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SAROGLITAZAR - MULTIVARIATE UV SPECTROPHOTOMETRIC QUANTIFICATION IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The aim of this research work was to develop a simple, accurate, sensitive and validated Ultra Violet (UV) spectrophotometric assay using multivariate regression method for the analysis of Saroglitazar. This multivariate calibration technique was based on equations constructed using linear regression analysis using the correlation between absorbance and concentration at five selected equidistant wavelengths. Saroglitazar had a maximum absorbance of 294 nm. The findings were statistically analysed for significance. A linear plot in the concentration range of 0.8-24µg/mL, with a regression co-efficient of 0.999 was obtained. The % RSD for intra-day and Inter-day precision were 0.539 and 0.452, respectively. The assay was determined and found to be 99.22% - 101.88% % w/w.

Keywords: Saroglitazar, antidiabetic agent, UV spectrophotometry, Multivariate calibration, Assay, ICH guidelines

INTRODUCTION

Saroglitazar (SAR) **(Figure 1)** is a drug for treating dyslipidaemia and type-2-diabetes Mellitus not controlled by statin therapy. SAR can reduce LDL cholesterol and reduction of triglycerides, LDL cholesterol, NON – HDL cholesterol, and

increase HDL cholesterol. SAR has Agonist action on PPARα lowers the high triglycerides and lower blood sugar levels. It can reduce fasting plasma glucose levels and gives control over glycaemia **[1]**. SAR chemically (2S)-2-ethoxy-3- [4- [2- [2-methyl-5-(4-methyl sulfanyl phenyl) pyrrol-1-yl] ethoxy] phenyl] propanoic acid. The chemical formula for SAR is $C_{25}H_{29}NO_{45}$, and molecular weight was found to be 439.56 g/mL **[2]**. Literature surveys reveal various methods as UV-Vis Spectrophotometry (UV) **[3-6]**, high performance liquid chromatography (HPLC) **[7-10]**, liquid chromatography and mass spectroscopy (LC-MS) **[11-12]**, high performance thin layer paper chromatography (HPTLC) **[13]**.

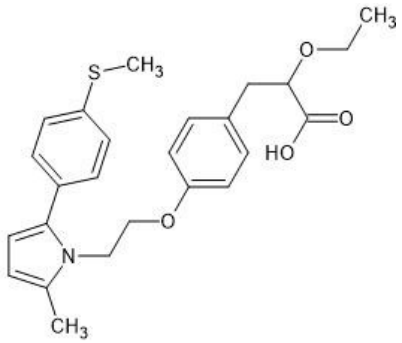

Figure 1: Chemical strucutre of Saroglitazar

The suggested technique provides higher confidence in results as it directly evaluates SAR and has been attested with greater accuracy and precision than a classical UV-Visible assay. This technique is more cost-effective, direct, and rapid than other methods and can be used for bulk drugs and various dosage forms. This multivariate standardization method simplifies the individual result and converts it into an "m" value as a reliant variable. Within optimized conditions, this analytical technique would provide excellent sensitivity, resolving power, expeditiousness, and cost-effectiveness for a validated quantification of SAR. The absorbance of an analyte (X), i.e., SAR, is scanned at 5 different absorbances (λ = 290, 292,294, 296, and 298 nm); the following formula can then be applied for any preferred wavelength **[14-21]**.

$$A_{\lambda 290} = a\ X\ C_x + \ k_1 \text{ ---------- (1)}$$

$$A_{\lambda 92} = b\ X\ C_x + \ k_2 \text{ ---------- (2)}$$

$$A_{\lambda 294} = c\ X\ C_x + \ k_3 \text{---------- (3)}$$

$$A_{\lambda 296} = d\ X\ C_x + \ k_4 \text{---------- (4)}$$

$$A_{\lambda 298} = e\ X\ C_x + \ k_5 \text{---------- (5)}$$

Where A_λ is the analyte's absorbance, a, b, c, d, and e being slopes of the analyte's linear regression functions; intercepts are denoted as k1, k2, k3, k4, k5 at the five specified wavelengths, and Cx is the analyte's concentration. The selected five equation systems (1–5) listed above can be summarised as follows:

$$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + \ d\ X\ C_x + \ e\ X\ C_x + \ K_T \text{---------- (6)}$$

The above equation can be further condensed to

$$A_T = C_x\ (a + b + c + d + e) + \ K_T =$$

------- (7)

A_T and K_T are the summations of the absorbance acquired cum totality of intercepts of regression equations at selected five wavelengths, respectively. The following formula computes the concentration of the analyte X.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

MATERIALS AND METHODS

Chemicals and reagents

- Methanol (MeOH) (Gradient grade, Finar Chemicals)
- SAR was obtained as a gift sample from Ideal Analytical and Research Institute, Pondicherry. The marketed tablet formulation used was Lipaglyn, Zydus Life Sciences, India, (Label claim – 4 milligram SAR) acquired from a local market.

