Chapter IV

Preformulation Studies

Design of new formulations of a drug depends on its various physicochemical properties. Preformulation studies are carried out to ensure the development of a stable, therapeutically effective and safe dosage form. It is a stage of development during which the physicochemical properties of drug substance and its interaction with various formulation components are studied (Well, 1988). Preformulation studies are performed to characterize and optimize the drug for its purity and other important properties before its fabrication in the delivery system. Thus, in order to establish optimum conditions for developing suitable drug delivery system, preformulation studies such as drug identification for its purity, melting point, solubility, partition coefficient, UV, IR spectroscopy were carried out.

4.1 DRUG SAMPLE(S)

Drug samples of Pioglitazone HCl (PIO) and Rosiglitazone Maleate (ROS-M) were received as gift sample(s) by courtesy of Alembic Pharmaceutical Ltd, Vadodara (Gujarat) India and Sun Pharmaceutical Industries Ltd, Mumbai, (MH) India, respectively. The drug sample of rosiglitazone obtained as maleate salt was further processed to get free base of Rosiglitazone (ROS-B).

4.2 PREPARATION OF ROSIGLITAZONE BASE

Rosiglitazone maleate was converted into rosiglitazone base by treating ROS-M (10.6 mg) with 6.2 mL of 0.1 N NaOH in a volumetric flask. This solution was kept for 24 hrs at room temperature under nitrogen after which the so formed ROS-B was extracted 5 times with diethylether using a separating funnel. Further, the combined etheral extract of ROS-B was evaporated using a rotary

vacuum evaporator to leave solid ROS-B which was collected and dried at RT for 24 hrs in a vacuum desiccator. The dried ROS-B so obtained was subjected to identification and preformulation studies.

4.3 DRUG IDENTIFICATION

The drug samples (PIO, ROS-M and ROS-B) were subjected to identification tests such as melting point, UV & IR scanning and the results so obtained were compared with those of standards as given in monographs. Further, other preformulation studies were performed.

4.3.1 PHYSICAL APPEARANCE

Drug sample(s) were subjected to physical appearance, checked by visual observation, which were recorded in **Table 4.1, 4.2 and 4.3**.

4.3.2 MELTING POINT

The melting point of drug sample(s) was determined using Thiele's tube melting point apparatus by filling in one side sealed capillary tube. Observations are depicted in **Table 4.1, 4.2, and 4.3** and compared with the standards reported in The Merck Index, 2006.

4.3.3 SOLUBILITY STUDIES

The solubility of drug sample(s) in different solvents was determined by taking 10 mg of drug sample(s) in successively increasing amounts of water, methanol, ethanol, acetone, acetonitrile, dichloromethane and dimethyl formamide (for PIO)/Chloroform (for ROS-M and ROS-B) in a series of 10ml volumetric flasks. The flasks were kept for stirring on magnetic stirrers for 24 hrs duration after which the content of each flask was filtered through 0.45

μM membrane filter and the drug concentration was determined by spectrophotometric method described in **Section 4.4.1, 4.4.3 and 4.4.4**. The volume of solvent required to dissolve the drug was determined and the calculated solubility was recorded in descriptive terms in **Table 4.4**.

4.3.4 FTIR-SPECTROSCOPIC STUDIES

Infrared spectrum of any organic compound or drug gives information about the chemical groups present in that particular compound. Infrared spectroscopy was performed by making pellets of the drug sample(s) in KBr. About 1-2 mg of finely powdered drug was intimately mixed with approximately 10-20 mg of KBr (spectroscopic grade) by using a pestle and mortar. This mixture was compressed into pellets with special dies using KBr press in a moisture free environment. The scanning was performed between 4000-400 cm^{-1} using FTIR spectrophotometer (Alpha T FTIR, Bruker, Germany), and the obtained IR spectrum was matched with the IR spectrum as reported, in official monograph (I.P., 2010). The characteristics bands at particular frequency which denotes characteristic groups were recorded in **Table 4.5, 4.6** and **4.7** for PIO, ROS-M and ROS-B, respectively. The standard IR spectra of PIO, ROS-M and ROS-B are shown in **Fig. 4.1, 4.3** and **4.5,** respectively. The IR spectral assignment of sample PIO, ROS-M and ROS-B are shown in **Fig. 4.2, 4.4** and **4.6,** respectively.

4.3.5 UV-SPECTROSCOPIC STUDIES

Organic molecules in solution form when exposed to light in the visible/ultra-violet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption (Silverstein et al., 2000). Solvent selection for the development of analytical method was based on the solubility of the drug molecule in that solvent and also

upon the solubility of the developed drug delivery system in the same solvent or a mixture of solvents in a specific ratio known as solvent system. Hence, ultraviolet absorption maxima (λ_{max}) of the drug sample(s) were determined in single solvent as well as in solvent systems depending upon the estimation of the drug sample(s) in drug delivery system or for drug release study.

4.3.5.1 Ultraviolet Absorption Maxima (λ_{max}) of Pioglitazone HCl

The solution of PIO in the concentration of 50 µg/ml was prepared in solvent system consisting of methanol : acetonitrile in ratio of 1:1 and scanned against solvent blank in the range of 200-350 nm using a UV Spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan). PIO exhibited a UV-light absorption maximum (λ_{max}) at 268.0 nm as shown in **Fig. 4.7.** Similarly, solution of PIO in the concentration of 50 µg/ml was prepared in solvent system of methanol : acetonitrile : PBS (pH 7.4) : 0.1N sodium hydroxide solution in ratio of 1:1:1:7 and scanned against solvent blank in the range of 200-300 nm using a UV Spectrophotometer. PIO exhibited a UV-light absorption maximum (λ_{max}) at 266.0 nm as shown in **Fig. 4.8.**

4.3.5.2 Ultraviolet Absorption Maxima (λ_{max}) of Rosiglitazone maleate and Rosiglitazone base

The solution of ROS-M in the concentration of 20µg/ml was prepared in methanol and scanned against solvent blank in the range of 220-350 nm using a UV Spectrophotometer. ROS-M exhibited a UV-light absorption maximum (λ_{max}) at 246.8 nm as shown in **Fig. 4.9.** Similarly, solution of ROS-B in the concentration of 24 µg/ml was prepared in solvent system of acetonitrile : PBS (pH 7.4) in ratio of 2:3 and scanned against solvent blank in the range of 220-350 nm using a UV

Spectrophotometer. ROS-B exhibited a UV-light absorption maximum (λ_{max}) at 246.2 nm **(Fig. 4.10).**

4.3.6 PARTITION COEFFICIENT

The partition behavior of PIO, ROS-M and ROS-B was examined in n-Octanol : Water and n-Octanol : PBS (pH 7.4) systems. It was determined by taking accurately weighed amount of drug (10 mg) in two separating funnels containing 10 mL each of two immiscible phases, n-Octanol and aqueous phase (distilled water/PBS-pH 7.4). The funnels were kept for drug partitioning in the two phases for 24 hrs with intermittent shaking following which the two phases were separated and the amount of drug in aqueous phase was determined by UV spectrophotometer method after suitable dilutions. The amount of drug in two phases was used to calculate log of partition coefficient of the drug in two selected phases **(Table 4.1, 4.2 and 4.3)** by using the formula -

$$\textbf{Log } P_{o/w} \textbf{ (Log Partition coefficient) = Log } [C_{octanol} / C_{water/PBS}]$$

