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# QUANTITATIVE UV SPECTROPHOTOMETRIC ANALYSIS OF RIZATRIPTAN IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS BY MULTIVARIATE CALIBRATION TECHNIQUE

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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 Rizatriptan. 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. Rizatriptan had a maximum absorbance of 226 nm. The findings were statistically analysed for significance. A linear plot in the concentration range of 14-16 µg/mL, with a regression co-efficient of 0.999 was obtained. The % RSD for intra-day and Inter-day precision were 0.2342 and 0.1537, respectively. The assay was determined and found to be 98.13% - 101.50 % w/w.

**Keywords: Rizatriptan, $5HT_{1B}$ and $5HT_{1D}$ receptors, UV spectrophotometry, Multivariate calibration, ICH guidelines**

## INTRODUCTION

Rizatriptan Benzoate (RIZ) **(Figure 1)**, N, N-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethana-mine mono benzoate is applied to the management of migraines. The empirical formula of RIZ $C_{22}H_{25}N_5O_2$ and its molecular weight is 391.4 **[1]**. RIZ stimulates the 5-HT1B and 5-HT1D serotonin receptors **[2]**. RIZ appears to exert its effects by stimulating the vasoconstrictive and inhibitory activity

of trigeminal perivascular nerve terminals via activation of postsynaptic 5-HT1B receptors in cerebral and dural vessel walls **[3-4]**. Physical properties include white or off-white crystalline powder with a melting point between 178 and 180 degrees and a high degree of stability under normal conditions, and it is soluble in water **[5]**. It is officially found in Indian Pharmacopoeia **[6]**. It is sold under the trade name Maxalt as orally disintegrating tablets. In this literature review, several analytical techniques have been reported for the estimation of RIZ, including High-performance liquid chromatography (HPLC) **[7-10]**, UV-Spectroscopy **[11-13]**, High-performance Thin layer chromatography (HPTLC) **[14]** and liquid chromatography-tandem mass spectrometry (LC-MS/MS) **[15]**, among others.

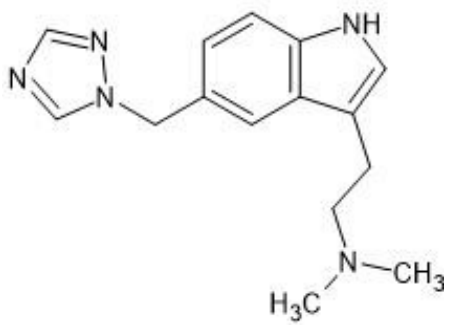


**Figure 1: Chemical strucutre of Rizatriptan**

The suggested technique provides higher confidence in results as it directly evaluates RIZ 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 RIZ. The absorbance of an analyte (X), i.e., RIZ, is scanned at 5 different absorbances (λ = 220, 223, 226, 229, 232 nm); the following formula can then be applied for any preferred wavelength **[16-23]**.

$A_{\lambda 220} = a\ X\ C_x +\ k_1$ ---------- (1)

$A_{\lambda 223} = b\ X\ C_x +\ k_2$ ---------- (2)

$A_{\lambda 226} = c\ X\ C_x +\ k_3$---------- (3)

$A_{\lambda 229} = d\ X\ C_x +\ k_4$---------- (4)

$A_{\lambda 232} = e\ X\ C_x +\ k_5$---------- (5)

Where $A_\lambda$ 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:

$A_T = a\ X\ C_x +\ b\ X\ C_x +\ c\ X\ C_x +\ d\ X\ C_x +\ e\ X\ C_x +\ K_T$---------- (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)
- RIZ was obtained as a gift sample from Ideal Analytical and Research Institute, Pondicherry. The marketed tablet formulation used was Rizact-10, Cipla Pharma, India, (Label claim – 10 milligram RIZ), 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, RIZ was found to be freely soluble. Hence, MeOH was used for further dilutions of both standard and sample.

