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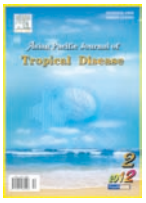

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Gradient HPLC method development and validation for Simultaneous estimation of Rosiglitazone and Gliclazide.

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ABSTRACT

Objective: The aim of present work was to develop a gradient RP-HPLC method for simultaneous analysis of rosiglitazone and gliclazide, in a tablet dosage form. **Method:** Chromatographic system was optimized using a hypersil C18 (250mm x 4.6mm, 5 μm) column with potassium dihydrogen phosphate (pH-7.0) and acetonitrile in the ratio of 60:40, as mobile phase, at a flow rate of 1.0 ml/min. Detection was carried out at 225 nm by a SPD-20A prominence UV/Vis detector. **Result:** Rosiglitazone and gliclazide were eluted with retention times of 17.36 and 7.06 min, respectively. Beer's Lambert's Law was obeyed over the concentration ranges of 5 to 70 μg/ml and 2 to 12 μg/ml for rosiglitazone and gliclazide, respectively. **Conclusion:** The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of both drugs in a tablets dosage form. Statistical analysis proves that the method is sensitive and significant for the analysis of rosiglitazone and gliclazide in pure and in pharmaceutical dosage form without any interference from the excipients. The method was validated in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible.

1. Introduction

TRosiglitazone (ROSI) and Gliclazide (GLC) a combination of drugs belong to thiazolidine dione and sulfonyl urea groups of oral hypoglycemic drugs.

Chemically rosiglitazone is (±)-5-{p-[2-(Methyl-2-pyridylamino)ethoxy]benzyl}-2,4-thiazolidinedione[1] . The structure of rosiglitazone shown in figure-1. Gliclazide is 1-(3-azabicyclo [3.3.0]oct-3-yl)-3-(p-tolylsulfonyl) urea[2]. The structure of rosiglitazone shown in figure-2. Rosiglitazone is a selective agonist for the nuclear peroxisome proliferator-activated receptor γ (PPAR γ) which enhances the transcription of several insulin responsive genes. They tend to reverse insulin resistance by stimulating GLUT4 expression and translocation: entry of glucose into muscle and fat is improved. Gliclazide provokes a brisk release of insulin from pancreas. It acts on sulfonylurea receptors on pancreatic β-cell membrane cause depolarization by reducing conductance of ATP sensitive K+channels. This enhances Ca^{2+} influx which causes degranulation. The rate of insulin secretion at any glucose concentration is increased [3].

The literature reveals few reported methods for rosiglitazone and gliclazide individually on spectrophotometry[4-6] , MEKC[7] , HPLC [7, 8] in pharmaceutical dosage form. Methods are also available for the determination of both drugs in biological fluids and there are also few methods for simultaneous estimation of rosiglitazone and gliclazide in combination with other drugs by HPLC [8-12] . As the combination of rosiglitazone and gliclazide is one of the successful combination therapies of diabetes mellitus type II and highly potent for patients. So there is a need for the development of some novel sensitive and specific method for estimation of rosiglitazone and Gliclazide. In present study we are optimizing the method by using fewer amount of organic solvent. Due to this reducing cost of analysis, so that formulation become economic. Hence it is necessary to develop a method to determine the combination in pure and formulations. The present paper describes a simple, sensitive, validated and economic method for the simultaneous determination of rosiglitazone and gliclazide by HPLC.

2. Materials and methods

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Pharmaceutical grade working standards rosiglitazone and gliclazide were obtained from Ranbaxy Laboratories, Dewas, India. All chemicals were of AR-grade. All reagents were of HPLC-grade. All chemicals and reagents were purchased from Merck pharmaceuticals. The formulation was purchased from the local pharmacy.

