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Original Research Article

Stability indicating gradient liquid chromatographic technique for the simultaneous estimation of rosiglitazone, glimepiride and metformin HCl in pharmaceutical dosage forms

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ABSTRACT

Background: A stability indicating high performance liquid chromatographic method has been developed and validated for the anti-diabetic drugs Rosiglitazone, Glimepiride, and Metformin HCl in pharmaceutical dosage forms.

Methods: Chromatographic separation was achieved on Zorbex SB C-8 (250 X 4.6 mm) 5μ and Hypersil BDS C18 (200 × 4 mm), 5μ column as stationary phase. Mobile phase consisting of 0.023M potassium dihydrogen phosphate and acetonitrile (60:40, v/v) supplied at a flow rate of 1ml/min. Detection was performed using a SPD-20A prominence UV/VIS detector at 230 nm.

Result: The retention time of rosiglitazone, glimepiride and metformin hydrochloride was achieved at 2.4 min, 4.5 min and 5.6 min respectively. The method was validated for linearity, precision, accuracy, toughness, specificity, and forced degradation studies and the relative response factor values of rosiglitazone, glimepiride, and metformin determined from linearity study were 0.998 in the combined form. Rosiglitazone, glimepiride, and metformin HCl showed percentage recoveries of 99.73%, 99.81and 100.31%, respectively. The propesd method found to be very effective and stable for the routine analysis of mentioned antidiabetic drugs in pharmaceutical dosage forms.

1. Introduction

Rosiglitazone maleate (ROSI) is considered as highly prescribed oral antidiabetic drug which is chemically(±)-5-[p-[2-(methyl-2-pyridylamino)ethoxy]benzyl}-2,4-thiazolidinedionemaleate comes under the category of thiazolidinediones which primarily work to increase the sensitivity of insulin to control the elevated blood sugar level.[1–3] Glimepiride(GLIM) is a sulphonyl urea antidiabetic drug which is chemically 3,4-dimethyl-N-(4-(N-((4-methylcyclohexyl)carbamoyl)sulfamoyl)phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-carboxamide and commonly known as insulin secretagogues.[4,5] Metformin HCl (MET) is chemically 1,1-dimethyl biguanide hydrochloride. It comes under Biguanides which primarily show glucose-lowering effect via inhibiting gluconeogenesis and inhibiting the effect of glucagon.[6–8]

Several Literature surveys have revealed several methods that has been developed so far to be used as standard methods for the analysis of ROSI, GLIM and MET individually, such as high performance liquid chromatography (HPLC) with ultraviolet (UV) detection[9–18] or fluorescence detection[19,20] and capillary electrophoresis (CE) with ultraviolet (UV) detection.[21,22] However, none of these methods was suitable for routine analysis of these drugs. In addition, some of them used solvent extraction in sample preparation[23,24] which is

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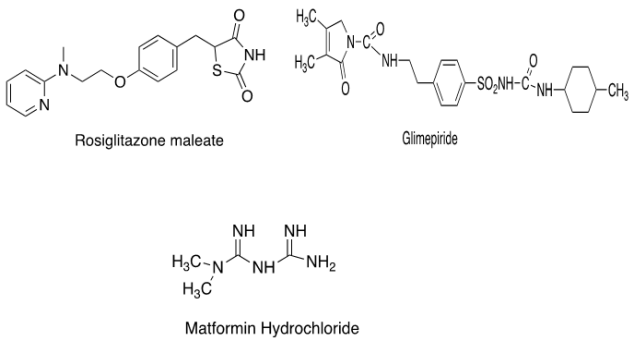

Fig. 1: Chemical structures of rosiglitazone maleate, glimepiride and metformin hydrochloride

tedious, and time-consuming involving complex sample preparation, such as equilibrium dialysis, ultrafiltration, solid phase extraction and liquid–liquid extraction. Some hyphenated techniques for analysis of ROSI, GLIM and MET like liquid chromatography/mass spectrometry or tandem mass spectrometry with improved sensitivity and efficiency have also been published.[25–30] but these are too expensive and the use of highly sophisticated instrument make them less affordable for the routine analysis of formulation. However, there is no method reported so far for the simultaneous estimation of ROSI, GLIM and MET in combined form using HPLC method. Also, The complexity of the multicomponent dosage forms includes multiple entities and excipients poses considerable challenge to the analytical chemist during the development of assay procedure. Traditionally, colorimetric and spectrophotometric methods were used for drug analysis due to reasons of economy and easy availability. These methods, however, are used to a lesser extent now a days because of lack specificity, sensitivity and accuracy. For the simultaneous estimation of the drugs present in multicomponent dosage forms, HPLC method is considered most suitable since this is a powerful and rugged method. It is also extremely specific, linear, precise, accurate, sensitive and rapid. The present work describes a simple, precise, accurate and validated HPLC method for the simultaneous routine analysis of ROSI, GLIM and MET in tablet dosages form.[31]