### Standard stock solution

RIZ standard stock solution was prepared by the dissolution of 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. 1 mL of this solution was transferred to another 10 mL volumetric flask and made up to 10 mL with diluent to obtain a 10 µg/mL concentration. Several concentrations (14 - 16 µg/mL) of solution were prepared from this standard stock solution.

### Determination of λmax

The standard stock solution was diluted in methanol to obtain 20 µg/mL. These solutions were measured in the Ultra-Violet region from 200 - 400 nm. The $\lambda_{max}$ was obtained as 226 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 226 nm, i.e., 220, 223, 226, 229, 232 nm, to enhance the correlation and diminish instrumental oscillations.

### Preparation of sample solution

Twenty tablets of RIZ were accurately weighed and powdered. A weight corresponding to 10 mg was measured into a 10 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 **[24]** guidelines this method was validated for

sensitivity, precision, accuracy, and linearity.

**Linearity**

The different concentrations over the 14-26 µg/mL range were prepared from the standard stock solution of RIZ. To minimize instrumental fluctuations and to better the correlation, these solutions were scanned over a range of wavelengths surrounding their absorbance maxima at 220, 223, 226, 229, and 232 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# | | | | |
|---|---|---|---|---|---|
| | 220 nm | 223 nm | 226 nm | 229 nm | 232 nm |
| 14 | 0.324 | 0.374 | 0.402 | 0.385 | 0.342 |
| 16 | 0.371 | 0.421 | 0.448 | 0.432 | 0.371 |
| 18 | 0.41 | 0.463 | 0.496 | 0.477 | 0.407 |
| 20 | 0.45 | 0.509 | 0.545 | 0.522 | 0.441 |
| 22 | 0.487 | 0.558 | 0.583 | 0.567 | 0.479 |
| 24 | 0.532 | 0.601 | 0.635 | 0.614 | 0.512 |
| 26 | 0.573 | 0.647 | 0.681 | 0.654 | 0.549 |

#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.

$$LOD = 3.3\ \sigma/S \quad \ldots\ldots\ldots\ldots\ldots (8)$$

$$LOQ = 10\ \sigma/S \quad \ldots\ldots\ldots\ldots\ldots (9)$$

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, 20 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**

The recovery studies for the suggested technique were resolved at 80%, 100%, and 120% using the standard addition technique. The standard and sample stock solutions were prepared. 1.4 mL of the standard was pipetted into three 10 mL volumetric flasks. 0.6, 1, and 1.4 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 RIZ present in the tablet formulation was calculated by measuring the absorbance of the extracted tablet solution at 226 nm.

## RESULTS AND DISCUSSION

The $\lambda_{max}$ of RIZ was found to be 226 nm with MeOH as the solvent as shown in **Figure 2**.

The technique is linear within the assigned concentration range of 14-26 µg/mL. The linear regression analysis shows an excellent linear relationship with $R^2$=0.9991 - 0.9998 for all the calibration plots. The % relative standard deviation for precision was found to be 0.2342 and 0.1537. The LOD and LOQ obtained are 0.3928 µg/mL and 1.1905 µg/mL, respectively. Therefore, the values fell according to ICH guideline limits of validation parameters.

### Linearity

The linearity was recorded at 220, 223, 226, 229, and 232 nm in the concentration range of 14-26 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 0.2342 and 0.1537, 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 RIZ was found to be from 98.13% - 101.50% 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 226 nm. The quantity and assay percentages are 9.95 mg and 99.53 % w/w, respectively, with % RSD values as in **Table 4**.