Where, $C_{octanol}$ = Amount of drug in organic phase and

$C_{water/PBS}$ = Amount of drug in aqueous phase

4.4 ANALYTICAL METHODS

4.4.1 Calibration curve of Pioglitazone HCl in Methanol : Acetonitrile (1:1)

Accurately weighed quantity of drug sample (10 mg) was dissolved in sufficient minimum volume of methanol in a 100 ml volumetric flask and volume was made upto the mark with solvent system consisting of methanol : acetonitrile (1:1), which gave stock solution of concentration 100 µg/mL. The stock solution was pipetted in aliquots of 1.0ml, 1.2ml, 1.4ml... 2.8ml into a

series of 10ml volumetric flasks and the volume was made up to 10 ml with methanol : acetonitrile (1:1) to give final concentrations of 10, 12, 14,...28 µg/ml. The solutions were shaken well and their absorbance was measured in UV range at λ_{max} 268.0 nm using UV Spectrophotometer against solvent blank. The above procedure was repeated three times and the mean absorbance values along with the regressed values (method of least squares) were recorded in **Table 4.8.** The data was processed (Microsoft Excel) and various statistical parameters were determined. The plot of absorbance against drug concentration is shown in **Fig. 4.11.**

4.4.2 Calibration curve of Pioglitazone HCl in Methanol : Acetonitrile : PBS (pH 7.4) : 0.1N Sodium Hydroxide Solution (1:1:1:7)

Accurately weighed quantity of drug sample (10 mg) was dissolved in 10 ml of methanol in a 100 ml volumetric flask and volume was made upto the mark with solvent system comprising of methanol : acetonitrile : PBS (pH 7.4) : 0.1 N Sodium Hydroxide Solution (in ratio of 1:1:1:7), which gave stock solution of concentration 100 µg/ml. The stock solution was pipetted in aliquots of 0.5ml, 1.0ml, 1.5ml,...5.0ml into a series of 10ml volumetric flasks and the volume was made up to 10 ml with methanol : acetonitrile : PBS (pH 7.4) : 0.1 N sodium hydroxide solution (1:1:1:7) to give final concentrations of 5, 10, 15,...50µg/ml. The solutions were shaken well and their absorbance was measured in UV range at λ_{max} 266.0 nm using UV Spectrophotometer against solvent blank. The above procedure was repeated three times and the mean absorbance values along with the regressed values (method of least squares) were recorded in **Table 4.11.** The data was processed (Microsoft Excel) and various statistical parameters were determined. The plot of absorbance against drug concentration is shown in **Fig. 4.12.**

Excel) and various statistical parameters were determined. The plot of absorbance against drug concentration is shown in **Fig. 4.14.**

4.4.5 Accuracy

Accuracy of an analytical method is the closeness of test result obtained by the method to the true value. For determination of PIO, ROS-M, ROS-B concentration, standard solutions were prepared. The absorbance of above solutions was taken at their respective λ_{max} which was recorded and compared for its accuracy. The method is said to be accurate for estimation if it gives < 1.0 % RSD at each concentration level.

$$RSD \approx \frac{SD}{Mean} X\,100$$

4.4.6 Precision

The precision is agreement between the results obtained when determinations are conducted. Precision provides an indication of random errors and is generally subdivided into two cases: repeatability and reproducibility, which were determined by calculating RSD of inter-day and intra-day determinations of the drug sample(s). RSD should be < 2.0 % for the method in both inter-day and intra-day determinations.

4.4.7 Linearity

The linearity of test procedure is defined as its ability (within a given range) to produce results which are directly proportional to the concentration of analyte in the sample. Linearity of a light absorption determinations should be examined to ensure that Beer's Law operates over the range of interest. For evaluation of linearity of the UV method of the given drug sample(s), the standard solutions were prepared at 10, 12, 14,...28 µg/ml, for PIO, and 2, 4,

6,...20 µg/ml for ROS-M and ROS-B concentrations (n = 3) and absorbance were taken at 268.0, 246.8 and 246.2 nm respectively, using UV-Spectrophotometer. The method is said to be accurate for estimation of drug sample(s) if it is linear over 10 to 28 µg/ml range for PIO and 2 to 20 µg/ml for ROS-M and ROS-B. Least square regression method was used to determine the regression coefficient **r**, and the equation of line $y = ax + b$ for the best fitting line.

4.4.8 Stability and selectivity

Stability of the solutions of PIO, ROS-M, and ROS-B, used for preparing the calibration curves was ascertained by observing the changes in the absorbance at 268.0, 246.8 and 246.2 nm respectively, over a period of 24 hours, at RT.

The selectivity of the method for the estimation of drug sample(s) was ascertained by carrying out the estimation procedure in the presence of various ingredients used in the preparation of nanoparticles and solution, at the concentrations in which they are present in the formulations.

Table 4.1: Tests for identification of Pioglitazone HCl

S.No.	Parameter		
1.	Physical appearance	**Reported**	**Observed**
		Odorless, white crystalline powder	Odorless, white crystalline powder
2.	Melting point	183° - 184° C	182.2° - 184.8°C
3.	UV -scanning (λ_{max})	Methanol : Acetonitrile	268.0 nm
		Methanol: Acetonitrile : PBS (pH 7.4)	266.0 nm
4.	Partition coefficient (Log $P_{o/w}$)	n-octanol/water	2.94
		n-octanol/PBS (pH 7.4)	1.89

Table 4.2: Tests for identification of Rosiglitazone maleate

S.No.	Parameter		
1.	Physical appearance	Reported	Observed
		A white to off-white, odorless crystalline powder	A white to off-white, odorless crystalline powder
2.	Melting point	122° - 123° C	124.7° - 126.6°C
3.	UV-Spectroscopic scanning (λ_{max}) (nm)	Methanol	246.8
4.	Partition coefficient (Log $P_{o/w}$)	n-octanol/water	1.93
		n-octanol/PBS (pH 7.4)	1.47

Table 4.3: Tests for identification of Rosiglitazone base

S.No.	Parameter		
1.	Physical appearance	Reported	Observed
		Odorless, off-white crystalline powder	Odorless, off-white crystalline powder
2.	Melting point	153° - 155° C	152.5° - 155.0°C
3.	UV - Spectroscopic scanning (λ_{max}) (nm)	Acetonitrile : PBS (pH 7.4)	246.2
4.	Partition coefficient (Log $P_{o/w}$)	n-octanol/water	3.19
		n-octanol/PBS (pH 7.4)	3.93

Table 4.4: Solubility profile of Pioglitazone HCl, Rosiglitazone maleate and Rosiglitazone base in various solvents

S. No.	Solvents	Solubility level		
		Pioglitazone HCl	Rosiglitazone maleate	Rosiglitazone base
1.	Water	Practically insoluble	Soluble	Practically insoluble
2.	Methanol	Soluble	Soluble	Soluble
3.	Ethanol (95%)	Slightly soluble	Soluble	Soluble
4.	Acetone	Very slightly soluble	Very slightly soluble	Soluble
5.	Acetonitrile	Very slightly soluble	Soluble	Soluble
6.	Dimethylformamide	Soluble	-	-
7.	Chloroform	-	Insoluble	Soluble
8.	Dichloromethane	Slightly soluble	Insoluble	Soluble

#Descriptive terms for solubility

Descriptive terms	Parts of solvent required for 1 part of solute	Solubility range (mg/ml)	Solubility assigned (mg/ml)
Very soluble	Less than 1	1000	1000
Freely soluble	From 1 to 10	100 - 1000	100
Soluble	From 10 to 30	33 - 100	33
Sparingly soluble	From 30 to 100	10 - 33	10
Slightly soluble	From 100 to 1000	1 - 10	1
Very slightly soluble	From 1000 to 10,000	0.1 – 1	0.1
Practically insoluble, or insoluble	More than 10,000	< 0.1	0.01

Table 4.5: Infrared spectral assignment of Pioglitazone HCl

Wave number(cm^{-1})	Functional group
3086	Aromatic C-H stretching
2927, 2740	Aliphatic C-H stretching
1742	C=O stretching
1239	-C-O- stretching
1150	-C-S stretching
1690, 1462	-C=N stretching
1317	-C-N- stretching