2. Materials and Methods

Rosiglitazone, Glimepiride and Metformin reference standard were kindly supplied by TORENT PHARMA (Ahmedabad, Gujarat, India). HPLC grade Solvents i.e. Acetonitrile, water and methanol were procured from Merck chemicals Mumbai, India. All other chemicals used were analytical grade reagents. The analysis was performed on a high performance liquid chromatographic (HPLC) system equipped with a prominence LC gradient pump, a manual injector with a 20-μl injection loop and a SPD-20A prominence UV/Vis detector which was set at 230nm of detection wavelength. The analytical column was a Phenomenex Luna, ODS, C-18 column (20mm×4mm). A gradient flow was consisting of 0.023M Potassium dihydrogen phosphate buffer (pH6.0) along with acetonitrile in ratio of 60:40v/v. The flow rate used was 1ml/min. Operation, data acquisition and analysis were performed using spinchrom software. Mobile phase was filtered through a 0.45 μm nylon Millipore membrane filter (Advantech MFS, Inc., CA, USA) under vacuum and degassed by ultrasonication (Sonorex, Bandelin, Germany).

2.1. Preparation of standard solutions and calibration curves

Standard stock solutions (1000 μgml^{-1}) of ROSI, GLIM and MET were prepared in methanol. Working standard solutions for the calibration curves were prepared in the concentration range of 2-50μg/ml for rosiglitazone, 2-50 μg/ml for glimepiride and 1-50μg/ml for Metformin hydrochloride. Calibration curves were represented by plotting the peak area ratio of ROSI,GLIM and MET versus the concentrations of the calibration standards.

2.2. Sample preparation for the assay

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to about 500mg of metformin hydrochloride, 2mg of rosiglitazone and 2mg glimepiride added to a 100ml volumetric standard flask and sonicated well for 20 minutes with 50ml methanol. The final volume was made up with methanol afterwards filtered a portion of this solution through a 0.45μm nylon membrane filter paper, and used the filtrate for the entire assay. The method was successfully applied on three brands, Voglimet (Wockhardt), ROM-G (Panjon)and Swimet (Ind Swift).The result was shown in table 1, 2 and 3.

3. Validation Studies for the Developed Method

3.1. System suitability

system suitability testing is an integral part of analytical method. The tests are based on the concept that the equipment, electronics, analytical operation and samples to be analysed constitute an integral system. It was determined by taking the coefficient of variation, peak asymmetry and theoretical plate of the five standard injections by using the above developed assay method. Statistical result was showed in Table 1.

3.2. Linearity and range

Linearity established by across the range of the analytical procedure. It should be established initially by visual

Table 1: System precision data forrosiglitazone, glimepiride and metformin

S. No	Parameters	Rosiglitazone	Glimepiride	Metformin
1	Theoretical plate	SD = 7.4162 RSD = 0.1381% SEM= 3.3166 PRE=6.5005 ± 5369	SD = 9.9900 RSD = 0.0186% SEM= 4.4676 PRE= 8.7565±3774.4	SD = 7.9498 RSD = 0.0093% SEM= 3.5552 PRE=6.9682±85747.2
2	Peak asymmetry	SD = 0.0008 RSD = 0.1966% SEM= 0.0004 PRE=0.0007±0.4276	SD = 0.0007 RSD = 0.1149% SEM= 0.0003 PRE=0.0006± 0.6137	SD = 0.0009 RSD = 0.1023% SEM= 0.0004 PRE=0.0008± 0.9236
3	Peak area	SD = 9.6695 RSD = 0.0513% SEM= 4.3243 PRE=8.4756±1885	SD = 13.1910 RSD = 0.0478% SEM= 5.8991 PRE=11.5622±2763	SD = 12.4419 RSD = 0.0007% SEM= 5.5641 PRE=10.9056 ± 7144

Where, *SD* = Standard Deviation; *RSD* = Relative standard Deviation; *SEM* = Standard error of mean, *PRE* = Percentage range of error (within 95% confidence limits)

Table 2: Statistical analysis of Inter day precision for rosiglitazone, glimepirideand metformin

