DEVELOPMENT AND VALIDATION OF HPTLC DENSITOMETRY METHOD FOR SIMULTANEOUS ESTIMATION OF ROSIGLITAZONE AND GLIMEPIRIDE IN FIXED TABLET DOSAGE FORM

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ABSTRACT

A new, simple, precise, and accurate high performance thin layer chromatography (HPTLC) densitometry method has been developed for simultaneous estimation of rosiglitazone maleate (ROSI) and glimepiride (GLIM) in tablet dosage form. Procedure does not require prior separation of components from the sample. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F₂₅₄ as the stationary phase and the solvent system consisted of methanol: toluene: ethyl acetate (1:8:1, v/v/v). Densitometric evaluation of the separated zones was performed at 228 nm. The drugs were satisfactorily resolved with R_f values 0.39 ± 0.03 and 0.20 ± 0.04 for ROSI and GLIM, respectively. Parameters such as linearity, precision, accuracy, recovery, specificity, and ruggedness were studied as reported in the International Conference on Harmonization (ICH) guidelines. The linearity regression analysis for calibration showed correlation coefficients R² value 0.9989 and 0.9996 for ROSI and GLIM, respectively with respect to peak area in the concentration range of 100-1500 ng/spot. The mean percentage recovery values were close to 100 %, it indicates that there is no interferences of additives with ROSI and GLIM present in tablet dosage form. Statistical analysis proved that the method is suitable for the quality control analysis of ROSI and GLIM as a bulk drug and in pharmaceutical formulations. The study may be extended to comprehend the degradation kinetics of these two drugs.

Keywords: Rosiglitazone maleate, Glimepiride, HPTLC, Densitometry, Validation.

INTRODUCTION

Type 2 diabetes is a long term metabolic disorder wherein the body becomes resistant to the effects of insulin, a hormone that regulates sugar absorption. Treatment of type-2 diabetes (non insulin dependent) is now possible with orally administered hypoglycemic agents that help to reduce blood sugar levels.¹ To control this disorder, combination therapy is often used. Rosiglitazone maleate (ROSI) and Glimepiride (GLIM) are widely used to treat type-2 diabetes. Rosiglitazone, chemically [(±)-5-[4-[2-[N-methyl-N-(2-pyridyl)amino]-ethoxy]benzyl]-2,4-dione thia- zolidine] (Fig. 1), is a potent oral antihyperglycemic agent that reduces insulin resistance in patients with type-2 diabetes by binding to peroxisomal proliferator-activated receptors gamma (PPAR-γ).^{2,3}

Fig. 1. Chemical structure of ROSI

Few capillary electrophoresis⁴, LC/MS HPLC in human plasma⁵, LC in pharmaceutical formulation and human plasma^{6,7}, MEKC and HPLC⁸, solid phase extraction coupled with LC- MS/MS⁹, HPTLC¹⁰ methods have been reported for analytical monitoring of ROSI individually and with other drugs. Glimepiride, chemically 3-ethyl-4-methyl-N-(4-(N-(1-(4-methylcyclohexyl)amino)vinyl)-sulfamoyl)phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide, is a sulphonyl-urea antidiabetic drug and its structure is shown in Fig. 2.¹¹

Fig. 2. Chemical structure of GLIM

Literature survey revealed that derivative spectrophotometric¹², HPLC¹³ and HPLC-ESI-MS-MS¹⁴, HPTLC^{15,16} methods are available for GLIM estimation alone and with other drugs. Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC thus reducing the analysis time and cost per analysis. Till date, to the best of our knowledge, no HPTLC method has been reported in the literature for estimation of ROSI and GLIM in combined dosage form.

In the present work, an attempt has been made to develop simple, precise, accurate HPTLC method for simultaneous estimation of ROSI and GLIM in tablet dosage form. The proposed method is validated as per ICH guidelines.¹⁷

EXPERIMENTAL

Chemicals and Reagents

Pharmaceutically pure sample of ROSI and GLIM were obtained as generous gifts from Panacea Biotec Ltd., Malpur, Solan (H.P.), India and Medley Pharmaceuticals Ltd., Bari Brahmna, Jammu, India, respectively.

A combination of ROSI (2.0 mg) and GLIM (1.0 mg) in tablet formulation (Rosicon-G, Glenmark Pharmaceuticals Ltd., Baddi, H.P., India) was obtained from local market commercially. All the chemicals used were of analytical grade, obtained from E.Merck, Mumbai, India.

Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample syringe (Hamilton, Switzerland) on aluminium plates precoated with silica gel 60 F₂₅₄ [10 cm X 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate of 0.1 µl/s was used. The space between two bands was 5 mm and 6 tracks were applied per plate. The slit dimension was kept at 5 mm X 0.45 mm and the scanning speed was 10 mm/s.

The mobile phase consisted of methanol: toluene: ethyl acetate in the proportion of 1:8:1, v/v/v and 10 ml of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm X 10 cm twin rough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C±2) at relative humidity of 60%±5. Each chromatogram was developed over a distance of 8 cm.

After the development the TLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed using a Camag TLC Scanner III in the reflectance absorbance mode at 228 nm and operated by WinCATS software. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

Preparation of standard solutions

Standard stock solutions of each concentration 1000 µg/ml of ROSI and GLIM were prepared separately using methanol. From the standard stock solution, the diluted mixed standard solution was prepared using the methanol to contain 200 µg/ml of ROSI and 100 µg/ml of GLIM, respectively.

Optimization of the HPTLC method

The TLC procedure was optimized with a view to develop a simultaneous assay method for ROSI and GLIM, respectively. The mixed standard stock solution (200 µg/mL of ROSI and 100 µg/mL of GLIM) was taken and 6 µl sample was spotted on to TLC plates and run in different solvent systems. After trying several permutation and combination, finally the mobile phase consisting of methanol: toluene: ethyl acetate in the proportion of 1:8:1 v/v/v was found optimum as it gave well separated band of the drugs, as compared to other mobile phases (Fig. 3).

In order to reduce the neckless effect, TLC chamber was saturated for 30 min using saturation pads. The mobile phase was run up to a distance of 8 cm; which takes approximately 15-20 min for complete development of the TLC plate.

After application and development of bands of the working standard solution, the separated bands on the HPTLC plate were scanned over the wavelength range 200-400nm. The wavelength selected for densitometric determination was 228 nm. The spectrums obtained are depicted in Fig. 4 and Fig. 5.

Fig. 3. Chromatogram of standard solution of ROSI and GLIM.

Fig. 4. Spectrum of ROSI (200–400 nm).

Fig. 5. Spectrum of GLIM (200–400 nm).

Analysis of tablet formulation

Twenty tablets were weighed and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 10 mg of ROSI was transferred to 10 ml volumetric flask and dissolved in 5 ml of methanol, sonicate for 15 min with occasional shaking and volume was adjusted up to 10 ml with same solvent. The solution was filtered through Whatman filter paper No.42 and aliquot portion of the above filtrate was diluted to obtain a solution of 200 µg /ml and 100 µg/ml of ROSI and GLIM, respectively. From the above sample solution 6 µl was applied on prewash TLC plate with standard solution which gave final concentration of 1200 ng/spot for ROSI and 600 ng/spot for GLIM, which was developed and scanned in optimized conditions. The amount of ROSI and GLIM present per tablet was calculated by comparing peak area of sample with that of standard.

Method validation

Validation of the optimized TLC method was carried out with respect to the following parameters.

Linearity

From the diluted standard stock solution 100µg/ml of each drug, 1 to 15 µl solution spotted on TLC plate to obtain final concentration 100 to 1500 ng/spot for ROSI and 100 to 1500 ng/spot for GLIM. Linearity of the method was studied by applying six concentrations of the drug (n=6) and each concentration was applied three times to the TLC plates. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Accuracy

The accuracy of the method was determined by calculating recoveries of ROSI and GLIM using standard addition method at different levels to the pre-analyzed sample. For that known amounts of standard solutions of ROSI and GLIM (80, 100, and 120 % of test concentration) were added to prequantified sample of tablet dosage form and determining their contents.

Precision

The precision of the method was verified by repeatability precision studies. Repeatability studies were performed by analysis the sample solutions of the drugs six times on the same day. Series of diluted standard solutions were prepared and analyzed by proposed method.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

$$\text{LOD} = \frac{3.3 \delta}{S} \quad \text{LOQ} = \frac{10 \delta}{S}$$

where, δ : standard of y-intercept and S: slope of calibration curve.

