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Research Article

SPECROPHOTOMETRIC ESTIMATION AND STATISTICAL CORRELATION FOR ROSIGLITAZONE IN RAT AND HUMAN PLASMA

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ABSTRACT

Objective: A rapid and sensitive Spectrophotometric method was developed for Rosiglitazone in rat and human plasma.
Method: The sample was prepared by simple extraction method without derivatization and no use of buffer .Methanol and acetonitrile were used as the solvents in the proposed methods.
Results: Calibration range extended for rosiglitazone from1mcg/ml to 10 mcg/ml in rat plasma and 1mcg/ml to 17mcg/ml in human plasma with good regression coefficients in both the cases. The Limit of Detection and Limit of Quantification were found out to be 0.726mcg/ml and 2.2mcg/ml in rat plasma and 0.414mcg/ml and 1.255mcg/ml in human plasma. Assay results from the proposed method were found to be 100.2% and 100.12% in rat and human plasma respectively. Stability of the drug in both the plasma was found to be suitable in both refrigerated and ambient conditions.
Conclusion: The current method implied no significance difference as for estimation in rat and human plasma as tested from ANOVA analysis and can be extended pharmacokinetic studies. The proposed method was found prudent to be used in routine QC analysis.

Keywords: Rosiglitazone, UV-Vis Spectrophotometer, Rat Plasma, Human Plasma, Statistical Correlation

INTRODUCTION

Among the metabolic disorders Type-2 diabetes has become a burning topic of disorder where the body becomes resistant to effects of insulin[1].Treatment for the disorder has been possible by oral administration of hypoglycemic agents which reduces blood sugar level in a controlled manner[2].Among the treatments available ,a most potent monotherapy in use is Rosiglitazone,which is an oral andiabetic agent available in formulation in 2mg or 4 mg[3,4,5].It is chemically 5-((4-(2-(methyl-2-pyridinylamino) ethoxy)phenyl)methyl)-2,4-thiazolidinedione (Figure 1). It exerts its effect through the peroxisome proliferators-activated receptor gamma, which facilitates the expression of genes responsible for glucose and lipid metabolism [6]. It has received regulatory approval for the treatment of type 2 diabetes mellitus (T2DM) in both the monotherapy and the therapy in combination with other oral antidiabetic agents for its advantages of the therapeutic profile[7,8].Till date no spectrophotometric method has been published for Rosiglitazone in plasma. Hence an optimized and validated Spectrophotometric method for Rosiglitazone in rat and human plasma is presented along with its statistical relation with each other.

Materials and Methods

A double beam UV-Vis Spectrophotometer was used of JASCO.Rosiglitazone was procured from Actavis pharmaceuticals LTD, Indrad, Dist. Mehsana (Gujarat).Formulations were available from the local market.Slovents ere procured from Merck, India. Male Albino rats were procured from the Department of Pharmacology, SPS; SOA University with prior permission from IAEC.Human plasma was collected from SUM Hospital, Kalinga Nagar; Bhubaneswar.

Preparation of the Stock Solution

The stock solution of Rosiglitazone was prepared in methanol and the volume was made up of with acetonitrile to get a concentration of 100 µg/mL.

For calibration the standard solution containing 0,1,2,5,7,10 µg/mL and 0,1,2,5,7,10,12,15,17 µg/mL were prepared by diluting the stock solution to a constant volume with acetonitrile.

Preparation of plasma solutions

A suitable amount of stock solution was spiked with 0.5mL of blank plasma and then extracted by liquid-liquid extraction method. The concentrations of plasma standards at respective points on the concentration graphs for plasma were 0,1,2,5,7,10 µg/mL in rat plasma and 0,1,2,5,7,10,12,15,17 µg/mL in human plasma. The quality control samples were prepared at 2.25 µg/mL, 5.5 µg/mL and 7.5 µg/mL and 5.75 µg/mL, 10.75 µg/mL and 14.5 µg/mL concentrations in both rat and human plasma respectively. The QCs for plasma sample were prepared accordingly. These samples were used in the daily analysis of plasma and standard samples as QCs for the purpose of checking recovery of analyte.

Extraction from plasma

The blood volume was centrifuged at 4000rpm for 30 minutes and the supernatant was added with acetonitrile and again centrifuged at 4000rpm for 15 minutes. A suitable amount of standard solution was added to 0.5mL plasma along with acetonitrile and vortexed around for 10minutes. It was the filtered through Whatmann filter paper and the transferred to quartz cells for analysis.

A blank solution was prepared in the same way using 0.5mL of plasma but without the addition of Rosiglitazone.

Method Validation

This was carried out by establishing linearity, range, specificity, Limit of Detection, Limit of Quantification, recovery studies and precision studies according to the International Conference on Harmonization Guidelines [9].

RESULTS

Rosiglitazone was dissolved with methanol and the volume was made upto the mark with acetonotrile to get a concentration of 100mcg/ml.