Instrumentation

- LAB INDIA 3092 UV-Visible double beam spectrophotometer
- Ultra Sonicator Bath
- Analytical balance
- Micropipette

Analytical method development

Choice of the solvent

In MeOH, SAR was found to be freely soluble. Hence, MeOH was used for further dilutions of both standard and sample

Standard stock solution

SAR standard stock solution was prepared by dissolving 10 mg of the standard drug in 10 mL of MeOH and then making up to the mark in a 100 mL standard flask with the same solvent. Several concentrations (8 - 24 µg/mL) of solution were prepared from this standard stock solution.

Determination of λmax

The standard stock solution was diluted in MeOH to obtain 16 µg/mL. These solutions were measured in the Ultra-Violet region from 200 - 400 nm. The λ_{max} was obtained as 294 nm **(Figure 2)**. The linear curve was obtained with a graph plotting the absorbance against the concentration **(Table 1)**. The solutions were scanned across the range surrounding 294 nm, i.e., 290, 292, 294, 296, 298 nm, to enhance the correlation and diminish instrumental oscillations.

Preparation of sample solution

Twenty tablets of SAR were accurately weighed and powdered. A weight corresponding to 10 mg was measured into a 100 ml volumetric flask, dissolved, and made up to the mark with MeOH to obtain 100 µg/mL. This solution was then filtered and used for further analysis.

Method Validation

According to ICH Q2B guidelines, this method was validated for sensitivity, precision, accuracy, and linearity.

Linearity

The different concentrations over the range of 8 - 24 µg/mL were prepared from the standard stock solution of SAR. To minimize instrumental fluctuations and to better the correlation, these solutions were scanned over a range of wavelengths surrounding their absorbance maxima at 290, 292, 294, 296, and 298 nm, respectively. The absorbances were recorded, and the standardizations were obtained by plotting a concentration vs. absorbance graph. **(Figure 3, Table 1)**.

Table 1- UV Calibration data at five distinct wavelengths

Concentration (µg/mL)	Absorbance				
	290 nm	292 nm	294 nm	296 nm	298 nm
8	0.269	0.282	0.299	0.278	0.258
12	0.411	0.421	0.455	0.421	0.371
16	0.534	0.541	0.599	0.555	0.478
20	0.667	0.688	0.744	0.682	0.589
24	0.798	0.811	0.888	0.818	0.698

[#]Average of 5 determinations; UV= Ultra violet

The sensitivity of the method was determined by calculating the detection and quantification limit using the below formula.

$$LOD = 3.3\ \sigma/S \quad \text{..... (8)}$$

$$LOQ = 10\ \sigma\ /S \quad \text{..... (9)}$$

Here, σ is the standard deviation (SD) of the lowermost concentration and S is the slope of the standard curve.

Precision

To assess the intra-day and inter-day precision, 16 µg/mL solution was scanned six times in a short interval on one day for intraday precision and six different days for inter-day precision.

Accuracy

Using the standard addition technique, the recovery study for the suggested technique was Using the standard addition technique, the recovery study for the suggested technique was resolved at 80%, 100%, and 120%. The standard and sample stock solutions were prepared. 0.8 mL of the standard was pipetted into three 10 mL volumetric flasks. 0.48, 0.8, and 1.12 mL of sample solution were added, respectively, making up to a capacity of 10 mL with MeOH. These solutions were measured with a UV spectrophotometer, and the percentage recovery was calculated.

Assay

The amount of SAR present in the tablet formulation was calculated by measuring the absorbance of the extracted tablet solution at 294 nm.

RESULTS AND DISCUSSION

The λ_{max} of SAR was found to be 294 nm with MeOH as the solvent as shown in **Figure 2**.