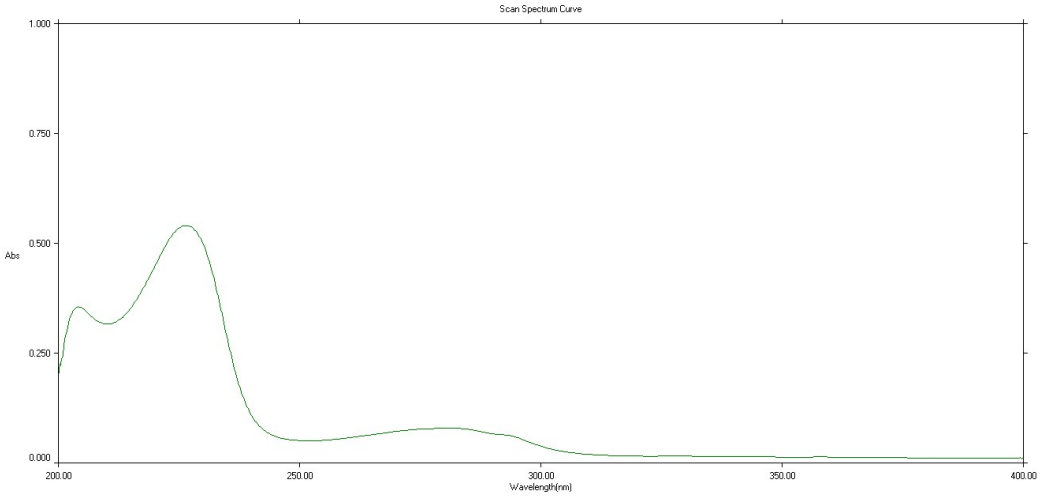


**Figure 2: UV spectrum of Rizatriptan (20 µg/mL), $\lambda_{max}$ at 226 nm**

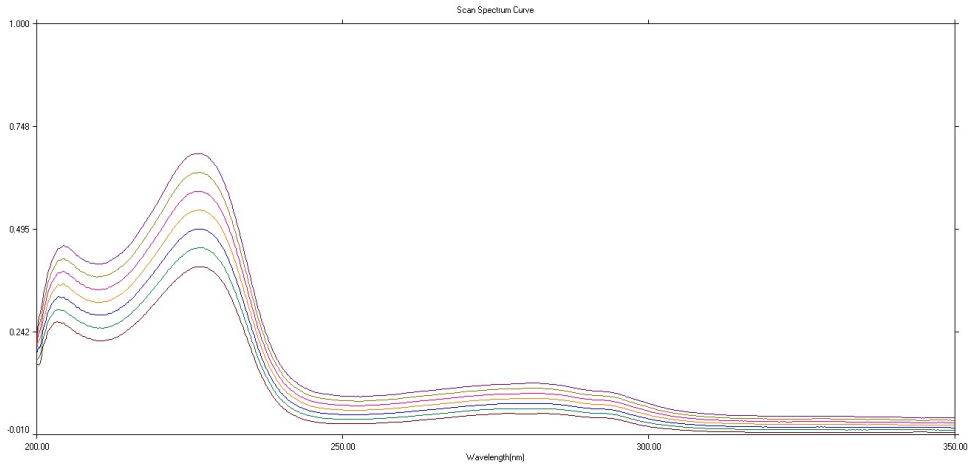


**Figure 3: UV Spectrum of rizatriptan showing linearity at 226 nm**

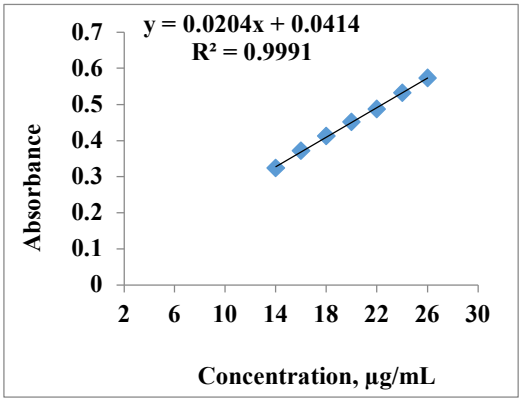


**Figure 4: Calibration curve at 220 nm**

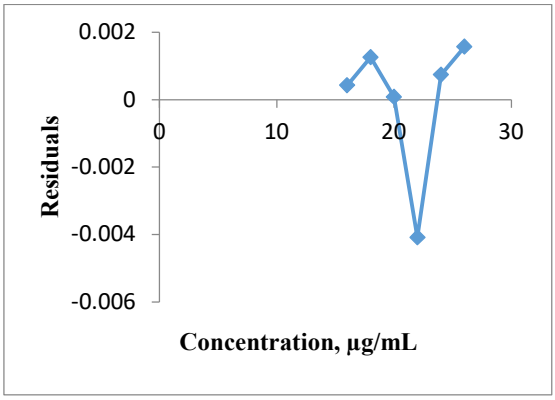


**Figure 9: Residual plot at 220 nm**

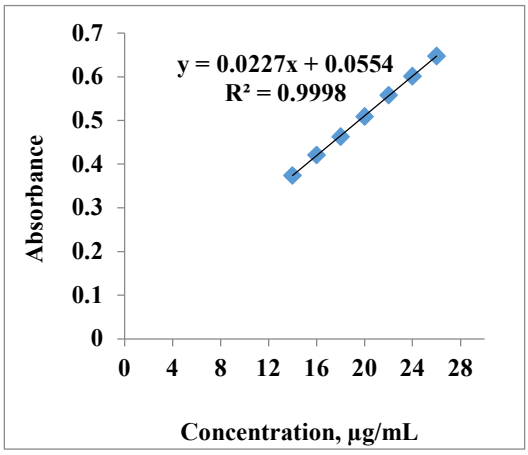


Figure 5: Calibration curve at 223 nm