Parameters	Rosiglitazone	Glimepiride
Standard Deviation	0.6139	0.6879
Coefficient of variation	0.6147	0.6881
Standard error of mean	0.2046	0.2293
Percentage range of error (within 95% confidence limits)	0.4011 ± 99.8779	0.4494 ± 99.9750

Table 3: Statistical analysis of intraday precision for rosiglitazone, glimepiride and metformin

Parameters	Rosiglitazone	Glimepiride
Standard Deviation	0.5430	0.3417
Coefficient of variation	0.5429	0.3407
Standard error of mean	0.1810	0.1139
Percentage range of error (within 95% confidence limits)	0.3547 ± 100.0191	0.2233 ± 100.2979

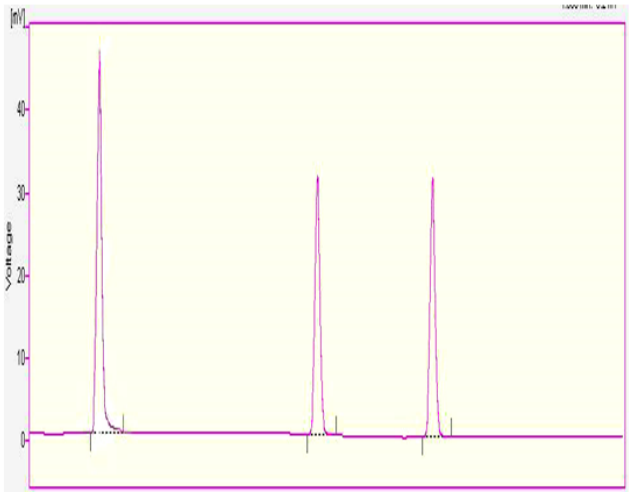

Fig. 2: Typical chromatogram of rosiglitazone, glimepiride and metformin.

examination of a plot of signals as a function of analyte concentration of content. It was determined at five levels over the range of 80% to 120% of test concentrations. A standard linearity solution was prepared to attain concentration of 80%, 90%, 100%, 110% and 120% of the test concentration. The area at each level is calculated and a graph of area versus concentration is plotted. The correlation co-efficient (r^2), was calculated and recorded.

3.3. Precision (Repeatability)

The precision of an analytical method was determined by adding a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation or Coefficient of variation. Repeatability was assessed by performing the nine determination, i.e., three concentrations and three replicates of each concentration of test solution. Statistical result for inter and intraday precision was showed in Tables 2 and 3.

Table 4: Recovery data for rosiglitazone, glimepiride and metformin

Name of the Drug	Labeled amount (in mg)	Amount added (in %)	Amount recovered (in mg) n=3*	Percentage Recover	Average percentage recovery
MET	500	90	= 950.3605±2.4806 SD =2.1921 R.S.D. = 0.2307%	100.04%	100.02%
		100	= 1000.176±2.1709 SD = 1.9184 R.S.D. = 0.1918%	100.02%	
		110	= 1049.864±2.1597 SD = 1.9085 R.S.D. = 0.1818%	99.99%	
ROSI	2	50	=3.0063 ±0.0317 SD = 0.028 R.S.D. = 0.932%	100.21%	99.87%
		100	= 3.9802±0.043 SD = 0.038 R.S.D. = 0.9546%	99.51%	
		150	= 4.9938±0.0782 SD = 0.0691 R.S.D. = 1.3844%	99.88%	
GLIM	2	50	= 2.9835±0.0266 SD = 0.0235 R.S.D. = 0.7887%	99.45%	100.04%
		100	= 4.0181±0.0253 SD = 0.0224 R.S.D. = 0.557%	100.45%	
		150	= 5.0113±0.0901 SD = 0.0797 R.S.D. = 1.5896%	100.23%	

S.D. = Standard Deviation,
R.S.D.= Relative Standard Deviation.
*Average of 3 samples

Table 5: Statistical analysis for Reproducibility of rosiglitazone, glimepiride and metformin

Parameters	Rosiglitazone	Glimepiride	Metformin
Standard Deviation	0.6436	0.5530	0.4379
Coefficient of variation	0.6434	0.5539	0.4398
Standard error of mean	0.2275	0.1955	0.1548
Percentage range of error (within 95% confidence limits)	0.4460 ± 100.0319	0.3832± 99.8344	0.3035 ± 99.5748

Table 6: Dataforced degradation study

Sample condition	Percentage Degradation		
	Rosiglitazone	Glimepiride	Metformin
Acid degradation	16.37	17.32	13.43
Alkali hydrolysis	9.13	7.11	8.32
H_2O_2-induced degradation	0.21	0.42	0.13
Photochemical degradation	0.22	0.17	0.06