Table 4.6: Infrared spectral assignment of Rosiglitazone maleate

Wave number (cm^{-1})	Functional group
3396	-OH stretching of COOH
3130	Aromatic C-H stretching
2930, 2781	Aliphatic CH stretching
1752	C=O stretching
1380, 1570	Characteristic C=C aromatic peaks
1247	-C-O stretching
1154	-C-S stretching

Table 4.7: Infrared spectral assignment of Rosiglitazone base

Wave number(cm^{-1})	Functional group
3315	N-H stretching
3080	Aromatic C-H stretching
2928	Aliphatic C-H stretching
1131	-C-O- stretching
1694, 1472	-C=N stretching
1311	-C-N- stretching
1165	-C-O-C- stretching

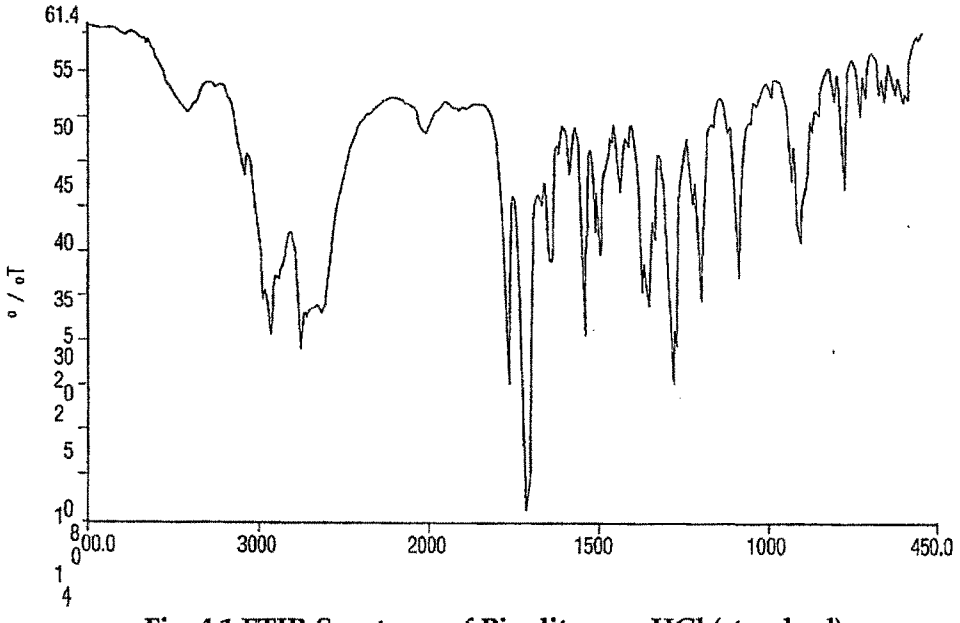

Fig. 4.1 FTIR Spectrum of Pioglitazone HCl (standard)

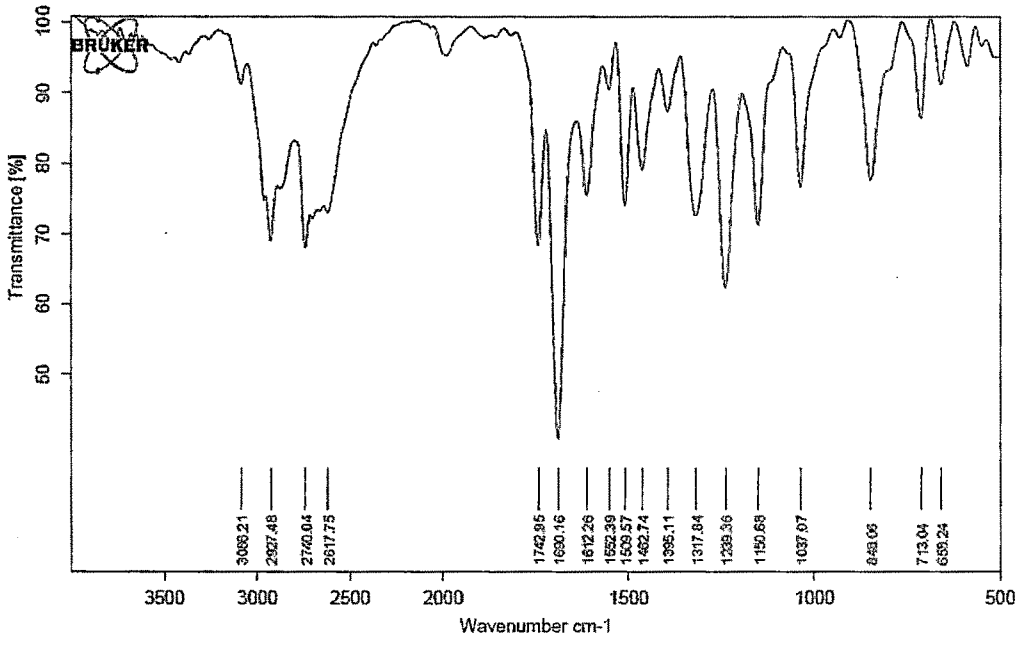

Fig. 4.2 FTIR Spectrum of Pioglitazone HCl (sample)

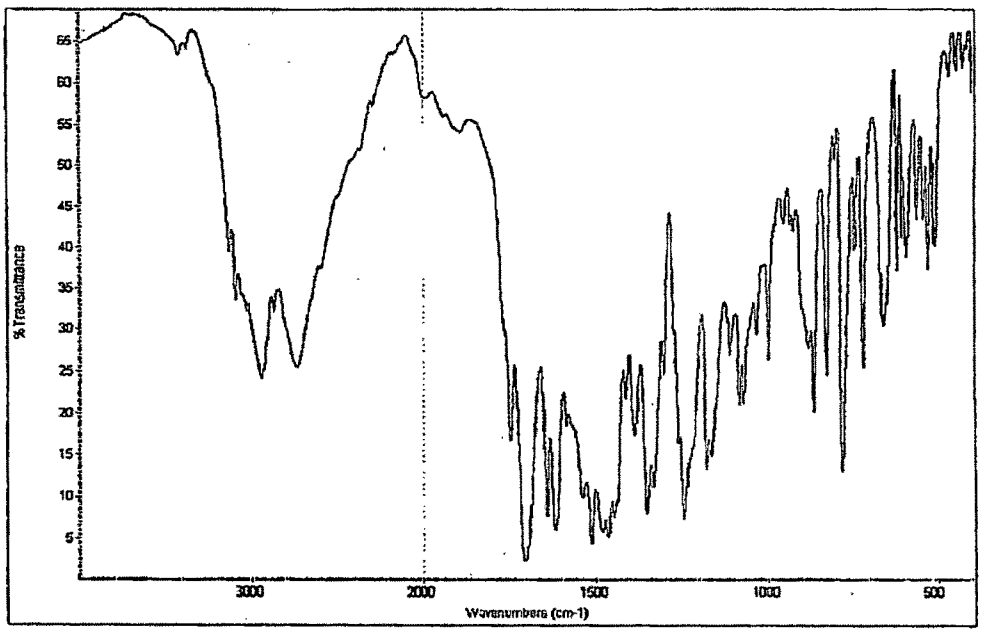

Fig. 4.3 FTIR Spectrum of Rosiglitazone maleate (standard)