Ruggedness

Ruggedness of the proposed method was determined by analysis of sample solution prepared by proposed method between different time intervals, days, and analysts.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spots for ROSI and GLIM in the samples were confirmed by comparing the R_f and spectrum to the spots with that of a standards. The peak purity of ROSI and GLIM was determined by comparing at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

RESULTS AND DISCUSSION

Literature survey reveals that no HPTLC method has been reported for simultaneous determination of ROSI and GLIM in combined tablet dosage form. So, the proposed HPTLC method was optimized with several solvent systems. The mobile phase consisting of methanol: toluene: ethyl acetate (1:8:1, v/v/v) and aluminium plate precoated with silica gel 60 F₂₅₄ (10 cm × 10 cm) as stationary phase were used which gave good resolution with R_f values of 0.39 and 0.20 for ROSI and GLIM, respectively. The well defined peaks were obtained only when the chamber was saturated with the mobile phase for 30 min at a controlled temperature before plate development and UV detection was carried out at 228 nm. Resolution of the peaks for mixture of standard drugs with clear baseline was obtained (Fig. 3).

The validation parameters were studied for proposed HPTLC method and summary of validation parameters is shown in Table 1.

Table 1. Validation parameters of the method.

Parameters	ROSI	GLIM
Linearity range (ng/spot)	100 to 1500 ng/spot	100 to 1500 ng/spot
Precision (CV)	0.4659%	0.4876%
% Recovery	99.55±0.1868	99.63±0.2138
LOD (ng/spot)	35	90
LOQ (ng/spot)	30	85
Ruggedness		
Intraday (n=3)	99.94	99.64
Interday (n=3)	99.78	99.54
Analysts(n=3)	99.66	99.82

LOD: Limit of Detection, LOQ: Limit of Quantification.

Linearity

Linear relationships were observed by plotting the concentration of each analyte versus peak area. ROSI and GLIM showed linear response in the concentration range of 100-1500 ng/spot. The standard calibration graph and data for regression analysis are shown in Fig. 6, Fig. 7 and Table 2, respectively.

Table 2. Regression analysis data of the method.

Parameters	ROSI	GLIM
Linear Range (ng/spot)	100-1500 ng/spot	100-1500 ng/spot
Slope	0.1887	0.1469
Intercept	16.218	13.407
Regression Co-efficient R ²	0.9989	0.9996

Fig. 6. Calibration graph for ROSI.

Fig. 7. Calibration graph for GLIM.

Precision

The results of the precision experiments are shown in Table 2. The developed method was found to be precise as the %RSD values for repeatability precision studies was < 2%, as recommended by ICH guidelines.

LOD and LOQ

The LOD and LOQ were found to be 35 ng/spot and 90 ng/spot for ROSI, 30 ng/spot and 85 ng/spot for GLIM, respectively.

Ruggedness

The standard deviation was calculated for each parameter and the % RSD were found to be less than 2%. The low values of the %RSD, as shown in Table 2 indicated the ruggedness of the method.

Specificity

The peak purity of ROSI and GLIM was assessed by comparing their respective standard and sample chromatogram.

Accuracy

Accuracy was investigated by means of recovery studies using the proposed method. The mean recoveries (n=9) 99.55% and 99.63% obtained for ROSI and GLIM, respectively which demonstrate an adequate accuracy (Table 3).

Analysis of formulation

The results of analysis of pharmaceutical dosage form by the proposed method (Table 4), expressed as percentage of label claim 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.

Table 3. Results of recovery studies.

Drug	Level (%)	Amount taken	Amount added	Total amount found*	Recovery* (%± SD)	Average recovery (%± S.D.)
ROSI	80	20	16	15.93	99.58	99.55± 0.1868
	100	20	20	19.94	99.72	
	120	20	24	23.84	99.35	
GLIM	80	10	8	7.984	99.80	99.63± 0.2138
	100	10	10	9.94	99.39	
	120	10	12	11.96	99.70	

*Average of three determinations, SD: Standard deviation.

Table 4. Analysis of formulation using the HPTLC method.

Drug	Amount Labelled (mg/tablet)	Amount Estimated*	% Label* claim	CV
ROSI	2	1.9926	99.63	0.4659
GLIM	1	0.9976	99.76	0.4876

*Average of six determinations, CV: Coefficient of variance.

CONCLUSION

The proposed HPTLC method is precise, specific and accurate. Statistical analysis proves that the method is suitable for the simultaneous analysis of ROSI and GLIM in pharmaceutical formulation without any interference from the excipients. It was concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps and improved sensitivity made it specific, reliable and easily reproducible in any quality control analysis providing all the parameters are followed accurately for its intended use.

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