metformin		Rosiglitazone	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	10% less	2022	1.7	2952.5	1.3
2	*Actual	2955	1.7	2356.5	1.4
3	10% more	2983	1.7	2886.4	1.3

CONCLUSION

The proposed RP-HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Metformin and Rosiglitazone in combined tablet dosage form. The method was validated as per ICH guidelines. The sample recoveries in the formulation was in good agreement with their respective label claims and they suggested non –interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Metformin and Rosiglitazone in combined tablet dosage form.

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