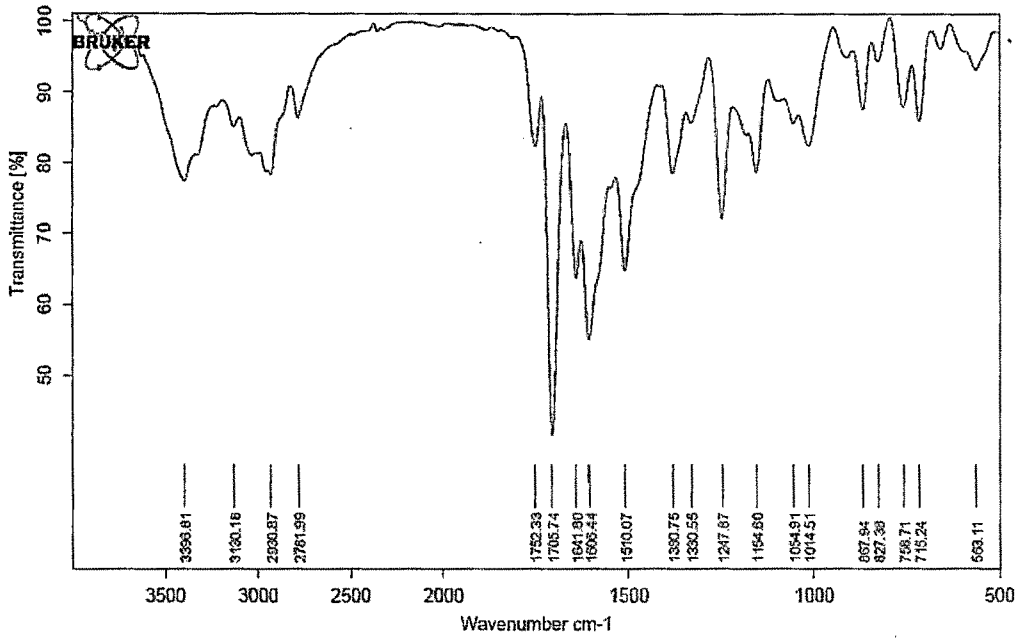

Fig. 4.4 FTIR Spectrum of Rosiglitazone maleate (sample)

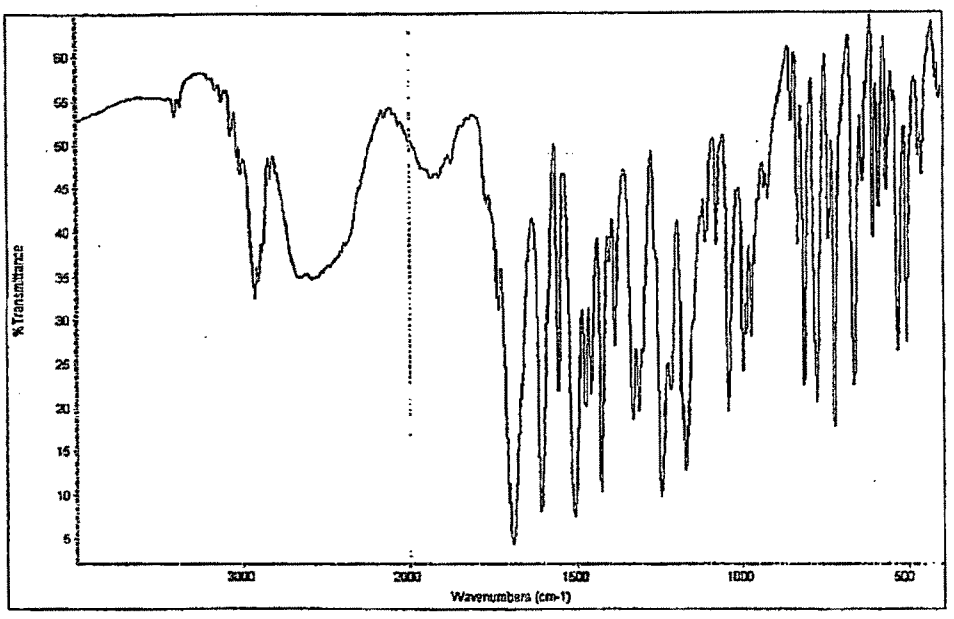

Fig. 4.5 FTIR Spectrum of Rosiglitazone base (standard)

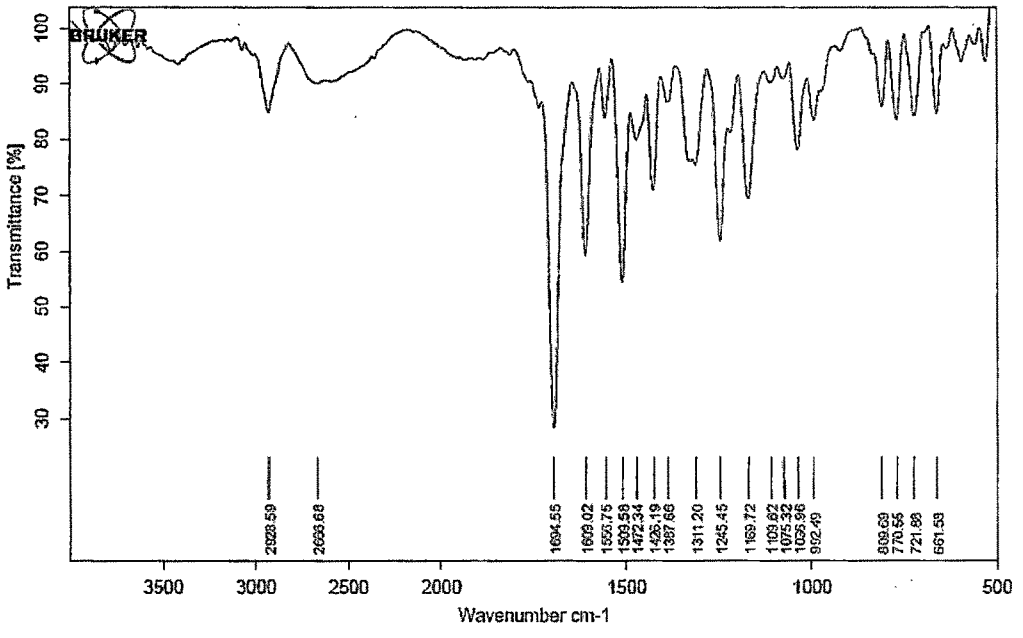

Fig. 4.6 FTIR Spectrum of Rosiglitazone base (sample)

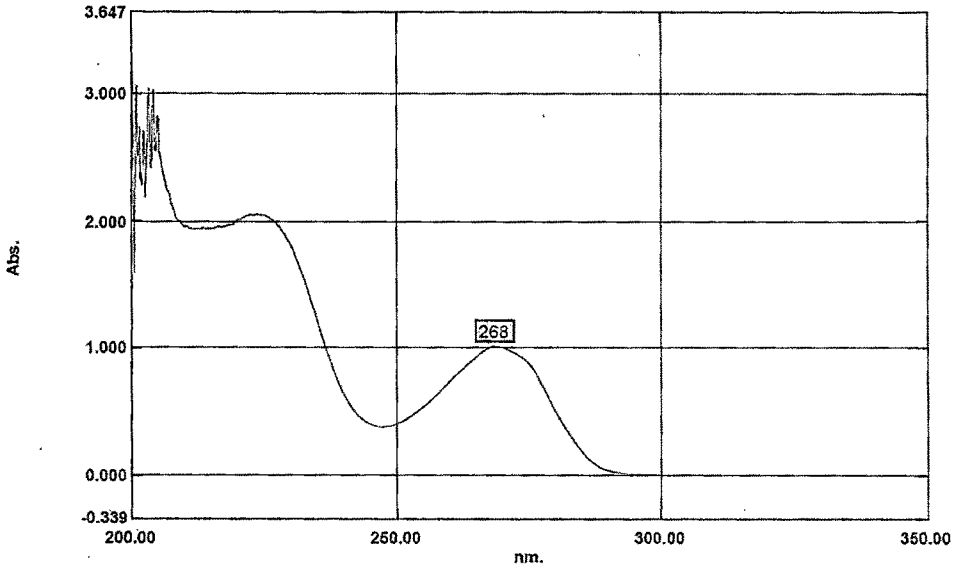

Fig. 4.7 Ultraviolet absorption spectrum of Pioglitazone HCl in Methanol : Acetonitrile