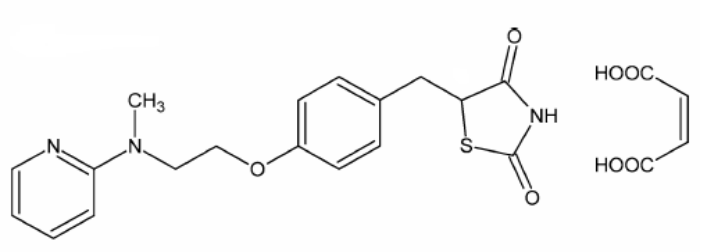
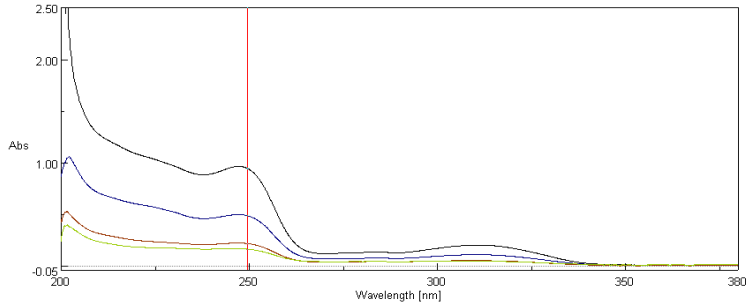
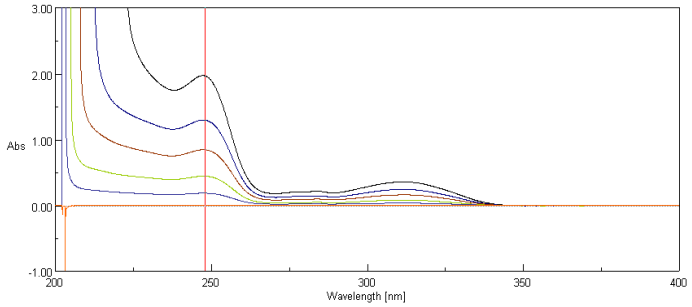


Fig.1:Structure of Rosiglitazone Maleate

Typical overlain spectra for Rosiglitazone in rat plasma and human plasma are shown in Fig:2



[a]



[b]

Fig.2: Overlain spectra for Rosiglitazone in(a) rat plasma(b)human plasma

Linearity: Calibration Curve for Rosiglitazone in both rat plasma and human plasma are represented in fig:

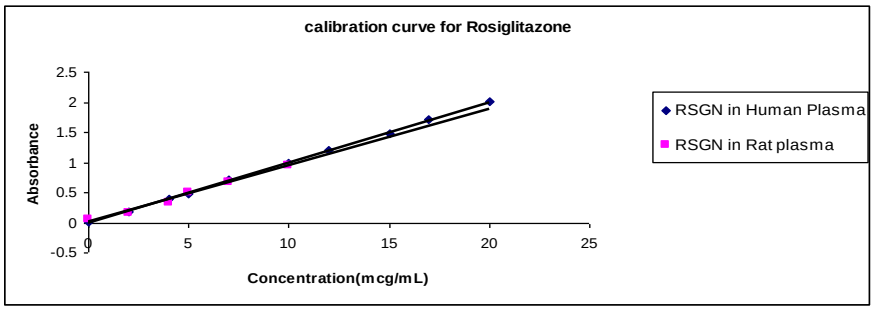


Fig.3: Calibration Curve for Rosiglitazone in rat and human plasma

Accuracy and Precision: The within day and between day studies were RSDs were calculated and are given in Table No: 1

Table.1: Precision of method in rat plasma and human plasma

Sample	Concentration added(mcg/mL)	Within day			Between Day		
		Mean	±S.D	%RSD	Mean	±S.D	%RSD
Rat Plasma	2.25	98.15	0.022	0.023	101.80	0.002	0.004
	5.5	98.73	0.016	0.018	100.75	0.02	0.24
	7.5	99.62	0.011	0.01	100.39	0.02	0.23
Human Plasma	5.75	99.58	0.016	0.278	101.17	0.009	1.63
	10.75	99.90	0.104	0.097	98.32	0.11	0.94
	14.5	99.86	0.0104	0.072	99.25	0.05	1.83

Table.2: Recovery studies of Rosiglitazone in rat plasma and human plasma

Sample	Quality Control Concentration(µg/mL)	Amount added(µg/mL)	Recovery±S.D
Rat Plasma	2.25	1.8	101.2036±0.002
		2.25	98.61063±0.002
		2.7	97.13998±0.007
	5.5	4.4	100.0602±0.002
		5.5	99.42687±0.002
		6.6	100.0602±0.002
	7.5	6	99.84907±0.003
		7.5	100.267±0.001
		9	100.0426±0.002
Human Plasma	5.75	4.6	99.86±0.04
		5.75	99.91±0.03
		6.9	99.93±0.03
	10.75	8.6	100.01±0.03
		10.75	100.08±0.02
		12.9	99.82±0.25
	14.5	11.6	99.97±0.04
		14.5	100.02±0.04
		17.4	99.99±0.04

