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# DEVELOPMENT AND VALIDATION OF LC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ROSIGLITAZONE MALEATE AND GLIMEPIRIDE IN PHARMACEUTICAL DOSAGE FORM

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*A simple, precise and accurate high performance liquid chromatography (HPLC) method was developed for simultaneous quantitative determination of rosiglitazone maleate and glimepiride in pure forms and in pharmaceutical formulation. The separation was achieved by $C_{18}$ column using methanol:20 mM ammonium dihydrogen phosphate [78:22 (v/v); pH 3.85] as mobile phase at a flow rate of 1 mL/min and detection at 240 nm. Separation was complete in less than 10 min. Linearity, accuracy and precision were found to be acceptable over the ranges 0.8–4.0 µg/mL for rosiglitazone maleate and 0.4–2.0 µg/mL for glimepiride. This method was found to be specific, reproducible, precise and accurate. Due to its simplicity and accuracy the method is particularly suitable for routine pharmaceutical quality control.*

**Keywords:** HPLC, Rosiglitazone maleate, Glimepiride, Multicomponent formulation

## INTRODUCTION

Diabetes mellitus is characterised by chronic hyperglycemia with disturbances in fat, carbohydrate and protein metabolism due to abnormal insulin secretion and/or action (Granner and Davis 2001). Several drugs are available for the treatment of Type 2 diabetes mellitus (T2DM) like rosiglitazone, chemically known as (±)-5-[4-[2-[*N*-methyl-*N*(2-pyridyl)amino]ethoxy]benzyl]-2,4-dione thiozolidine (Fig. 1). It is a potent new oral antihyperglicemic agent that reduces insulin resistance in patients with T2DM by binding to peroxisome proliferator-activated receptors gamma (PPAR-γ) (Gale 2001; Balfour and Plosker 1999). Glimepiride is 1-[{p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl}sulfonyl]-3-(trans-4-methylcyclohexyl)urea (Fig. 2), which belongs to second generation sulphonylurea used for the treatment of T2DM (O'Neil 2001). The drugs are prescribed individually as well as in multi component dosage forms.

Few methods are available in the literature for the analysis of rosiglitazone and glimepiride. Some methods have been reported for the determination of rosiglitazone in human plasma (Pedersen, Brosen and Nielsen 2005; Hruska and Frye 2004; Mamidi *et al.* 2003) in pharmaceutical dosage form (Gomes 2006; Gomes *et al.* 2004; Sankar, Kumar and Reddy 2004; Gayatri, Shantha and Vaidyalingam 2003; Gumieniczek *et al.* 2003; Sane *et al.* 2002) and some analytical methods for determination of glimepiride have been reported like estimation of glimepiride in plasma (AbuRuz, Millership and Elnay 2005; Kim *et al.* 2004; Maurer *et al.* 2002, 1990) and in combinations with other drugs; glimepiride (Lad *et*

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*al.* 2003; Nadkarni *et al.* 1997) and rosiglitazone (Yardimci and Zaltin 2007; Zhang *et al.* 2007; Vasudevan *et al.* 2001). A literature survey revealed that no HPLC method has been reported for the simultaneous analysis of rosiglitazone and glimepiride in pharmaceutical preparations. Therefore, an HPLC method was developed for simultaneous analysis of rosiglitazone and glimepiride in fixed dose combination tablet preparations. The methods described are rapid, economical, precise and accurate and can be used for routine analysis of rosiglitazone and glimepiride, simultaneously in fixed dose combination tablets in quality control laboratories. The method was validated as per International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (ICH 2007).