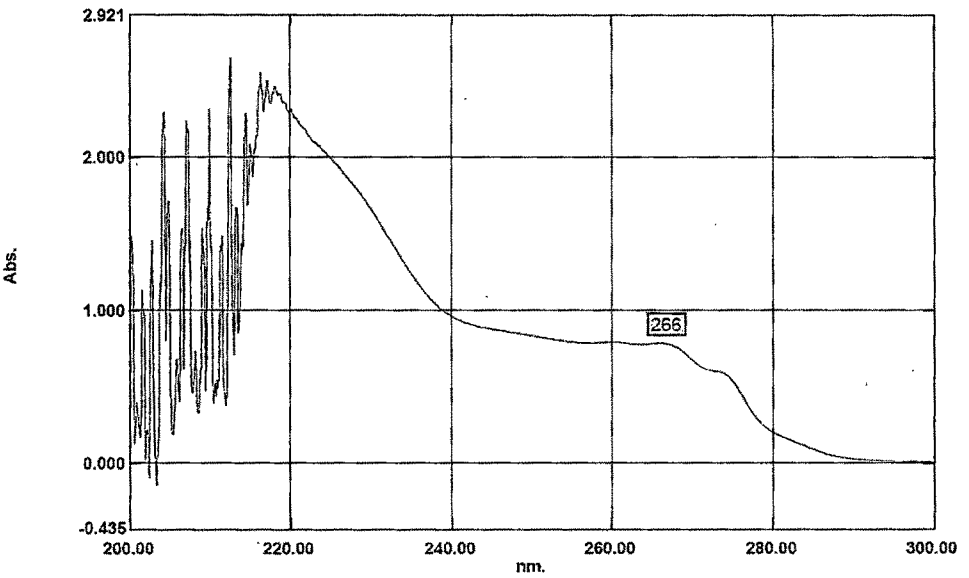

Fig. 4.8 Ultraviolet absorption spectrum of Pioglitazone HCl in Methanol : Acetonitrile : PBS (pH 7.4) : 0.1 N NaOH Solution

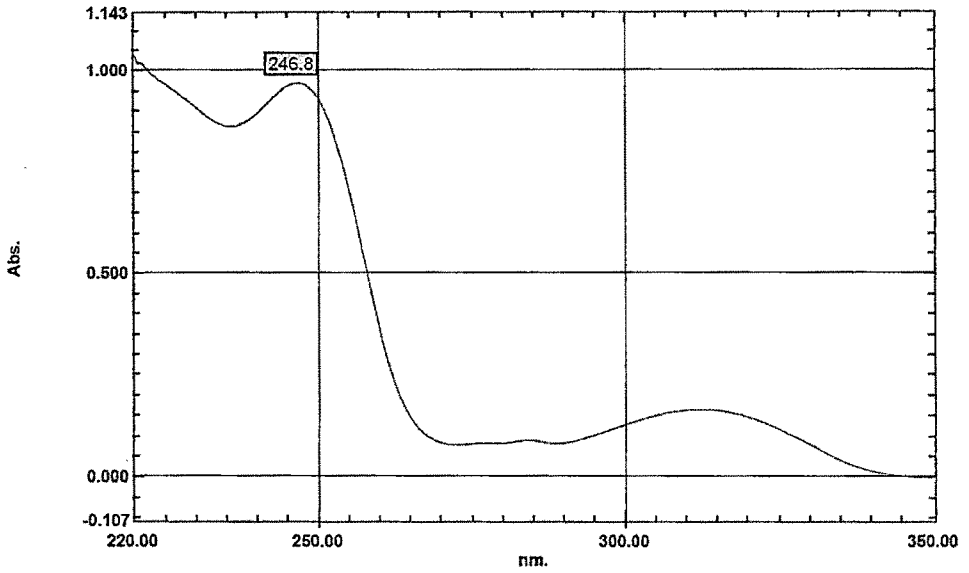

Fig. 4.9 Ultraviolet absorption spectrum of Rosiglitazone maleate in Methanol

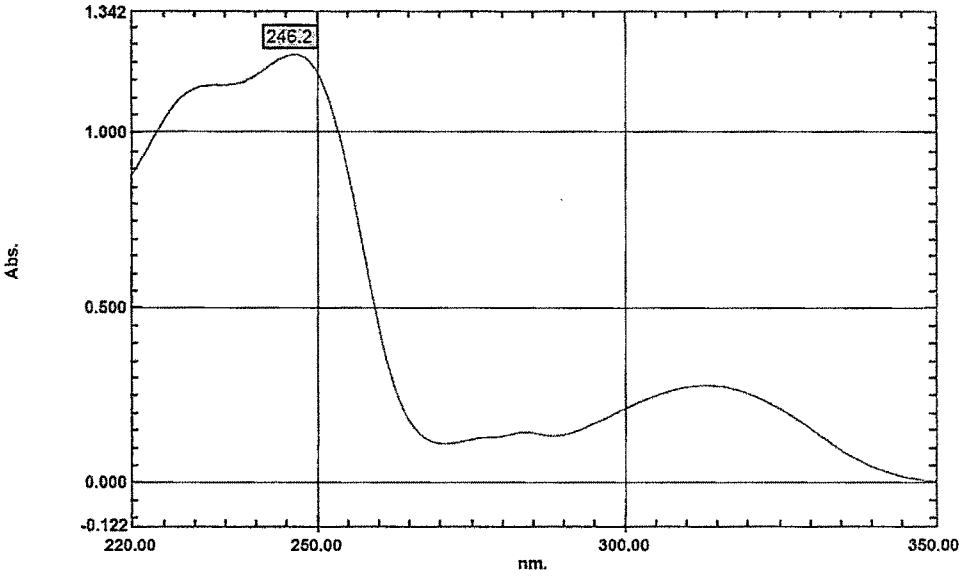

Fig. 4.10 Ultraviolet absorption spectrum of Rosiglitazone base in Acetonitrile : PBS (pH 7.4)

Table 4.8: Mean absorbance values, regressed values and statistical data of the calibration curve for the estimation of Pioglitazone HCl in MeOH-ACN at λ_{max} = 268.0 nm.

S. No.	Concentration (µg / ml)	Mean Absorbance#	Regressed Absorbance	RSD	Regression Analysis
1.	0	0	0	0	Equation of line: y = 0.021x Correlation coefficient : R^2 = 0.99
2.	10	0.223 ± 0.010	0.210	0.887	
3.	12	0.248 ± 0.012	0.252	0.833	
4.	14	0.329 ± 0.014	0.294	0.957	
5.	16	0.350 ± 0.015	0.336	0.816	
6.	18	0.383 ± 0.011	0.378	0.820	
7.	20	0.425 ± 0.016	0.420	0.819	
8.	22	0.467 ± 0.014	0.462	0.821	
9.	24	0.492 ± 0.015	0.504	0.901	
10.	26	0.516 ± 0.019	0.546	0.943	
11.	28	0.590 ± 0.011	0.588	0.856	