The technique is linear within the assigned concentration range of 8 - 24 µg/mL. The linear regression analysis shows an excellent linear relationship with R^2=0.9992 - 0.9999 for all the calibration plots. For precision,

the % relative standard deviation was found to be 0.5390 and 0.4521. The LOD and LOQ obtained are 0.3782 and 1.1463 µg/mL, respectively. Therefore, the values found fell according to ICH guideline limits of validation parameters.

Linearity

The linearity was recorded at 290, 292, 294, 296, and 298 nm in the concentration range of 8 - 24 µg/mL and depicted in **Figure 3**, and corresponding calibration curves and residual plots are presented in **Figures 4 to 8 & 9-13** respectively. For each wavelength, the low values of % relative standard deviation show that the technique is accurate and precise. The LOD and LOQ were calculated and reported in **Table 2**.

Precision

The low standard deviation values indicate that this technique is specific, and % RSD for the intra-day and inter-day precision were found to be 0.5390 and 0.4521, respectively. It lies within the limits of less than 2% at each wavelength. The low percentage value of relative standard deviation reveals that the suggested technique is accurate and precise **(Figure 14, 15)**.

Recovery

As per ICH guidelines, the % recovery of SAR was from 99.22% to 101.88% w/w. The recovery was within the acceptable range of 97 - 103 % w/w **(Figure 16, Table 3)**.

Assay:

The UV absorbance of the tablet formulation was recorded at 294 nm. The quantity and assay percentages are 3.99 mg and 99.67 % w/w, respectively, with % RSD values as in **Table 4**.