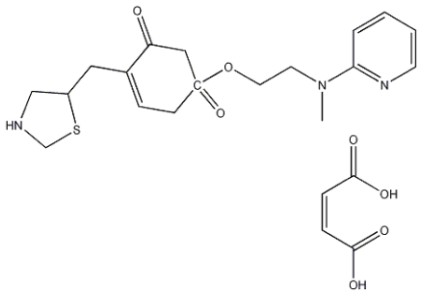

**Fig. 1:** Chemical structure of rosiglitazone.

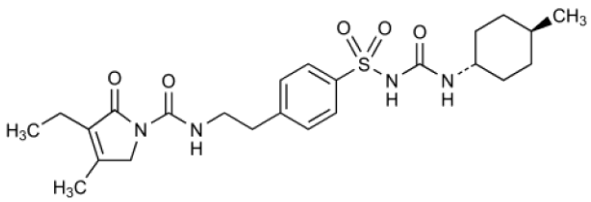

**Fig. 2:** Chemical structure of glimepiride.

## EXPERIMENTAL

### Materials and Methods

Pharmaceutical grade rosiglitazone maleate (rosiglitazone) [batch no. 758001] and glimepiride (glimepiride) [batch no. 2088581] working standard were obtained as generous gifts from Ranbaxy Pvt. Ltd. (Indore, India). Fixed-dose combination tablets Rosicon-G (batch no. 7829800) containing 2 mg of rosiglitazone and 1 mg glimepiride were purchased from Glenmark (T-I; Healtheon, Nasik, Maharashtra, India) and Enseline-2G (batch no. 00012930) containing 2 mg of rosiglitazone and 1 mg glimepiride were purchased from Torrent Pharmaceuticals Pvt. Ltd. (T-II; Jasco Analytical Instruments, Japan). All chemicals and reagents were of HPLC grade and were purchased from Merck Chemicals (Mumbai).

**Instrumentation**

The LC system consisted of a pump (model Jasco PU1580, intelligent LC pump, Jasco Analytical Instruments, Japan) with auto injecting facility (AS-1555 sampler) programmed at 20 µL volume per injection was used. The detector consisted of a UV–VIS (Jasco UV 1575, Jasco Analytical Instruments, Japan) model operated at a wavelength of 240 nm. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system (Jasco Analytical Instruments, Japan). The column used was HiQ Sil C18HS (250 mm × 4.6 mm, 5.0 µm; Kya Technologies Corporation, Japan). The mobile phase and samples were filtered using 0.45 µm membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature.

**Preparation of Standard Stock Solutions and Mixed Standard Solutions**

Standard stock solutions of a concentration of 1 mg/mL of rosiglitazone and 1 mg/mL of glimepiride were prepared separately using methanol. From the standard stock solution, the mixed standard solutions were prepared by dilution of the stock solution with mobile phase to reach a concentration range 0.8–4.0 µg/mL for rosiglitazone and 0.4–2.0 µg/mL for glimepiride.

**Sample Preparation**

For the analysis of tablets, 20 tablets of each T-I and T-II were weighed and finely ground in a mortar. For T-I and T-II, the portion equivalent to 4 mg of rosiglitazone and 2 mg of glimepiride were transferred in 25 mL volumetric flask, volume was made up to 25 mL with methanol and sample was sonicated for 45 min with swirling. After sonication, the solution was filtered through Whatman filter paper 41. For both T-I and T-II, six determinations were performed.

**Optimisation of Chromatographic Methods**

The HPLC procedure was optimised with a view to develop a simultaneous estimation of glimepiride and rosiglitazone in fixed dose combined dosage form. Initially methanol and water in different ratios were tried. But glimepiride gave broad peak shape, so water was replaced by potassium dihydrogen phosphate buffer (20 mM), and mixture of methanol and potassium dihydrogen phosphate buffer in different ratios and pH were tried.

**Method Validation**

Validations of optimised chromatographic methods were carried out with respect to the following parameters.

*Linearity*

Linearity of the method was studied by taking five calibration points for both rosiglitazone and glimepiride. The mixed standard solutions in the concentration range of 0.8–4.0 µg/mL for rosiglitazone and 0.4–2.0 µg/mL for glimepiride injected 6 times into the LC system keeping the injection volume constant.

*Precision*

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability was performed by analysis of 3 different concentrations: 0.8, 2.4, 4.0 µg/mL for rosiglitazone and 0.4, 1.2, 2.0 µg/mL for glimepiride, 6 times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

*Limit of detection and quantification*

Limits of detection (LOD) and limits of quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise (S/N) ratios of 3 for LOD and 10 for LOQ, respectively. The LOD and LOQ were determined by measuring the magnitude of analytical background by injecting a blank and calculating the signal-to-noise ratio for rosiglitazone and glimepiride by spotting a series of solutions until the S/N ratio 3 was obtained for the LOD and 10 for the LOQ.

