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Research Article

DEVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR THE ESTIMATION OF ROSIGLITAZONE ENANTIOMERS IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, fast, specific and precise LC-MS/MS method was developed and validated for determination of rosiglitazone enantiomers in pharmaceutical formulation. The interface used was Atmospheric Pressure Chemical Ionisation Technique. Analysis was performed using a ACI cellu 1 column (150 x 4.6 mm I.D., particle size 5 µ) by isocratic elution with 0.025% formic acid (pH 6): Acetonitrile (15:85) and flow rate was 0.5 ml/min. The calibration plot was linear over the range of 30 - 70 ng/ml of R-Rosiglitazone and 90 - 210 ng/ml of S-Rosiglitazone with a correlation of 0.998 for R and S Rosiglitazone. The method was also validated for the precision and recovery. This optimized mobile phase separated R-Rosiglitazone at 3.09 min and S-Rosiglitazone at 5.3 min respectively. The proposed LC-MS/MS method is suitable for analysis of rosiglitazone enantiomers in pharmaceutical formulations and quality control analysis.

KEY WORDS: Rosiglitazone, LC-MS/MS, Atmospheric Pressure Chemical Ionisation, Enantiomers, Pharmaceutical formulation

INTRODUCTION

Human diabetes is currently classified into two general categories: Type I, or insulin-dependent diabetes mellitus, and Type II, or non-insulin-dependent diabetes mellitus (NIDDM). Peripheral insulin resistance is one of the pathogenic factors that contribute to the hyperglycemic state in NIDDM. Rosiglitazone maleate chemically known as [(RS)-5-4-[2-[*N*-methyl-*N*(2-pyridyl)amino]ethoxy]benzyl]-2,4-dione thiozolidine maleate[1] is a potent and orally active insulin sensitizing agent that was shown to improve glycemic control in animal models of NIDDM (Fig: 1). It was reported that analysis of Rosiglitazone metabolites by semi micro column liquid chromatography with ultraviolet absorption and pulsed ampherometric detectors[2,3]. Furthermore, Pierina SB et al. have reported enantioselective determination of rosiglitazone in plasma by high-performance liquid chromatography electrospray mass spectrometry[4]. Stereospecific analysis of the major metabolites of rosiglitazone in urine by sequential achiral-chiral high-performance liquid chromatography has been described by Tan SC et al.[5]. Chiral HPLC methods[6-12] for the analysis of rosiglitazone and its enantiomers by using AGP, Chiralcel OD-R and Cyclobond I SP columns. However, there are no reports concerning the analysis of rosiglitazone enantiomer in pharmaceutical formulations. So it is felt necessary to develop a liquid chromatography mass spectroscopy (LC-MS/MS) procedure which would serve as a rapid and reliable method for the determination of rosiglitazone enantiomer in pharmaceutical formulations.

EXPERIMENTAL

Solvents and chemicals

Reference standards of enantiomers of Rosiglitazone were procured from Sigma Aldrich limited, Mumbai, India. Working standard of Rosiglitazone RS (98.67%) was obtained as gift sample from Sun Pharmaceutical Industries Ltd., Bharuch, and Gujarat, India. Commercially available tablets Rosiglitazone were purchased commercially from the local market, Ooty, Tamilnadu, India. Chromatographic grade solvents like acetonitrile and formic acid were obtained from Qualigens chemicals, Mumbai, India.

Apparatus and instrument conditions

The LC-MS/MS was performed using a Shimadzu API 3000 LC-MS/MS with auto injector and Analyst 1.31 data solution. ACI cellu 1 column (150 x 4.6 mm I.D., particle size 5 µ) was used. Sample volume of 10 µl was injected. LC separation was carried out using mobile phase of 0.025% formic acid (pH 6): Acetonitrile (15:85), and flow rate was 0.5 ml/min. The working conditions for APCI MS/MS were as follows: The probe temperature was set at 510 ºC and the polarity was maintained at positive ion mode, ion at m/z 357.43 was assigned to (M+H) of Rosiglitazone. This ion was monitored and quantified.

Preparation of Standard solution

The stock solutions containing 1 mg/ml of R and S form of Rosiglitazone were prepared in methanol. These stock solutions were stored in light resistant containers. Aliquots of R-Rosiglitazone (30 - 70 ng/ml) and S-Rosiglitazone (90 - 210 ng/ml) were prepared in the mobile phase for analysis.

Preparation of sample solution

Twenty tablets were weighed; the average weight was determined and finely powdered. The powder equivalent to 5 mg of R and S form of Rosiglitazone (equivalent to 10 mg of racemic Rosiglitazone) was accurately weighed and transferred into a 10 ml volumetric flask. To this 5 ml of mobile phase was added and sonicated for 10 min. The resulting solution was made up to 10 ml with mobile phase and filtered using whatmann filter paper No. 42. The components R and S enantiomers of rosiglitazone from one formulation (Reglit Tablets containing 2 mg of Rosiglitazone) were extracted in mobile phase. The standard and sample solutions were analysed by the optimized chromatographic conditions, the chromatograms were recorded.