2.1. Instrumentation and chromatographic condition

The analysis was performed on Binary Shimadzu HPLC system. It is equipped with two prominence LC 20AD pump, and a SPD-20A prominence UV/Vis detector. Data acquisition was performed by using sphinocrome software. Hypersil, C18 column (250mm x 4.6mm, 5 μm.) was used as a stationary phase for analysis. Injections was performed by a manual-injector with 20 μl, loop. Different mobile phases were tested in order of their polarity to find out the best conditions for separation of ROSI and GLC. The selected mobile phase containing 20mM potassium dihydrogen phosphate (pH-7.0) and acetonitrile in the ratio of 60:40 gave acceptable retention time(RT) and good resolution between ROSI and GLC. The flow rate was maintained at 1.0 ml/min, with run time 20min. The mobile phase was filtered by using 0.45 μm millipore nylon filter paper. Mobile phase was degassed by sonication prior to use. All determinations were performed at ambient temperature.

2.2. Standard solutions and calibration curve prepration

ROSI (100mg) and GLC (100mg) were weighed and transferred separately to 100 ml volumetric flask. Both drugs are dissolved in HPLC-grade methanol and volume was made up with same to prepare 1000 μg/ml. Pipette out 10ml of above and transfer in 100 ml volumetric flask and make up volume till mark with methanol to make 100 μg/ml standard stock solutions Calibration standard were prepared by taking aliquots and further diluted standard stock solutions in the concentration ranges of 5 to 70 μg/mL and 2 to 12 μg/mL for ROSI and GLC, respectively and peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.3. Sample preparation

For the analysis of a tablet dosage form, 20 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. Transferred an accurately weighed portion of the powder, equivalent to about 2mg of ROSI and 80mg GLC to a 100 ml volumetric flask and diluted with methanol till the mark, and sonicate for 20 minutes. The solution was filtered through a Whatman filter paper no.1. Filtrate was then appropriatly diluted with mobile phase to get a final concentration. Before the assay of tablet formulations, 3 replicate aliquot of the appropriately diluted tablet stock solution were sonicated for 15 min, then injected into the chromatographic system, and analyzed quantitatively.

2.4. Optimization of hplc method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for ROSI and GLC. Preliminary experiments were carried out to optimize the parameters affecting simultaneous estimation of the two drugs. Reverse phase column [hypersil C18 (250mm x 4.6mm, 5 μm.) column] was selected on the basis of polarity of drugs for analysis. Following parameters were optimized for the development of method i.e. column, wavelength, mobile phase concentration, solvent, flow rate, concentration of buffer. The solvent type, solvent strength, detection wavelength, and flow rate were varied to determine the best chromatographic conditions for the separation of ROSI and GLC in chromatogram. The mobile phase conditions were optimized to avoid interference from solvent and formulation excipients. Other criteria, for example, time required for analysis, flow rate of mobile phase, symmetry of the eluted peaks, assay sensitivity and solvent noise during drug analysis were also considered. The UV spectra of the analytes were determined independently and in combination. It was observed that at wave length 225nm both drugs could be detected simultaneously with no mobile phase interference, good separation, sensitivity and consistent baseline. The feasibility of various combinations of solvents such as acetonitrile, methanol, buffer and water with altered flow-rates (in the range 0.8-1.2mL/min), was investigated for complete chromatographic resolution of above used drugs with best sensitivity, efficiency, and peak shape.

2.5. Method validation

The method was validated according to the ICH guidelines [13]. The following validation characteristics were addressed: linearity, accuracy, precision, specificity, and robustness.

2.5.1. System suitability

System suitability parameter was calculated before starting validation parameters. It was determined by the taking the Coefficient of variation of peak area, peak asymmetry, and theoretical plate of the five standards injections by using the same standard method which given on assay method.

2.5.2. Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Linearity established across the range of the analytical procedure. 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 (r2) was calculated and recorded.

2.5.3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical method was determined by assaying a

sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation and relative standard deviation.