#: Mean of 3 values

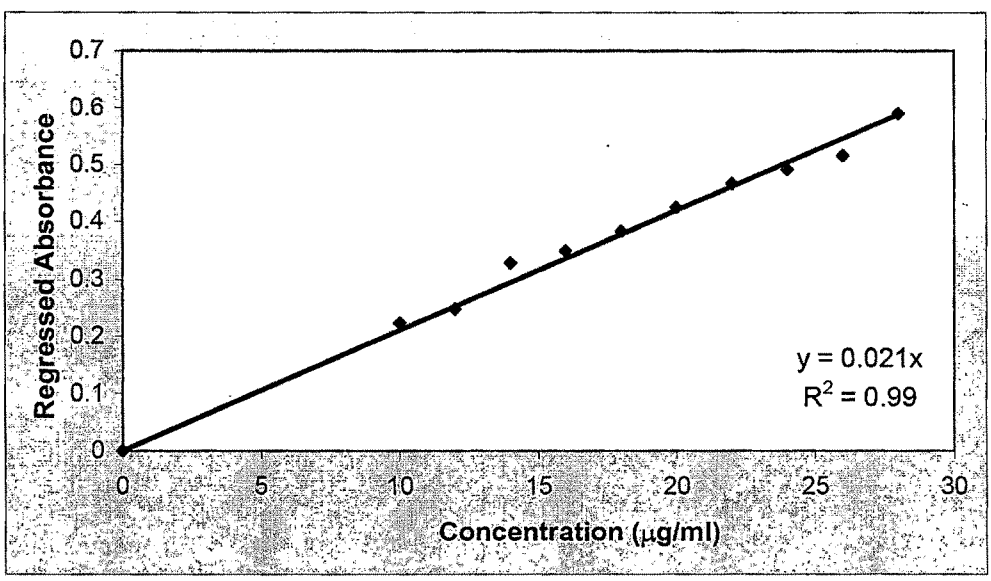

Fig. 4.11 Linearly regressed calibration curve of Pioglitazone HCl in MeOH-ACN at λ_{max} = 268.0 nm

Table 9: Intra-day accuracy and precision of calibration curve of Pioglitazone HCl in MeOH-ACN at λ_{max} = 268.0 nm

Intra-day accuracy and precision			
Theoretical concentration (µg)	20.0	20.0	20.0
Observed concentration (mean) µg	20.012	20.019	19.957
S.D.	0.236	0.278	0.215
R.S.D.	1.18	1.39	1.08
N	3	3	3
Accuracy (%)	100.06	100.095	99.785

Table 10: Inter-day accuracy and precision of calibration curve of Pioglitazone HCl in MeOH-ACN at λ_{max} = 268.0 nm

Inter-day accuracy and precision			
Theoretical concentration (µg)	20.0 (Day I)	20.0 (Day II)	20.0 (Day III)
Observed concentration (mean) µg	20.012	19.817	20.138
S.D.	0.236	0.253	0.292
R.S.D.	1.18	1.28	1.45
N	3	3	3
Accuracy (%)	100.06	99.08	100.69

Table 4.11: Mean absorbance values, regressed values and statistical data of the calibration curve for the estimation of Pioglitazone HCl in MeOH - ACN - PBS(pH 7.4) - 0.1 N NaOH at λ_{max} = 266.0 nm

S. No.	Concentration (µg / ml)	Mean Absorbance#	Regressed Absorbance	RSD	Regression Analysis
1.	0	0	0	0	
2.	5	0.076 ± 0.011	0.075	0.812	Equation of line: y = 0.015x
3.	10	0.154 ± 0.014	0.150	0.805	
4.	15	0.228 ± 0.015	0.225	0.811	
5.	20	0.300 ± 0.012	0.300	0.805	
6.	25	0.375 ± 0.014	0.375	0.808	
7.	30	0.451 ± 0.016	0.450	0.813	Correlation coefficient : R^2= 0.9999
8.	35	0.527 ± 0.013	0.525	0.807	
9.	40	0.597 ± 0.018	0.600	0.801	
10.	45	0.677 ± 0.015	0.675	0.810	
11.	50	0.748 ± 0.013	0.750	0.816	

#: Mean of 3 values

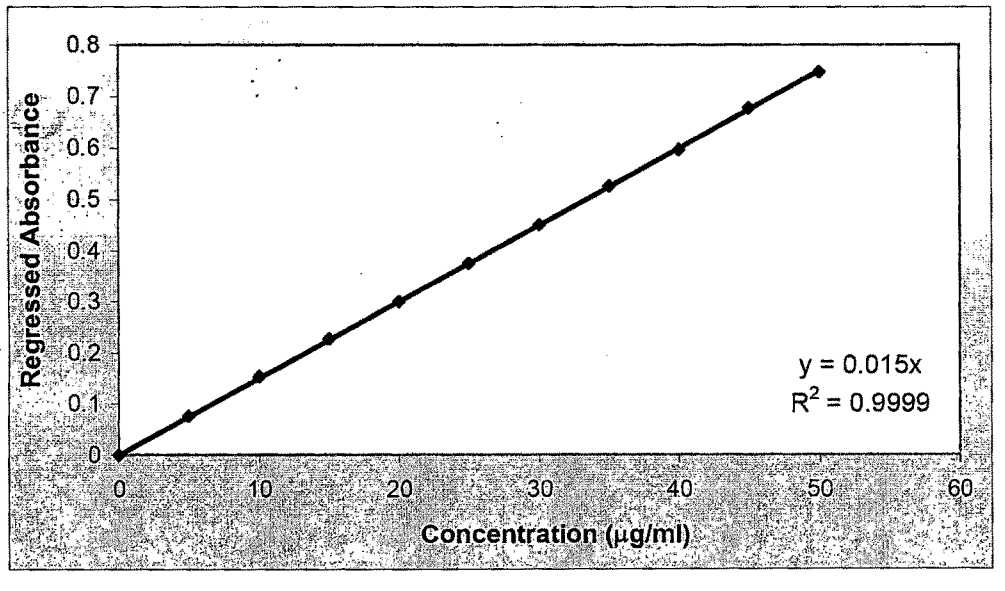

Fig. 4.12 Linearly regressed calibration curve of Pioglitazone HCl in MeOH - ACN - PBS(pH 7.4) - 0.1 N NaOH at λ_{max} = 266.0 nm

Table 4.12: Intra-day accuracy and precision of calibration curve of Pioglitazone HCl in MeOH - ACN - PBS(pH 7.4) - 0.1 N NaOH at λ_{max} = 266.0 nm

Intra-day accuracy and precision			
Theoretical concentration (µg)	25.0	25.0	25.0
Observed concentration (mean) µg	24.938	25.013	24.847
S.D.	0.317	0.465	0.296
R.S.D.	1.27	1.86	1.19
N	3	3	3
Accuracy (%)	99.72	100.05	99.39

Table 4.13: Inter-day accuracy and precision calibration curve of Pioglitazone HCl in MeOH - ACN - PBS(pH 7.4) - 0.1 N NaOH at λ_{max} = 266.0 nm

Inter-day accuracy and precision			
Theoretical Concentration (µg)	25.0 (Day I)	25.0 (Day II)	25.0 (Day III)
Observed concentration (mean) (µg)	24.938	25.179	25.128
S.D.	0.317	0.466	0.269
R.S.D.	1.27	1.85	1.07
N	3	3	3
Accuracy (%)	99.72	100.71	100.51

Table 4.14: Mean absorbance values, regressed values and statistical data of the calibration curve for the estimation of Rosiglitazone maleate in MeOH at λ_{max} = 246.8 nm

S. No.	Concentration (µg / ml)	Mean Absorbance#	Regressed Absorbance	RSD	Regression Analysis
1.	0	0	0	0	
2.	2	0.107 ± 0.014	0.098	0.814	Equation of line: y = 0.0489x
3.	4	0.205 ± 0.017	0.196	0.813	
4.	6	0.306 ± 0.013	0.293	0.816	
5.	8	0.401 ± 0.012	0.391	0.810	
6.	10	0.510 ± 0.019	0.489	0.865	
7.	12	0.593 ± 0.011	0.587	0.833	Correlation coefficient : R^2 = 0.998
8.	14	0.691 ± 0.010	0.685	0.809	
9.	16	0.783 ± 0.012	0.782	0.805	
10.	18	0.880 ± 0.015	0.880	0.808	
11.	20	0.946 ± 0.018	0.978	0.879	