*Robustness*

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 ± 0.1 mL/min), mobile phase composition (methanol ± 2 mL). These chromatographic variations were evaluated for resolution between rosiglitazone and glimepiride. Robustness of the method was done at 3 different concentration levels: 0.4, 1.2, 2.0 µg/mL for rosiglitazone and 16, 48, 80 µg/mL for glimepiride.

*Solution stability*

To assess the solution stability mixed solution of rosiglitazone and glimepiride (2 µg/mL each) was prepared from stock solution and was kept at room temperature for 24 h. This solution was compared with freshly prepared standard solution.

*System suitability*

The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between rosiglitazone and glimepiride peaks were defined for HPLC.

*Specificity*

Extracts of commonly used placebo were injected to demonstrate the absence of interference with the elution of the rosiglitazone and glimepiride. For determining selectivity of the method, a powder blend of typical tablet excipients containing lactose monohydrate, mannitol, maize starch, povidone K30, citric acid anhydrous granular, sodium citrate, natural lemon and lime flavour, acesulfame, potassium and magnesium stearate was prepared and analysed. All chromatograms were examined to determine if compounds of interest co-eluted with each other or with any additional excipients peaks.

*Accuracy*

Recovery studies were carried out by applying the method to drug samples to which known amount of drug corresponding to 80%, 100% and 120% of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared with expected results.

## RESULTS

### Method Development and Optimisation

UV scanning at 200–400 nm for rosiglitazone and glimepiride showed that 240 nm (Fig. 3) is the suitable wavelength for detection of combination. The chromatographic methods were optimised with a view to develop a suitable LC method for the analysis of rosiglitazone and glimepiride in fixed dose combined dosage form. It was found that methanol:20 mM ammonium dihydrogen phosphate [78:22 (v/v); pH 3.85] with ortho phosphoric acid, gave acceptable retention time ($t_R$ 3.32 min for rosiglitazone and $t_R$ 8.42 min for glimepiride) [Fig. 4], number of theoretical plates, and good resolution for glimepiride and rosiglitazone at the flow rate of 1.0 mL/min.

### Validation

*Linearity*

Linearity was evaluated by analysis of working standard solutions of rosiglitazone and glimepiride of five different concentrations. The range of linearity was from 0.8–4.0 µg/mL for rosiglitazone and 0.4–2.0 µg/mL for glimepiride. The regression data obtained are represented in Table 1.

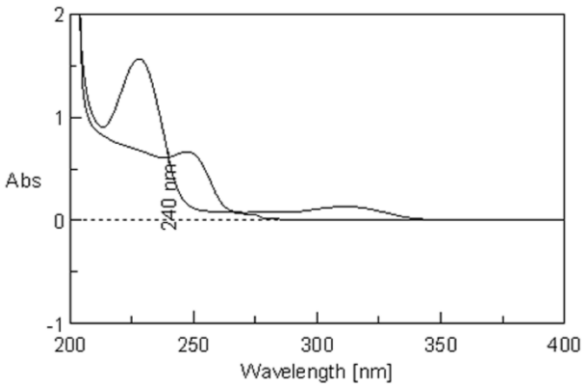


**Fig. 3:** Overlain UV spectra of rosiglitazone and glimepiride measured from 200–400 nm.

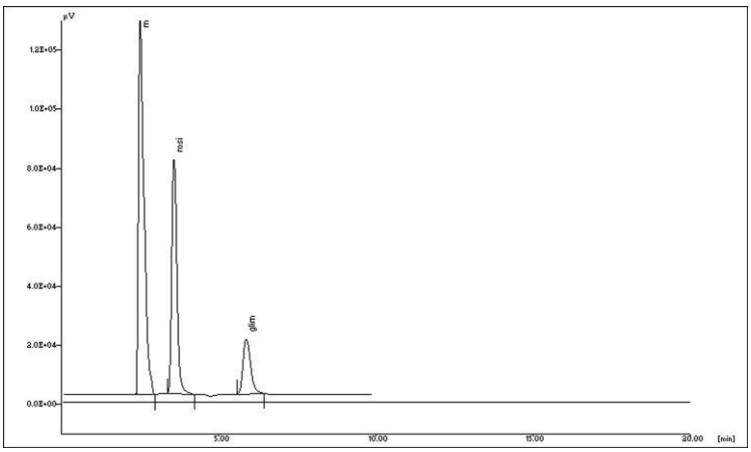


**Fig. 4:** Overlay chromatogram obtained for the commonly used excipients and rosiglitazone (16 µg/mL), $t_R$: 3.32 min; glimepiride (8 µg/mL), $t_R$: 8.42 min; measured at 240 nm, mobile phase: methanol:potassium dihydrogen phosphate buffer (20 mM) (78:22 v/v).