Stability of plasma samples

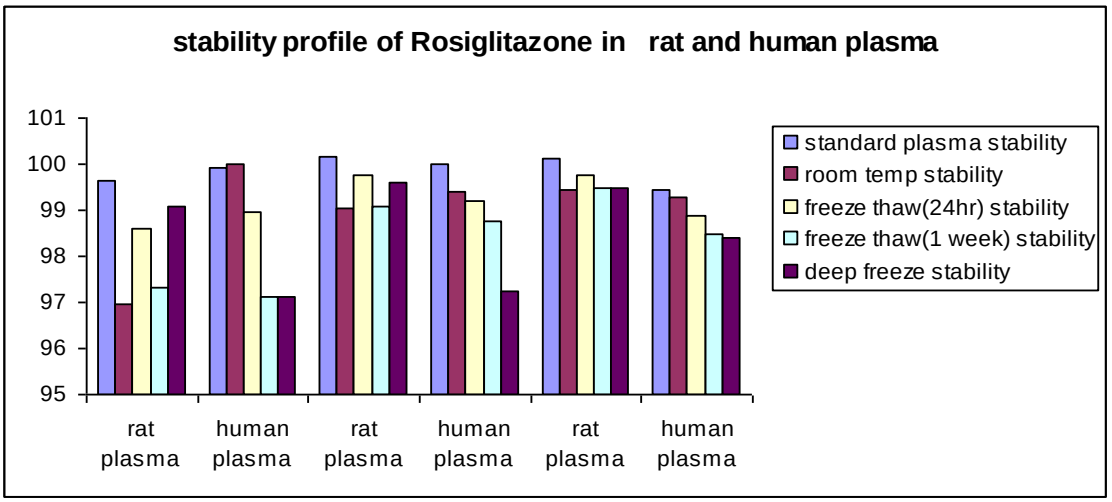


Fig.4: Graphical representation of stability of Rosiglitazone in rat and human plasma at different conditions

Table.3: One way ANOVA test for Rosiglitazone in rat and human plasma

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Rat Plasma	6	24.041798	4.006966333	0.00018635
Human Plasma	6	24.276	4.046	0.029144

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.004570881	1	0.004570881	0.31168269	0.588930076	4.964602701
Within Groups	0.146651755	10	0.014665176			
Total	**0.151222637**	**11**				

DISCUSSION

The standard solutions were scanned at the wavelength range of 200nm to 400nm and the wavelength maxima for Rosiglitazone was found at 247nm in both rat and human plasma respectively. This wavelength was used for all measurements.

Linearity was determined by plotting the absorbance at its wavelength maxima against the extracted sample concentration from rat and human plasma. Linear regression analysis of the data was found to be 0.9879 and 0.9996 in rat and human plasma respectively. The slope and intercept values were very small indicating a high precise method.

The Limit of Detection were analyzed from a series of solutions containing a decrease of amounts of Rosiglitazone in both rat and human extracted plasma and the Relative Standard Deviation computed was not more than 10% and Limit of Quantification did not exceed 20%.

Assay Precision and accuracy were determined by taking three QC samples (2.25,5.5 and 7.5 mcg/ml in rat plasma and 5.75,10.75 and 14.5 mcg/ml in human plasma)in six replicates for within day precision and between day precision an the %RSD was found to be less than 2% in all cases.

To determine the stability of Rosiglitazone in rat and human plasma, 4 sets of quality control concentrations of the spiked calibration standards were divided in to 16 tubes. One was taken as standard (100%).Two of the sets were stored and taken the readings after freeze thaw cycles for 24hours and 1week.The remaining set was stored at -20ºC for 1 week. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and the results are summarized in Fig no.: 4.The plasma samples were found to be stable in all the conditions, for only the percentage found was comparatively less in stability at -20ºC.

Statistical correlation

To correlate the difference between the two methods for Rosiglitazone in rat and human plasma, six different samples in the two cases were taken and quantified individually**.** To test difference between the proposed HPLC methods statistical tests were performed for the level of confidence 95% (*P = 0.05*).One way ANOVA was applied to test both method – sample interaction and differences in method precision. In both the cases ***F stat*** **is less than** ***F crit,*** signifying the method – sample interaction and the differences between the methods are not significant as shown in table: 3

CONCLUSION

The present study reveals that method development for Rosiglitazone in rat plasma and human plasma can be used for routine QC analysis. The method also indicates that there is no significant difference in analysis for the drug in rat plasma and human plasma and the stability of the drug in plasma indicates that it can be considered fair for pharmacokinetic studies.

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