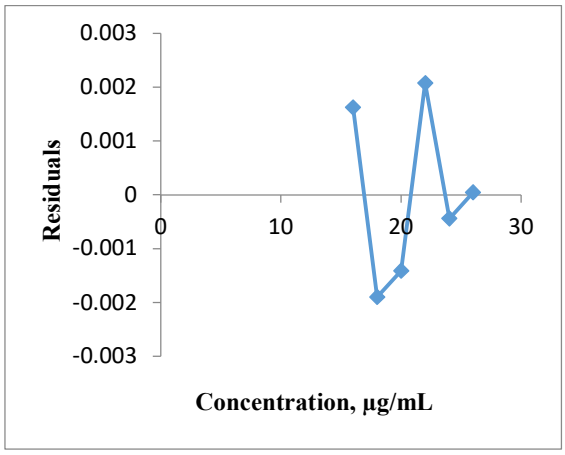


Figure 10: Residual plot at 223 nm

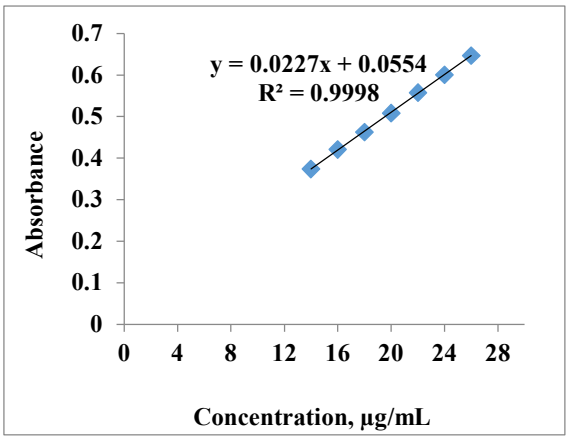


Figure 6: Calibration curve at 226 nm

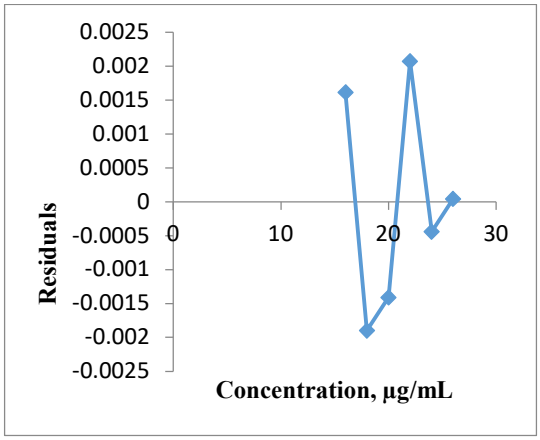


Figure 11: Residual plot at 226 nm

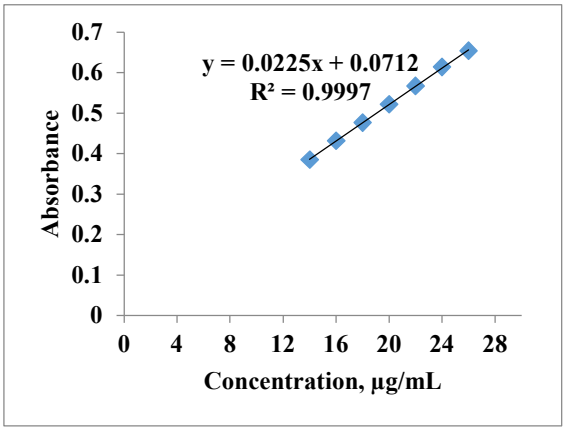


Figure 7: Calibration curve at 229 nm

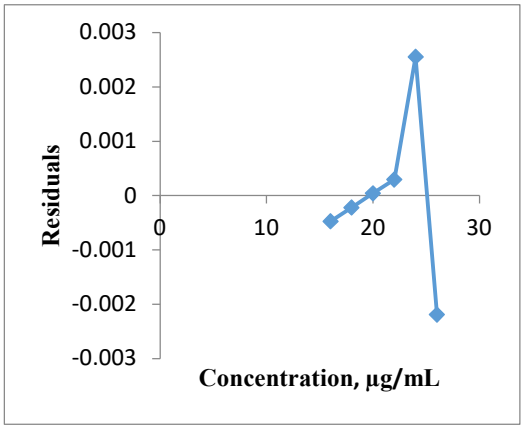


Figure 12: Residual plot at 229 nm

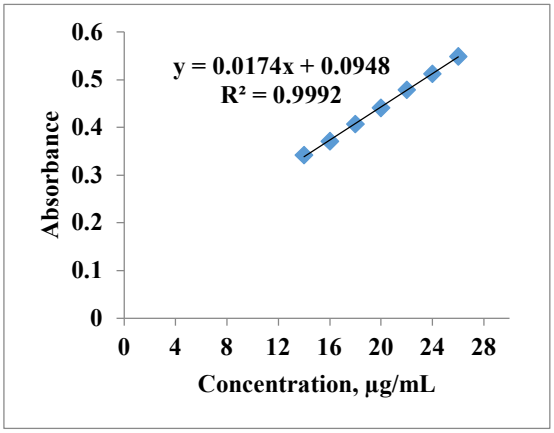


Figure 8: Calibration curve at 232 nm