Method validation

Linearity

Standard solutions of 30 - 70 ng/ml of R-Rosiglitazone and 90 - 210 ng/ml of S-Rosiglitazone were analyzed to check the linearity of response (Fig: 2).

Specificity

The specificity of the method was ascertained by analyzing the standards and the samples. The peaks of R and S Rosiglitazone in samples were confirmed by comparing the retention time and M+H peak.

Precision

Six injections at three different concentrations of R-Rosiglitazone (30, 50, 70 ng/ml) and S-Rosiglitazone (90, 150, 210 ng/ml) enantiomers were made and analyzed to examine the precision of the method. The mean peak area, standard deviation and % RSD were calculated.

Accuracy (Recovery)

Accuracy of the method was determined by recovery experiments. The recovery of the method was determined at single level by adding a known quantity of Rosiglitazone R and S enantiomers to the drug products of pre analyzed samples and the mixtures were reanalyzed. The average recoveries obtained from each sample were calculated.

Ruggedness and Robustness

The ruggedness of the proposed method was determined by carrying out the experiment on different operators. Robustness of the method was determined by making small changes in the chromatographic conditions as stated in ICH guidelines.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the developed method were performed as stated in ICH guidelines.

Table: 1. Linearity and range for R and S Rosiglitazone enantiomers by LC-MS/MS

S. No	R – Rosiglitazone		S – Rosiglitazone	
	Concentration ng/ml	Peak area	Concentration ng/ml	Peak area
1	30	13980	90	41921
2	40	18329	120	54786
3	50	23670	150	70892
4	60	28952	180	86825
5	70	33325	210	99980

Table: 2. Precision studies for R and S Rosiglitazone enantiomers by LC-MS/MS

S. No	R – Rosiglitazone (ng/ml)			S – Rosiglitazone (ng/ml)		
	30	50	70	90	150	210
1	13980	23670	33325	41921	70892	99980
2	13985	23587	33387	41865	70799	99498
3	14191	23602	33211	41893	70853	99987
4	13889	23591	33305	41975	70875	99643
5	13897	23685	33427	41906	70902	99454
6	13995	23732	33298	41942	70823	99455
Mean	13989.5	23644.5	33325.5	41917	70857.33	99669.5
SD	108.99	59.86	75.32	38.48	40.27	252.89
% RSD	0.779	0.253	0.226	0.091	0.0564	0.253

Table: 3. Results of analysis of drug products and recovery studies for R and S Rosiglitazone enantiomers by LC-MS/MS

Label* claim (mg)	Amount present (mg/tablet) ± % RSD*			% Label Claim			% Recovery ± % RSD**
	R&S form	R-form	S-form	R&S form	R-form	S-form	
2	2.01 ±0.3468	0.50 ±0.8481	1.50 ±0.6987	100.73 ±0.8303	25.24 ±0.9394	75.49 ±0.3635	98.65 ±0.7641

*Reglit Tablets containing 2 mg of Rosiglitazone (RS)

** RSD of three determinations

Table: 4. System suitability studies for estimation of R and S Rosiglitazone enantiomers by LC-MS/MS

S. No	Parameters	R - Rosiglitazone	S – Rosiglitazone
1	Linearity range	30 -70 ng/ml	90 - 210 ng/ml
2	Regression equation Y = mx + c	y = 493.1x - 1005	y = 493.8x – 3197
3	Correlation coefficient	0.998	0.998
4	Resolution factor	1.8	
5	Asymmetric factor	1.02	1.01
6	LOD (ng/ml)	0.2086	0.2086
7	LOQ (ng/ml)	0.6324	0.6324