2.5.3.1. Repeatability (intraday and interday study)

Repeatability expresses the precision under the same operating conditions. Repeatability was assessed by performing the determination, of three concentrations and three replicates of working standard solution in intraday and interday study.

2.5.3.2. Reproducibility

Reproducibility expresses the precision between laboratories. 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 (80% and 100%) and two replicator of each concentration in two Laboratories.

2.5.4. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by +0.2 ml/min).

2.5.5. Accuracy and recovery study

The accuracy of an analytical procedure expresses the closeness of agreement between value which is accepted either as a conventional true value or an accepted reference value and value found. Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte (10%, 20%, and 30%) in the sample. Accuracy assayed by using nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration.)

2.5.6. Specificity

In case of the assay, demonstration of specificity requires that it can be shown by the presence of impurities or excipients. It was done 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 and Observed any significant peak area (not more than 1%) at the analyte RT.

3. Results

3.1. Development and optimization of hplc method

The proposed method was optimized with a view to develop a suitable analytical method to for the analysis of rosiglitazone and gliclazide in combined pharmaceutical dosage form. It was found that the mobile phase containing 20mM potassium dihydrogen phosphate (pH-7.0) and acetonitrile in the ratio of 60:40 in gradient elution mode at a flow rate of 1 ml/min gave optimum and adequate peak separation, with less tailing, and resulted in the best resolution. All experiments were performed at ambient temperature. Run time was taken 20 min for each run. Under the optimum chromatographic conditions, the retention times obtained 17.36 min and 7.059 min for ROSI and GLC respectively. Resolution between ROSI and GLC was found more than 2.

3.2. Validation

3.2.1. System suitability

System suitability parameters such as retention time, number of theoretical plates, peak area, resolution and peak asymmetry were determined. The results obtained were statistically analyzed and found within range (table-1).

Table 1.
System suitability tests(S.D.- Standered Deviation, R.S.D.- Relative Standered Deviation, S.E.M.- Standered Error of Mean, P.R.E.- Percentage Range of Error)

Parameter	Compound	
	Rosiglitazone	Gliclazide
Retention time	S.D.-0.080829	S.D.-0.072528
	R.S.D.-0.467941	R.S.D.-1.018699
	S.E.M.-0.046667	S.E.M.-0.041874
	P.R.E.-0.091467	P.R.E.-0.082073
Peak area	S.D.-14.62874	S.D.-16.69431
	R.S.D.-.07851	R.S.D.-.000818
	S.E.M.-6.542077	S.E.M.-7.465816
	P.R.E.-12.82247 ± 18633	P.R.E.-14.633 ± 2040631
Peak asymmetry	S.D.-0.000981	S.D.-0.000709
	R.S.D.-0.092045	R.S.D.-0.093607
	S.E.M.-0.000439	S.E.M.-0.000317
	P.R.E.-0.00086 ± 1.06614	P.R.E.-0.000622 ± 0.75766
Theortical plates	S.D.-5.80517	S.D.-9.607289
	R.S.D.-0.154879	R.S.D.-0.020278
	S.E.M.-2.596114	S.E.M.-4.296449
	P.R.E.-5.088383 ± 3748.2	P.R.E.-8.421039 ± 47377.6

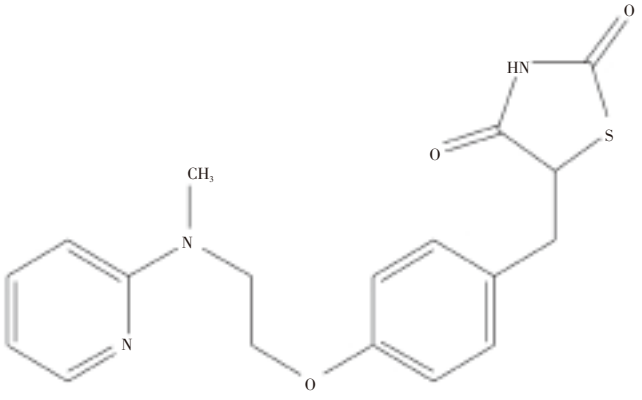

Figure 1. Structure of Rosiglitazone.