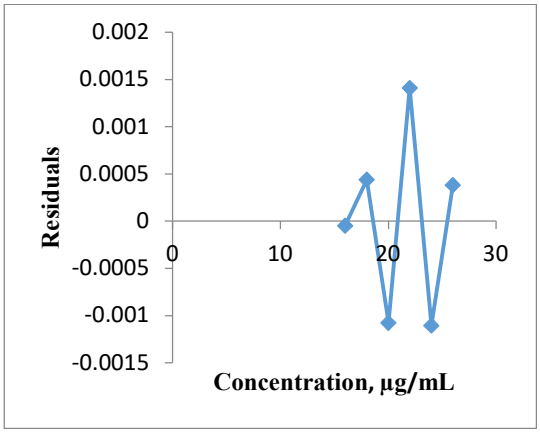


Figure 13: Residual plot at 232 nm

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 |
|---|---|---|---|---|---|
| 220 | y = 0.0204x + 0.0414 | 0.9991 | 0.4767 | 1.4446 | 0.6549 |
| 223 | y = 0.0227x + 0.0554 | 0.9998 | 0.2318 | 0.7024 | 0.3124 |
| 226 | y = 0.0232x + 0.0779 | 0.9994 | 0.3928 | 0.1905 | 0.5101 |
| 229 | y = 0.0225x + 0.0712 | 0.9997 | 0.2597 | 0.7870 | 0.3395 |
| 232 | y = 0.0174x + 0.0948 | 0.9992 | 0.4415 | 1.3381 | 0.5255 |