*Precision*

The average of standard deviation (SD) and percentage of relative standard deviation (RSD) of all three concentrations of rosiglitazone and glimepiride for repeatability and intermediate precision study are shown in Table 2.

*LOD and LOQ*

The LOD and LOQ values for rosiglitazone were found to be 0.01 and 0.025 µg/mL respectively and for glimepiride 0.002 and 0.006 µg/mL respectively.

*Robustness of the method*

None of the alterations caused a significant change in resolution between rosiglitazone and glimepiride, peak area, RSD, tailing factor and theoretical plates (Table 3).

*Solution stability studies*

Three different concentrations of rosiglitazone and glimepiride (2, 4 and 6 µg/mL) were prepared from sample solution and stored at room temperature for 8 days. They were then injected into the HPLC system, no additional peak was found in the chromatogram (Table 4).

*System suitability*

System suitability parameters such as the number of theoretical plates, resolution, and assymetry were determined. The results obtained are shown in Table 5.

**Table 1:** Linear regression data for the calibration curves[a].

| Parameters | | Rosiglitazone | Glimepiride |
|---|---|---|---|
| Linearity (µg/mL) | | 0.8–4.0 | 0.4–2.0 |
| SD | | 4007 | 221 |
| y = A + Bx | A | –2933 | 599.9 |
| | B | 33797 | 66491 |
| $r^2$ | | 0.999 | 0.999 |

*Note:* [a]n=6; [a]$r^2$=coefficient of correlation

**Table 2:** Repeatability and intermediate precision of methods[a].

| Compound | Repeatability | | Intermediate | |
|---|---|---|---|---|
| | SD | % RSD | SD | % RSD |
| Rosiglitazone | 1506 | 4581 | 0.69 | 0.72 |
| Glimepiride | 647 | 146 | 0.19 | 0.89 |

*Note:* [a]n=6, (0.8, 2.4, 4.0 µg/mL for rosiglitazone and 0.4, 1.2, 2.0 µg/mL for glimepiride)

*Specificity*

Extracts of commonly used placebo were injected to demonstrate the absence of interference with the elution of the drugs. These results demonstrate that there was no interference from other materials in the tablet formulation (Fig. 4).

*Recovery studies*

Good recoveries of the rosiglitazone and glimepiride were obtained at various added concentrations for T-I and T-II as shown in Table 6.

*Analysis of a commercial formulation*

Two different brands of fixed dose combination tablets were analysed using the proposed procedures Table 7.

## DISCUSSION

It was found that methanol:20 mM ammonium dihydrogen phosphate [78:22 (v/v); pH 3.85] with ortho phosphoric acid, gave acceptable retention time, number of theoretical plates, and good resolution for glimepiride and rosiglitazone.

The result shows that there was an excellent correlation between peak area and concentration of each drug. The developed methods was found to be precise, with RSD values for repeatability and intermediate precision <2%, as recommended by ICH guidelines. Separation of the drugs was found to be similar when analysis was performed

on different chromatographic systems on different days. The low values of the (%) RSD indicated robustness of the method.

Three different concentrations of rosiglitazone and glimepiride (2, 4 and 6 µg/mL) were injected into the HPLC system, no additional peak was found in the chromatogram indicating the stability of rosiglitazone and glimepiride in the solution. These results demonstrate that there was no interference from other materials in the tablet formulation; therefore, confirm the specificity of the method. When we compared this method with already reported methods such as method reported by AbuRuz, Millership and McElnay (2005), Sankar, Kumar and Reddy (2004), Gayatri, Shantha and Vaidyalingam (2003) and Maurer *et al.* (1990). Our method showed good recoveries of the rosiglitazone (100.13%–100.34%) and glimepiride (99.85%–99.92%) from tablet at various added concentrations for T-I and T-II. And also this method was found to be sensitive as low the LOD and LOQ values were obtained for rosiglitazone and glimepiride. Experimental results of the amount of rosiglitazone and glimepiride in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients, which are normally present in tablets.