Table 7: Summary of validation data for rosiglitazone, glimepiride and metformin by HPLC method

S. No	Parameters	Acceptance criteria	Result obtained		
			Rosiglitazone	Glimepiride	Metformin
1	System suitability				
1.1	% RSD	% RSD of standard NMT 2.0	0.0513	0.0477	0.0007
1.2	Peak Asymmetry	Not more than 2	0.4276	0.6137	0.9236
1.3	Theoretical plates	Not less than 1800	5369	53774.4	85747.2
2	Linearity	r^2 = 0.995 to 1.0	0.998	0.998	0.998
3	Precision (Repeatability)				
3.1	Interday	RSD NMT 2.0%	0.6146	0.688062	0.5964
3.2	Intraday	RSD NMT 2.0%	0.5429	0.3407	0.6566
4	Specificity	NMT 1%	0.0058	0.0006	0.0001
5	Accuracy	Recovery : 98.0 to 102.0%	99.87	100.04	100.02
6	Reproducibility	RSD NMT 2.0%	0.6434	0.5539	0.4398
7	Robustness	NMT 1%	0.927	0.5223	0.6341

3.4. Accuracy

Accuracy determined by application of the analytical method to synthetic mixtures of the drug product components to which known amounts of analytes have been added within the range of the method. Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample. Accuracy assayed by using a minimum of nine determination over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration. Statistical result are shown in Table 4.

3.5. Reproducibility

The reproducibility of an analytical method was determined by analysis of aliquots, from homogenous lots in different Laboratory. Reproducibility was assayed by performing eight determination i.e. two concentration and two replicator of each concentration in two Labs. Statistical result are shown in Table 5.

3.6. Specificity

The study was performed by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials. Placebo (sample without analyte) was prepared in the same way as the sample under the conditions prescribed in the assay method and duplicate injection was taken. The excipient mixture of the tablet hasn't shown any specific peak at the RT of the analyte peak. This shows that the excipient do not interfere with the analyte peak. Therefore, This method was found to be specific for Rosiglitazone, Glimepiride and Metformin.

3.7. Robustness

The Robustness of method was established by making deliberate minor variation in the flow rate. Method was performed twice first by same method as the described in assay method of rosiglitazone, glimepiride and metformin by HPLC and second time same as usual first only changed the flow rate from 1 ml / min to 1.2 ml/min, calculated the % deviation.

4. Determination of Forced Degradation Stability Study

A stock solution containing 1 mg/ ml drug in methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method.

4.1. Preparation of acid-induced degradation product

To 15 ml of methanolic stock solution, 5 ml of 1N HCl was added and mixture was refluxed for 1 h at 70 °C. The forced degradation in acidic basic media was performed in the dark in order to exclude the possible degradative effect of light.

4.2. Preparation of base-induced degradation product

To 15 ml of methanolic stock solution, 5 ml of 1 N NaOH was added and mixture was refluxed for 1 h at 70 °C.

4.3. Preparation of hydrogen peroxide-induced degradation product

To 15 ml of methanolic stock solution, 5 ml of 3.0% v/v hydrogen peroxide was added. The solution was heated in boiling water bath for 10 min to remove the excess of hydrogen peroxide completely and then refluxed for 30 min. at70 °C on water bath.

4.4. Photochemical degradation effect

The photochemical stability of the drug was also studied by exposing the stock solution (1 mg/ml) to direct sunlight for 48 h on a wooden plank and kept on a terrace. In this study, The sample was subjected to acid, base, oxidation, dry heat and Photochemical degradation. Each degradation were injected and the separation of degraded impurities from main peak was checked and recorded in Table 6.

5. Result and Discussion

The method was found to be accurate, simple and rapid, for routine simultaneous analysis of the formulations without prior separation. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation, percent relative standard deviation and standard error. The percent range of error within 95% confidence limits. The specificity indicates non-interference from the excipients used in the formulations. Thus the method developed in the present investigation found to be simple, sensitive, accurate and precise and can be successfully applied for the simultaneous estimation of rosiglitazone, glipermide and metformin hydrochloride in tablets. The results for the developed method are mentioned in table no. [1–7]

6. Conclusion

The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatograms of ROSI, GLIM and MET showed clear resolution with retention time of 2.4, 4.5 and 5.6 minutes respectively.

7. Source of Funding

None.

8. Conflict of Interest

None.

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