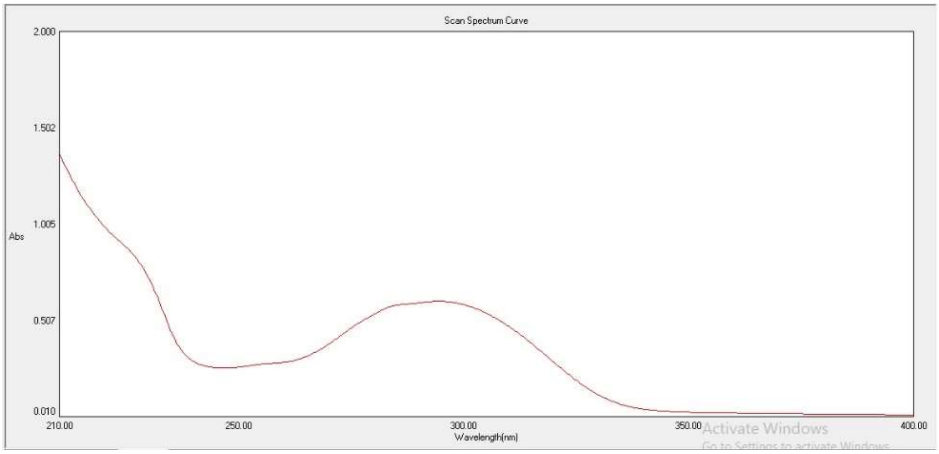

Figure 2: UV spectrum of Saroglitazar (16 µg/mL), λ_{max} at 294 nm

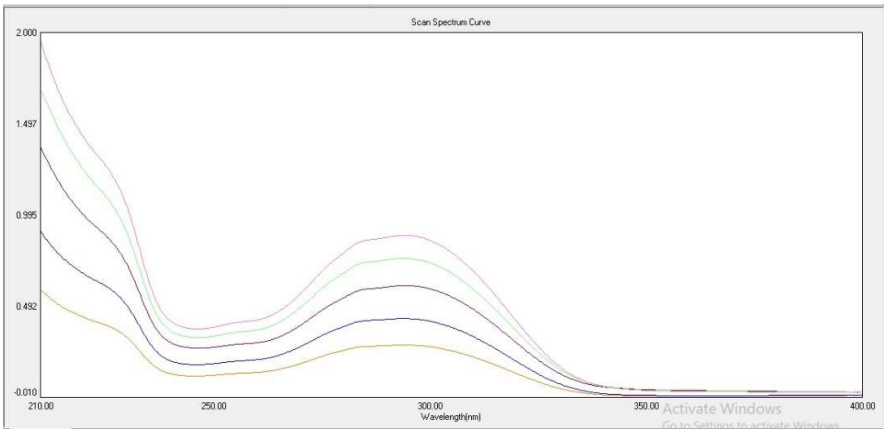

Figure 3: UV Spectrum of Saroglitazar showing linearity at 240 nm

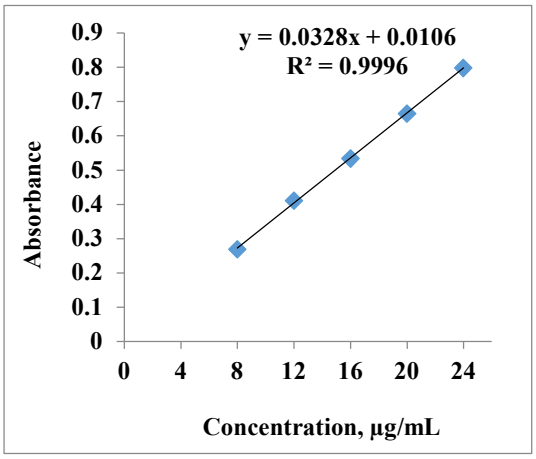

Figure 4: Calibration curve at 290 nm

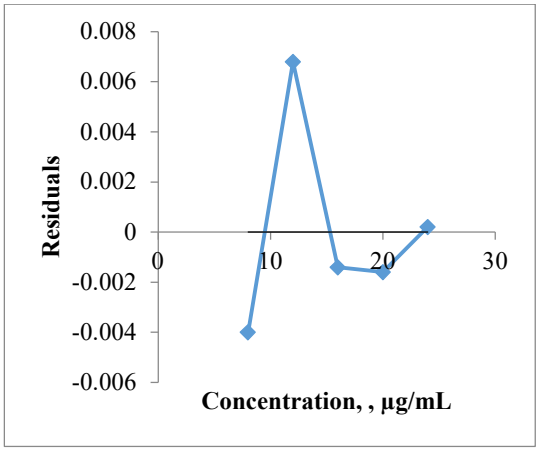

Figure 9: Residual plot at 290 nm

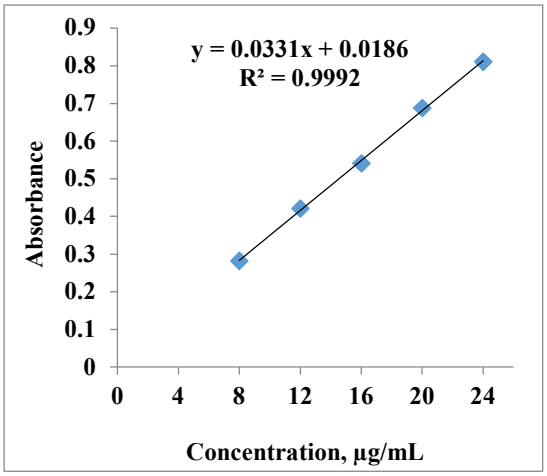

Figure 5: Calibration curve at 292 nm

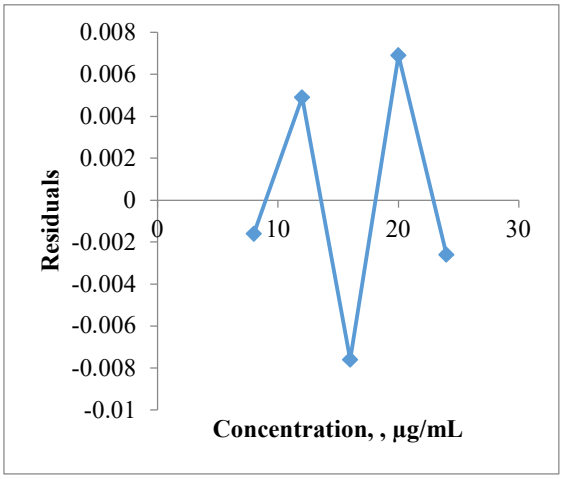