: Mean of 3 values

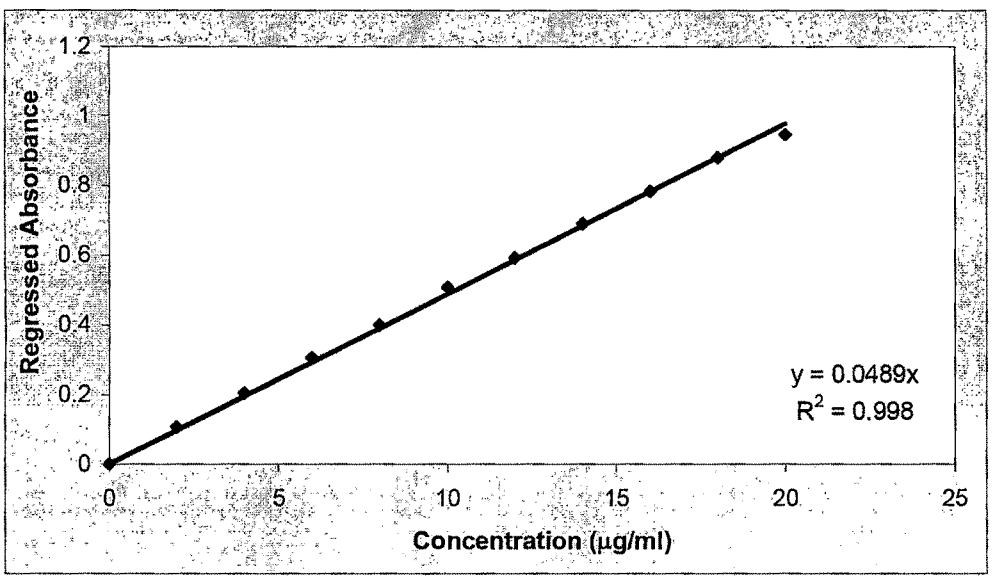

Fig. 4.13 Linearly regressed calibration curve of Rosiglitazone maleate in MeOH at λ_{max} = 246.8 nm

Table 4.15: Intra-day accuracy and precision of calibration curve of Rosiglitazone maleate in MeOH at λ_{max} = 246.8 nm

Intra-day accuracy and precision			
Theoretical concentration (µg)	10.0	10.0	10.0
Observed concentration (mean) (µg)	10.045	10.016	9.971
S.D.	0. 139	0.157	0.126
R.S.D.	1.38	1.57	1.26
n	3	3	3
Accuracy (%)	100.45	100.16	99.71

Table 4.16: Inter-day accuracy and precision calibration curve of Rosiglitazone maleate in MeOH at λ_{max} = 246.8 nm

Inter-day accuracy and precision			
Theoretical concentration (µg)	10.0 (Day I)	10.0 (Day II)	10.0 (Day III)
Observed concentration (mean) µg	10.045	9.908	9.929
S.D.	0. 139	0.129	0.092
R.S.D.	1.38	1.31	0.93
n	3	3	3
Accuracy (%)	100.45	99.08	99.29

Table 4.17: Mean absorbance values, regressed values and statistical data of the calibration curve for the estimation of Rosiglitazone base in ACN - PBS (pH 7.4) at λ_{max} = 246.2 nm

S. No.	Concentration (µg / ml)	Mean Absorbance#	Regressed Absorbance	RSD	Regression Analysis
1.	0	0		0	Equation of line: y = 0.0455x Correlation coefficient : R^2 = 0.9991
2.	2	0.080 ± 0.012	0.091	0.813	
3.	4	0.171 ± 0.014	0.182	0.815	
4.	6	0.274 ± 0.013	0.273	0.816	
5.	8	0.377 ± 0.018	0.364	0.844	
6.	10	0.453 ± 0.012	0.455	0.809	
7.	12	0.552 ± 0.010	0.546	0.813	
8.	14	0.632 ± 0.013	0.637	0.809	
9.	16	0.744 ± 0.017	0.728	0.845	
10.	18	0.817 ± 0.015	0.819	0.823	
11.	20	0.901 ± 0.014	0.910	0.818	

#: Mean of 3 values

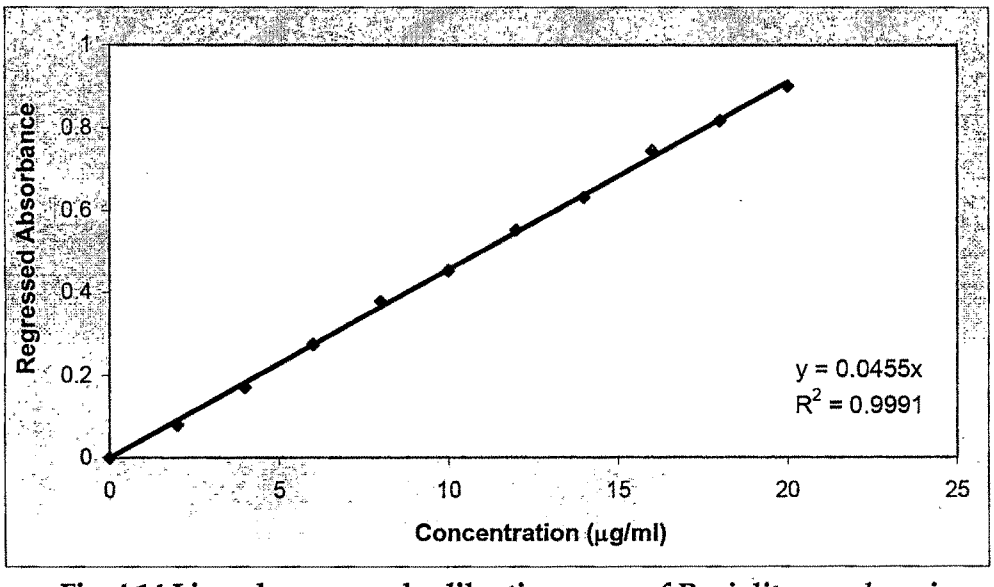

Fig. 4.14 Linearly regressed calibration curve of Rosiglitazone base in ACN-PBS(pH 7.4) at λ_{max} = 246.2 nm

Table 4.18: Intra-day accuracy and precision of calibration curve of Rosiglitazone base in ACN-PBS(pH 7.4) at λ_{max} = 246.2 nm

Intra-day accuracy and precision			
Theoretical concentration (µg)	10.0	10.0	10.0
Observed concentration (mean) (µg)	10.052	10.061	9.937
S.D.	0.107	0.124	0.117
R.S.D.	1.07	1.23	1.18
n	3	3	3
Accuracy (%)	100.52	100.61	99.37

Table 4.19: Inter-day accuracy and precision of calibration curve of Rosiglitazone base in ACN-PBS(pH 7.4) at λ_{max} = 246.2 nm

Inter-day accuracy and precision			
Theoretical concentration (µg)	10.0 (Day I)	10.0 (Day II)	10.0 (Day III)
Observed concentration (mean) (µg)	10.052	10.085	9.952
S.D.	0.107	0.140	0.141
R.S.D.	1.07	1.39	1.42
n	3	3	3
Accuracy (%)	100.52	100.085	99.52

4.5 RESULTS AND DISCUSSION

The preformulation studies were performed in order to ensure the authenticity of drug sample and development of formulation. The drugs pioglitazone HCl and rosiglitazone maleate were obtained as gift samples from Alembic Pharmaceuticals Ltd, Vadodara (Gujarat) India and Sun Pharmaceutical Industries Ltd, Mumbai, (MH) India, respectively. The drug sample of rosiglitazone obtained as maleate salt was further processed to get free base of rosiglitazone. Thus, drug samples viz. pioglitazone HCl, rosiglitazone maleate and rosiglitazone base were used in the formulation of drug delivery systems and hence were subjected to preformulation studies for identity and purity. Various standardization parameters studied included: IR spectra, λ_{max}, solubility profile and partition coefficient.