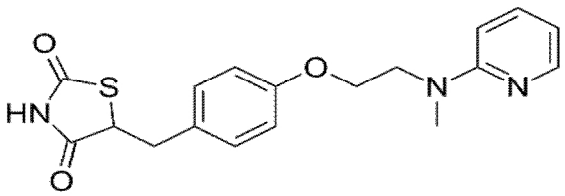

Fig: 1. Structure of Rosiglitazone

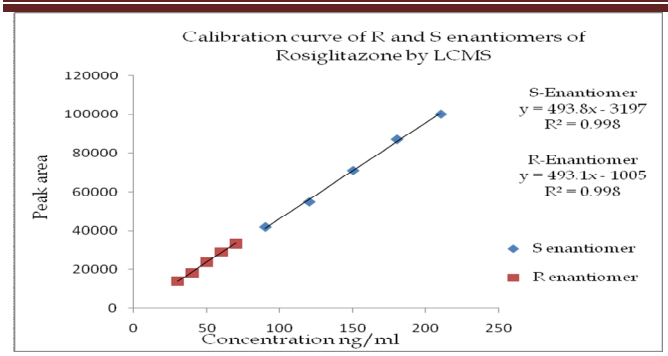

Fig: 2. LC-MS/MS Calibration curve of R and S Rosiglitazone

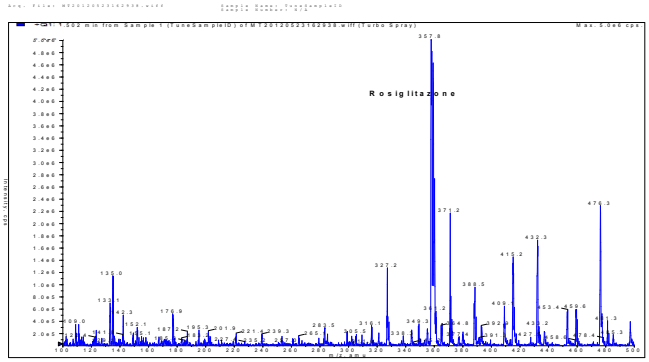

Fig: 3. Typical MS chromatogram of R and S Rosiglitazone

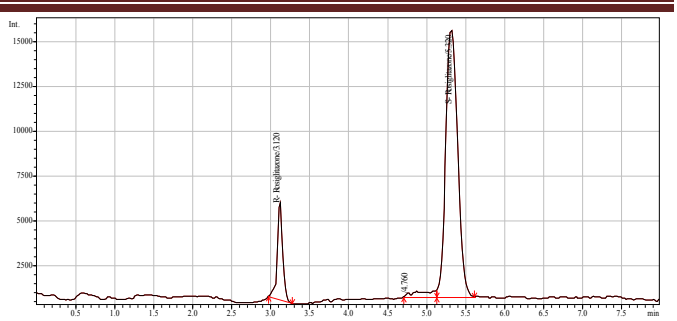

Fig: 4. Typical LC-MS/MS chromatogram of R and S Rosiglitazone standard solution

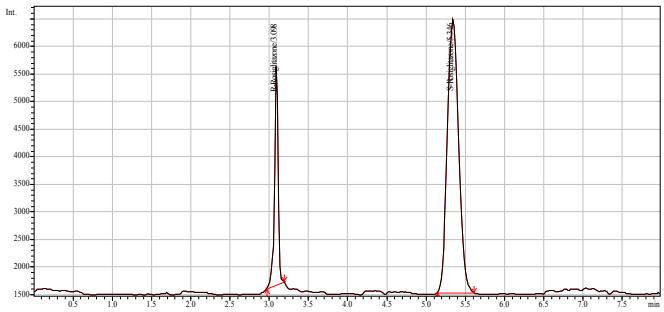

Fig: 5. Typical LC-MS/MS chromatogram of sample I containing R and S Rosiglitazone

RESULTS AND DISCUSSION

In the spectral investigation by LC/MS/MS in the SCAN mode, standard solution of rosiglitazone showed major peak at m/z of 359, which was assigned to the [M+H] ion of rosiglitazone (Fig: 3). Optimization of the method was carried out using various concentrations of acetonitrile while keeping the aqueous phase constant. A solvent combination of 0.025% formic acid (pH 6): acetonitrile (15: 85 % v/v) gave a satisfactory separation of the enantiomers of interest. This optimized mobile phase separated R-Rosiglitazone at 3.09 min and S-Rosiglitazone at 5.3 min respectively. The typical chromatograms of the standard and the sample solutions are shown in Fig: 4-5.

The calibration curves of R-Rosiglitazone and S-Rosiglitazone were linear in the range of 30–70 ng/ml and 90–210 ng/ml respectively (Table-1). Linear regression equation and correlation coefficient are shown in Table-4.

The precision of the method was demonstrated by reproducibility studies. The mean, standard deviation and % RSD were calculated and are presented in Table-2. The % RSD values of less than 2% revealed that the methods were precise.

The accuracy of the optimized method was determined by absolute recovery experiments. The percentage recovery values for R and S Rosiglitazone was found to be between 25.24 % and 75.49 %. An analysis of the results showed that the percentage recovery values were close to 100 % thus establishing that the developed method is accurate and reliable (Table-3).

Detection limits and quantification limits of R-Rosiglitazone and S-Rosiglitazone were found to be 0.2086 ng/ml and 0.6324 ng/ml respectively (Table-4).

No marked changes in the chromatogram occurred on changing the operator and chromatographic conditions indicating that the developed method was rugged and robust.

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and are presented in Table-4. The values obtained demonstrated the suitability of the system for the analysis of R-Rosiglitazone and S-Rosiglitazone in combined form in pharmaceutical formulation.

CONCLUSION

The developed LC-MS/MS method in the present study for the estimation was found to be simple, rapid, accurate, precise, specific, linear and rugged. It is thus suitable for the estimation of Rosiglitazone enantiomers in raw materials and formulations.

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