Figure 10: Residual plot at 292 nm

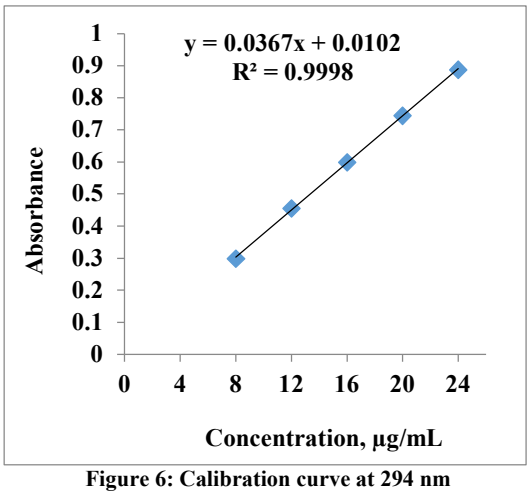

Figure 6: Calibration curve at 294 nm

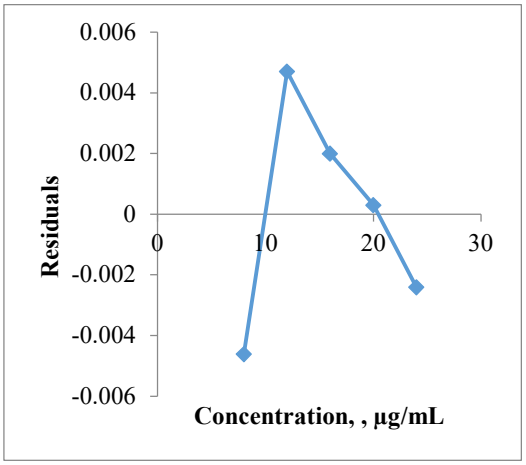

Figure 11: Residual plot at 294 nm

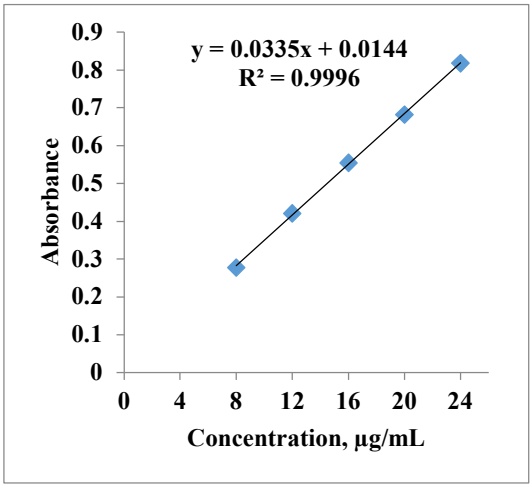

Figure 7: Calibration curve at 296 nm

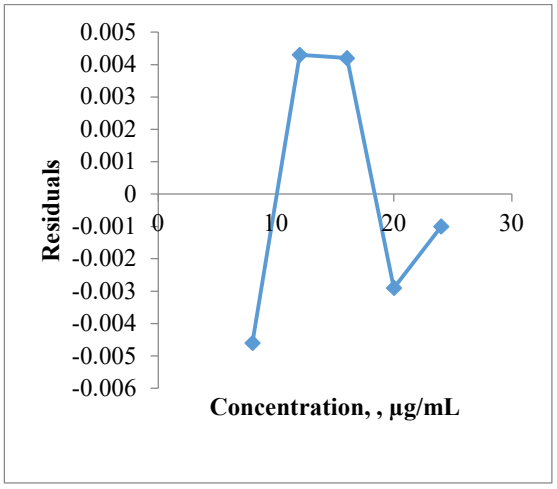

Figure 12: Residual plot at 296 nm

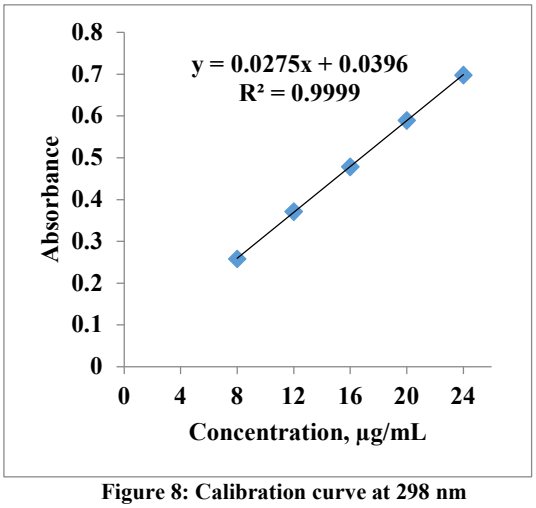

Figure 8: Calibration curve at 298 nm

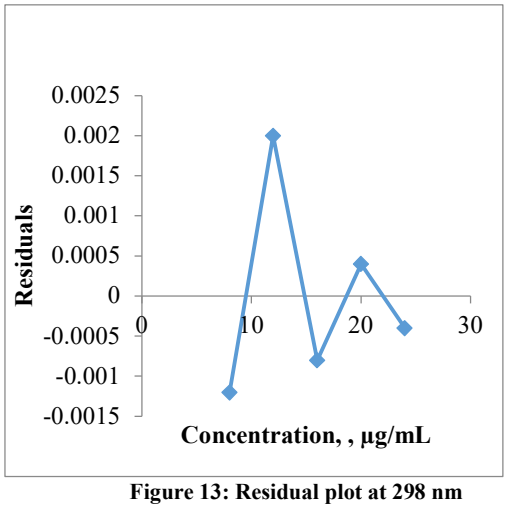

Figure 13: Residual plot at 298 nm

Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	Regression equation	R^2	LOD (µg/mL)	LOQ (µg/mL)	% RSD
290	**y = 0.0329x + 0.0102**	**0.9996**	**0.4747**	**1.4386**	**0.881**
292	**y = 0.0331x + 0.0186**	**0.9992**	**0.6778**	**2.0542**	**1.239**
294	**y = 0.0367x + 0.0102**	**0.9998**	**0.3782**	**1.1463**	**0.704**
296	**y = 0.0335x + 0.0144**	**0.9996**	**0.4644**	**1.4075**	**0.856**
298	**y = 0.0275x + 0.0396**	**0.9999**	**0.1752**	**0.5311**	**0.305**