The physical appearance, color and crystalline nature of the drug sample(s) matched with those mentioned in official monographs. The melting point of each drug sample was sharp and very close to that reported in The Merck Index, (2001) (**Table 4.1, 4.2, 4.3**) proving that the drug samples were pure.

Solubility profile of drug sample(s) was studied in water and various organic solvents. PIO was found to be soluble in methanol and dimethylformamide, slightly soluble in ethanol (95%) and dichloromethane, very slightly soluble in acetone and acetonitrile, and insoluble in water. ROS-M was found to be soluble in water, methanol, ethanol (95%) and acetonitrile, very slightly soluble in acetone, and insoluble in chloroform and dichloromethane. ROS-B was found to be soluble in methanol, ethanol (95%), acetone, acetonitrile, chloroform and dichloromethane, and insoluble in water. Hence, the solubility of each drug sample in various solvents was found to be very much similar to the reported values (**Table 4.4**).

The drug samples were further identified by IR and UV spectroscopy. The IR spectrum of PIO showed characteristic peaks at 3086 (Aromatic C-H stretching); 2927, 2740 (Aliphatic C-H stretching); 1742 (C=O stretching); 1239 (-C-O- stretching); 1150 (-C-S stretching); 1690, 1462 (-C=N stretching); 1317 (-C-N- stretching) **(Table 4.5, Fig. 4.2)** and was found to be identical to that reported in I.P., 2010 **(Fig. 4.1)**. The IR spectrum of ROS-M showed characteristic peaks at 3396 (-OH stretching of COOH); 3130 (Aromatic C-H stretching); 2930, 2781 (Aliphatic CH stretching); 1752 (C=O stretching); 1380, 1570 (Characteristic C=C aromatic peaks); 1247 (-C-O stretching); 1154 (-C-S stretching) **(Table 4.6, Fig. 4.4)** and was found to be identical to that reported in I.P., 2010 **(Fig. 4.3)**.

The IR spectrum of ROS-B showed characteristic peaks at 3315 (N-H stretching); 3080 (Aromatic C-H stretching); 2928 (Aliphatic C-H stretching); 1131 (-C-O- stretching); 1694, 1472 (-C=N stretching); 1311 (-C-N- stretching); 1165 (-C-O-C- stretching) **(Table 4.7, Fig. 4.3)**. In IR spectra of ROS-B, the disappearance of OH stretching peak for -COOH moiety at 3396.1 cm^{-1} confirms the formation of rosiglitazone base from rosiglitazone maleate.

When the solution of a compound is scanned in ultraviolet-visible range, it exhibits maximum absorbance at a specific wavelength which is called as λ_{max}. It serves as an identification parameter for a compound. The UV-scan of 50 µg/ml solution of PIO in solvent system of methanol : acetonitrile in ratio of 1:1, exhibited λ_{max} at 268.0 nm **(Fig. 4.7)** and absorbance was 1.017. The UV-scan of 20 µg/ml solution of ROS-M in methanol exhibited λ_{max} at 246.8 nm as shown in **Fig. 4.9** and absorbance was 0.968. The UV-scan of 24 µg/ml solution of ROS-B in acetonitrile : PBS (pH 7.4) in ratio of 2:3, exhibited λ_{max} at 246.2 nm **(Fig. 4.10)** and absorbance was 1.220. The observed λ_{max} of PIO, ROS-M and ROS-B matched close to the λ_{max} values reported in the literature (Mehta et al., 2005, Sripalakit et

al., 2006, Yamashita et al., 1996, Kolte et al., 2003). The spectroscopy studies further confirmed the identity and purity of drug sample(s).

Partition coefficient is defined as the ratio of unionized drug distributed between organic and aqueous phase in equilibrium. It is a measure of drug lipophilicity and an indication of its ability to cross biomembrane. Partitioning behavior of each of the drugs was studied in n-octanol : water as well as in n-octanol : phosphate buffer saline (pH 7.4) (PBS). The partition coefficient of pioglitazone in n-octanol : water and n-octanol : PBS were found to be 2.94 and 1.89, respectively **(Table 4.1)**. The partition coefficient of rosiglitazone maleate in n-octanol : water and n-octanol : PBS were found to be 1.93 and 1.47, respectively **(Table 4.2)**. Further, the partition coefficient of rosiglitazone base in n-octanol : water and n-octanol : PBS were found to be 3.19 and 3.93, respectively **(Table 4.3)**. This study revealed that both pioglitazone and rosiglitazone base are hydrophobic and lipophilic in nature and this property enhances entrapment of drug molecules in the nanoparticle matrix. Hence, rosiglitazone base is predicted to exhibit higher tendency for entrapment in hydrophobic interior of nanoparticles as compared to rosiglitazone maleate.

Calibration Curve of drug sample(s)

In order to estimate the drugs in experimental protocols, standard curves were prepared using ultraviolet spectroscopy. The first standard curve of pioglitazone HCl was made in methanol : acetonitrile (1:1) mixture and the other in methanol : acetonitrile : PBS (pH 7.4) : 0.1N sodium hydroxide solution (1:1:1:7). The first standard curve was used in determination of drug entrapment in the nanoparticles whereas second curve was used in determining the drug release from the drug delivery system. In the first standard curve, absorbance was taken at λ_{max} 268.0 and statistically analyzed (Microsoft Excel). The typical equation

describing the calibration curve of pioglitazone was y = 0.021x with a regression coefficient of 0.99 which confirmed an excellent linearity between concentration and absorbance. Pioglitazone followed Beer-Lambert's Law in the concentration range of 10-28 μg/mL in methanol: acetonitrile (1:1) mixture solvent system **(Fig.4.11).**

The standard curve of pioglitazone HCl in second solvent system was prepared in the concentration range of 5-50 μg/mL at λ_{max} 266.0 and statistically analyzed (Microsoft Excel). The typical equation describing the calibration curve of pioglitazone was y = 0.015x with a regression coefficient of 0.9999 which confirmed excellent linearity between concentration and absorbance. Pioglitazone followed Beer-Lambert's Law in the range 5-50 μg/mL in methanol : acetonitrile : PBS (pH 7.4) : 0.1N sodium hydroxide solution (1:1:1:7) solvent system **(Fig.4.11).**

The standard curve of rosiglitazone maleate was made in methanol in the concentration range of 2-20 μg/mL. Absorbance was taken at λ_{max} 246.8 and statistically analyzed (Microsoft Excel, USA). The typical equation describing the calibration curve of rosiglitazone maleate was 0.0489x with a regression coefficient of 0.998 which confirmed an excellent linearity between concentration and absorbance. Rosiglitazone maleate followed Beer-Lambert's Law in the range of 2-20 μg/mL in methanol **(Fig.4.13).**

Similarly, standard curve of rosiglitazone base was made in solvent system of acetonitrile : PBS (pH 7.4) (2:3) in the concentration range of 2-20 μg/mL. Absorbance was taken at λ_{max} 246.2 and statistically analyzed (Microsoft Excel, USA). The typical equation describing the calibration curve of rosiglitazone base was 0.0455x with a regression coefficient of 0.9991 which confirmed an excellent linearity between concentration and absorbance. Rosiglitazone base followed Beer-Lambert's Law in the range of 2-20 μg/mL in acetonitrile : PBS (pH 7.4) (2:3) solvent system **(Fig.4.14).**

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