3.2.2. Linearityand range

The statistical data obtained are represented in table-2. The result shows that within the concentration range 5 to 70 μg/ml and 2 to 12 μg/ml for rosiglitazone and gliclazid, respectively, there was an excellent correlation between

Table 2.
Linearity parameters for calibration curves of ROSI and GLC

Compound	Level of conc. In %	Slope		Intercept		Correlation coficient (r2)
		Mean ± SD	CV(%)	Mean ± SD	CV(%)	
ROSI	80–120	88.07661 ± 0.061101	0.069373	740.2667 ± 14.31689	1.934018	0.996
GLC	80–120	10059.67 ± 0.57735	.005739	28759.67 ± 4.50925	0.015679	0.998

(S.D.– Standered Deviation, C.V.–Coffecient of Variation)

Table 3.
Precision of method (S.D.– Standered Deviation, R.S.D.– Relative Standered Deviation, S.E.M.– Standered Error of Mean)

Level of conc. (ROSI)	Interday Precision			Intraday Precision		
	Mean area ± SD	SEM	RSD	Mean area ± SD	SEM	RSD
80%	6129.33 ± 13.2035	7.623	0.21541	6106.67 ± 4.16	2.404	0.0682
90%	7764.67 ± 54.629	31.54	0.70356	7768 ± 43.1	24.879	0.555
100%	9429.33 ± 27.574	15.92	0.29243	9498 ± 31.32	18.083	0.33
Level of conc. (GLC)	Interday Precision			Intraday Precision		
	Mean area ± SD	SEM	RSD	Mean area ± SD	SEM	RSD
80%	657903.3 ± 276.333	159.54	0.04200	658121.3 ± 57.047	32.936	0.0087
90%	828853.7 ± 835.776	482.536	0.10084	828730.7 ± 603.478	348.419	0.0728
100%	1017262 ± 1241.156	716.582	0.12201	1018580 ± 711.787	410.951	0.0699

Table 4.
Ruggudness Study.(S.D.– Standered Deviation, R.S.D.– Relative Standered Deviation, S.E.M.– Standered Error of Mean)

Level of conc. (ROSI)	LEB–1			LEB–2		
	Mean Area ± SD	SEM	RSD	Mean Area ± SD	SEM	RSD
80%	6091.5 ± 14.849	8.57322	0.244	6090 ± 7.071	4.082	0.116
100%	9570 ± 26.87	15.51344	0.281	9496.5 ± 6.364	3.674	0.067
Level of conc. (GLC)	LEB–1			LEB–2		
	Mean Area ± SD	SEM	RSD	Mean Area ± SD	SEM	RSD
80%	655866 ± 1630.588	941.421	0.2486	655138 ± 1687.157	974.081	0.257
100%	1024758 ± 4500.028	2598.093	0.4391	1016090 ± 357.089	206.165	0.035

Table 5.
Robustness Study.(S.D.– Standered Deviation, R.S.D.– Relative Standered Deviation, S.E.M.– Standered Error of Mean)

FlowRate	ROSI			GLC		
	Mean area ± SD	SEM	RSD	Mean area ± SD	SEM	RSD
1ml/min	9479.5 ± 13.435	7.7567	0.1417	1021176 ± 888.126	512.7601	0.086971
1.2ml/min	9419.5 ± 7.778	4.4907	0.0826	1015360 ± 21.213	12.24745	0.002089

Table 6.
Accuracy and Recovery study. (S.D.– Standered Deviation, R.S.D.– Relative Standered Deviation, S.E.M.– Standered Error of Mean, P.R.E.– Percentage Range of Error)