***nm = nanometre; µg/mL = Microgram per millilitre**

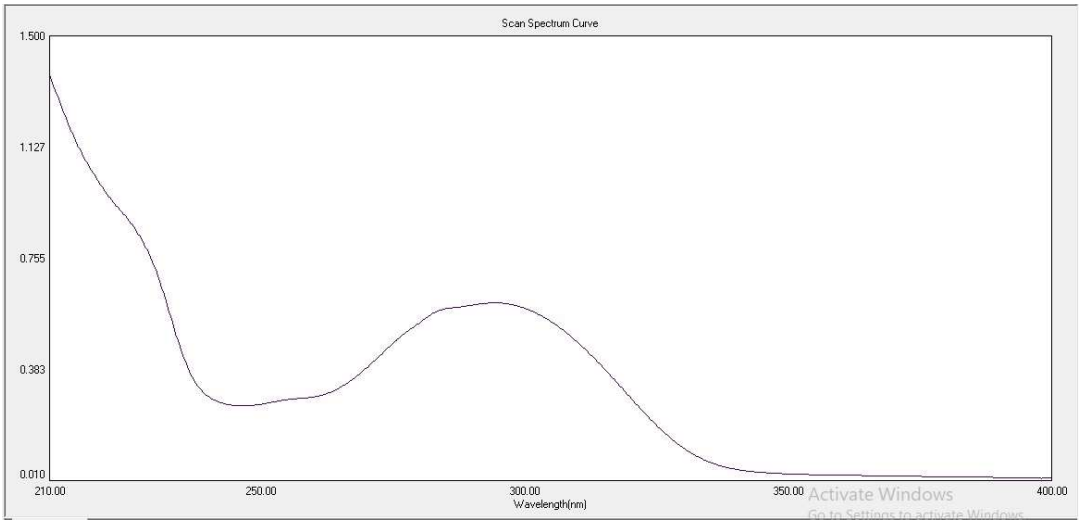

Figure 14: UV spectra showing intraday precision

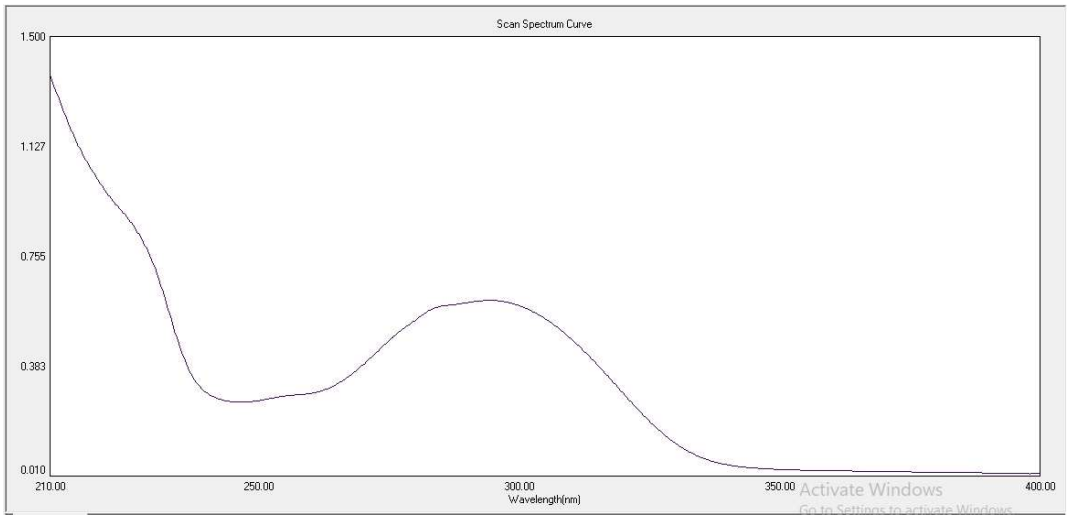

Figure 15: UV spectra showing interday precision

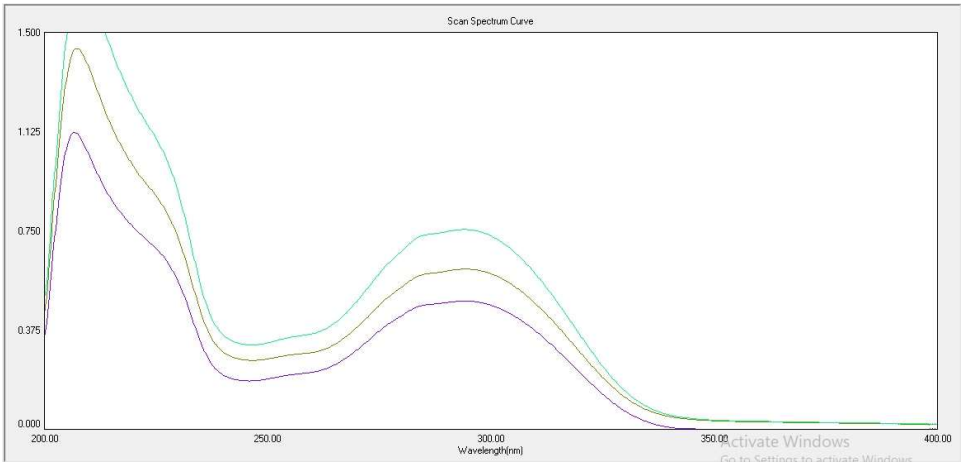

Figure 16: UV Spectrum showing accuracy of Saroglitazar

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
290nm	8	4.8	0.385	12.6	98.44
		8	0.486	16.1	100.63
		11.2	0.564	19.2	100.00
292 nm	8	4.8	0.396	12.7	99.22
		8	0.499	16	100.00
		11.2	0.587	19.1	99.48
294 nm	8	4.8	0.401	13	101.56
		8	0.509	15.9	99.38
		11.2	0.592	19.3	100.52
296 nm	8	4.8	0.397	12.9	100.78
		8	0.501	15.98	99.88
		11.2	0.588	19.3	100.52
298nm	8	4.8	0.386	12.7	99.22
		8	0.493	16.3	101.88
		11.2	0.574	19.5	101.56

Table 4: Assay of Saroglitazar

Label claim (mg)	Amount obtained (mg)	% Assay
4	3.98	99.50
4	4.01	100.25
4	3.97	99.25
Average	3.99	99.67
SD		0.5204
% RSD		0.5222

CONCLUSION:

This novel multivariate technique is more accurate, precise, reproducible, cost-effective, and sensitive than classical UV-Visible Spectrophotometry for SAR assay. This multilinear regression analysis is proven desirable for the testing standard drug and other dosage forms of SAR. This method is validated using ICH Quality Guidelines and found to be within the set limits of validation. This is a simple working procedure compared to expensive and

intricate techniques such as HPLC and HPTLC, and hence can be employed for routine analysis of SAR in bulk drugs and pharmaceuticals.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

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There is no funding to report.

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