## CONCLUSION

The developed and validated LC method enables specific, accurate, robust and precise simultaneous analysis of rosiglitazone maleate and glimepiride in tablet formulations. The method is sensitive enough for quantitative detection of the analytes in pharmaceutical preparations. The proposed method can thus be used for routine analysis, quality control and for studies of the stability of pharmaceutical tablets containing these drugs.

## CONFLICT OF INTEREST

The author declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

**Table 3:** Robustness testing.

| Chromatographic factors[a] | Level | Rosiglitazone | | | Glimepiride | | |
|---|---|---|---|---|---|---|---|
| | | $t_R$[b] | Rs[c] | As[d] | $t_R$[b] | Rs[c] | As[d] |
| Flow rate (mL/min) | 0.9 | 3.41 | 0 | 1.24 | 8.98 | 2.45 | 1.25 |
| | 1.0 | 3.32 | 0 | 1.29 | 8.42 | 2.43 | 1.27 |
| | 1.2 | 3.28 | 0 | 1.38 | 8.90 | 2.41 | 1.31 |
| % of methanol | 76 | 3.45 | 0 | 1.26 | 8.93 | 2.46 | 1.23 |
| | 78 | 3.35 | 0 | 1.27 | 8.47 | 2.42 | 1.26 |
| | 80 | 3.29 | 0 | 1.30 | 8.85 | 2.38 | 1.29 |

*Note:* [a](n=6), $t_R$[b]: Retention time, Rs[c]: Resolution, As[d]: Asymmetry

**Table 4:** Stability of drugs in sample solutions[a].

| Parameters | Rosiglitazone | Glimepiride |
|---|---|---|
| Average area | 40291004 | 720019 |
| SD of areas | 1487.64 | 529.27 |
| % RSD | 0.46 | 0.89 |

*Note:* [a]n=6

**Table 5:** System suitability parameters for rosiglitazone and glimepiride.

| Parameters | Rosiglitazone | Glimepiride |
|---|---|---|
| Number of theoretical plates | 6629.92 | 3969.86 |
| Resolution | – | 3.35 |
| Peak asymmetry | 1.29 | 1.27 |
| % RSD | 0.08 | 0.05 |

**Table 6:** Recovery studies[a].

| Label claim | Amount of drug added (%) | Total amount of drug present (µg/mL) | Amount found (µg/mL) | % recovery |
|---|---|---|---|---|
| T-I | | | | |
| Rosiglitazone 2 mg | 80 | 1.44 | 1.441 | 100.13 |
| | 100 | 1.60 | 1.59 | 99.38 |
| | 120 | 1.76 | 1.74 | 99.41 |
| Glimepiride 1 mg | 80 | 0.72 | 0.71 | 99.48 |
| | 100 | 0.80 | 0.79 | 99.66 |
| | 120 | 0.88 | 0.87 | 99.85 |
| T-II | | | | |
| Rosiglitazone 2 mg | 80 | 1.44 | 1.43 | 99.37 |
| | 100 | 1.60 | 1.59 | 99.40 |
| | 120 | 1.76 | 1.50 | 100.34 |
| Glimepiride 1 mg | 80 | 0.72 | 0.71 | 99.21 |
| | 100 | 0.80 | 0.79 | 99.26 |
| | 120 | 0.88 | 0.87 | 99.92 |

*Note:* [a]n=6

**Table 7:** Applicability of the method for the analysis of the pharmaceutical formulations.

| Sample | Label claim (mg) | Drug content (%) | % RSD |
|---|---|---|---|
| T-I | | | |
| Rosiglitazone | 2 | 100.49 | 0.17 |
| Glimepiride | 1 | 99.93 | 0.38 |
| T-II | | | |
| Rosiglitazone | 2 | 99.53 | 0.059 |
| Glimepiride | 1 | 99.28 | 0.44 |

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