Amount of drug added	Theoretical content (mg/ml)	Conc. Found (mg/ml) ± SD	Recovery (%)	SEM	PRE	RSD (%)
ROSI						
10	4.4	4.399 ± 0.013	99.9841	0 .0075	4.399 ± 0.01469	0.2952
20	4.8	4.812 ± 0.0156	100.259	0.0090	4.812 ± 0.01769	0.3248
30	5.2	5.218 ± 0.0074	100.356	0.00426	5.218 ± 0.00835	0.1414
GLC						
10	88	88.205 ± 0.149	100.234	0.08607	88.205 ± 0.1687	0.169
20	96	96.031 ± 0.034	100.033	0.01990	96.031 0.039	0.036
30	104	104.313 ± 0.468	100.301	0.27014	104.313± 0.529	0.448

Table 7.
Specificity Study (Placebo Interference).

Sample No.	Injections	Excipients area at the RT of ROSI peak	Excipients area at the RT of GLC peak	% deviation for ROSI	% deviation for GLC
01	01	0.1124	1.18	0.001183	0.000115
	02	0.2731	1.7114	0.002873	0.000167
	Avg. % deviation	0.002028	0.000141		

peak area and concentration of each drug.

3.2.3. Precision

3.2.3.1. Repeatability

The results of the interday and intraday precision experiments are shown in table–3. Separation of the drugs

was found to be similar when analysis was performed on different time (intraday) and on different days (interday). The developed method was found to be precise, with relative standered deviation (RSD) values less than 2%.

3.2.3.2. Reproducibility

The results of the reproducibility experiments (performed in different laboratories) are shown in table-4. The developed method was found to be precise, with RSD values less than 2%.

3.2.4. Robustness

Minor change in chromatographic condition (change in flow rate, altered by 0.2 ml/min) did not cause a significant change in, peak area, theoretical plates and RT of rosiglitazone and gliclazide. (table-5).

3.2.5. Accuracy and recovery study

Good recoveries of the ROSI (99.98 to 100.36) and GLC (100.03 to 100.30) were obtained at various added concentrations for the ROSI and GLC (table-6).

3.2.6. Specificity

Injections of Placebo (sample without analyte) were performed to confirm specificity of method. Obtained results show (table-7) that excipients mixture of the tablet shows no specific peak at the RT of the analyte peak. This shows that the excipients do not interfere with the analyte peak and the assay is specific for the simultaneous estimation of Rosiglitazone and Gliclazide tablet.

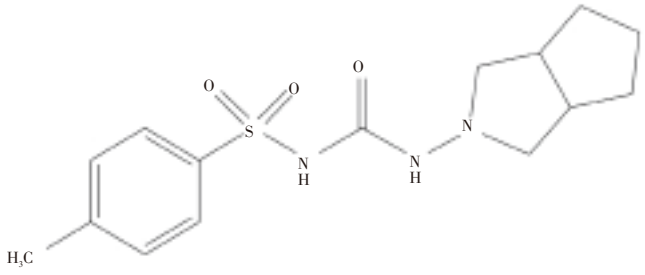

Figure 2. Structure of Gliclazide

4. Discussion

A suitable analytical procedure refers to the way of performing the analysis with accuracy and precision. This developed method is describes in detail the steps necessary to perform each parameter for validation. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The quality control laboratory requires analytical methods which are simple, robust, and rugged. Interpretation of results of validation parameters study shows that results of method is directly proportional to the concentration of analyte within a given range shows linearity of method. Different environmental condition and minor change in chromatographic condition doesn't cause any significant change in results shows stability and reproducibility of developed method. There was no interference by excipients with analyte peak shows proposed method is specific for analyte. As well as Recovery study shows the developed method is highly accurat. Hence the proposed HPLC method has been evaluated and validated for the accuracy, precision, and linearity and found to be convenient, sensitive and specific for the quality control of rosiglitazone and Gliclazide in tablet dosage form.

Conflict of interest statement

We declare that we have no conflict of interest.

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