*nm = Nano meter; µg/mL = Microgram per millilitre

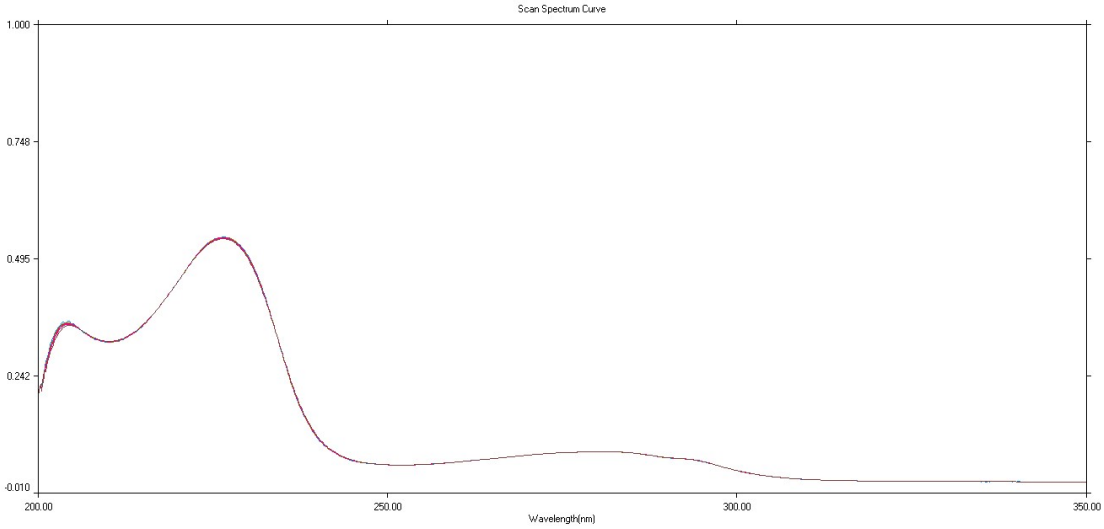


Figure 14: UV spectra showing intraday precision

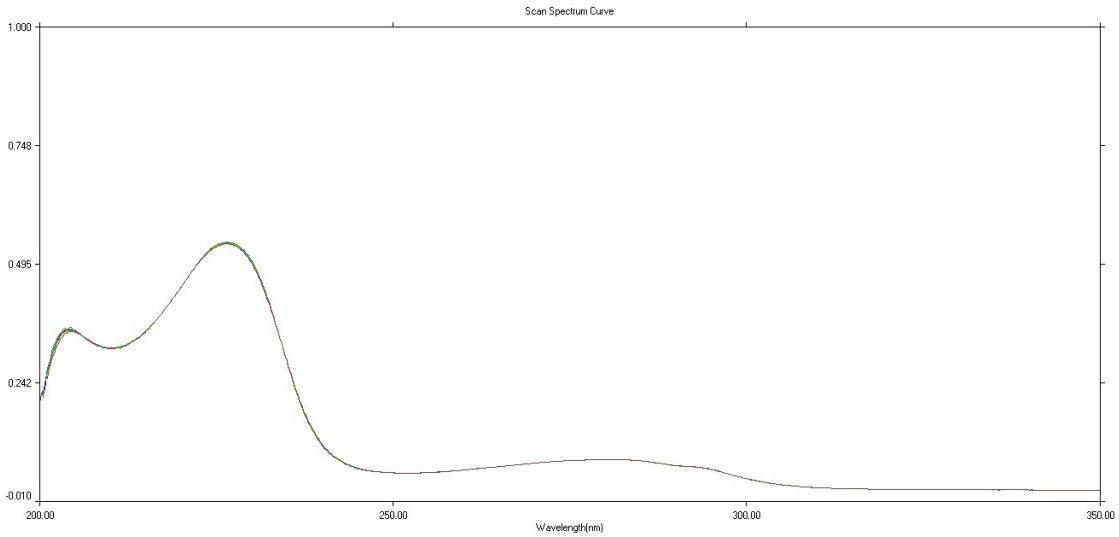


**Figure 15: UV spectra showing interday precision**

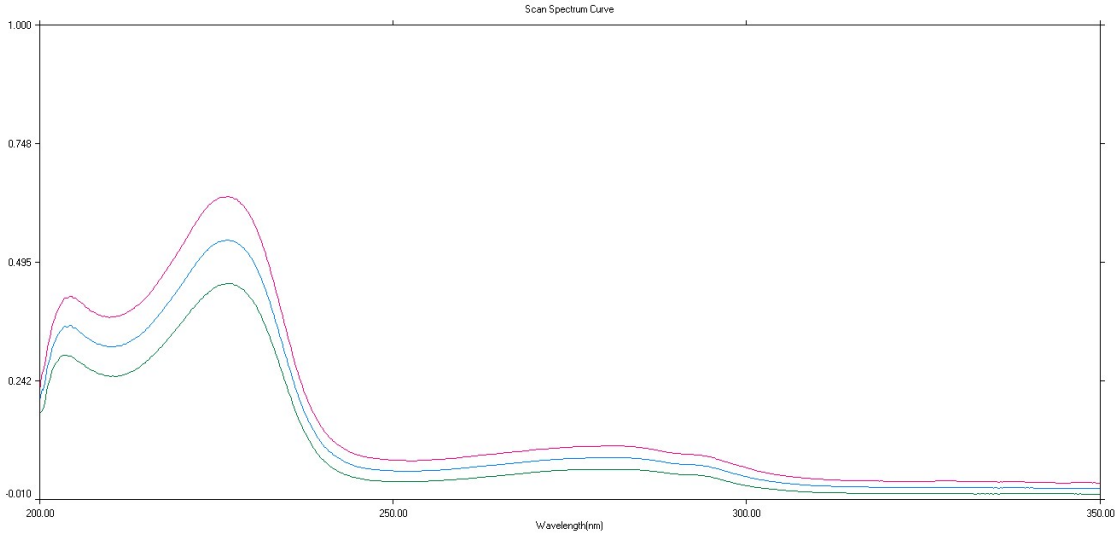


**Figure 16: UV Spectrum showing accuracy of rizatriptan.**

**Table 3: Recovery Studies**

| Wavelength (nm) | Amount present (µg/mL) | Amount added (µg/mL) | Absorbance | Amount recovered (µg/mL) | % Recovery |
|---|---|---|---|---|---|
| 220 nm | 10 | 6 | 0.371 | 15.7 | 98.13 |
| | | 10 | 0.451 | 19.8 | 99.00 |
| | | 14 | 0.532 | 23.7 | 98.75 |
| 223 nm | 10 | 6 | 0.421 | 15.8 | 98.75 |
| | | 10 | 0.509 | 19.9 | 99.50 |
| | | 14 | 0.601 | 23.8 | 99.17 |
| 226 nm | 10 | 6 | 0.448 | 16.2 | 101.25 |
| | | 10 | 0.545 | 19.7 | 98.50 |
| | | 14 | 0.635 | 23.9 | 99.58 |
| 229 nm | 10 | 6 | 0.432 | 15.9 | 99.38 |
| | | 10 | 0.522 | 20.3 | 101.50 |
| | | 14 | 0.614 | 23.8 | 99.17 |
| 232 nm | 10 | 6 | 0.371 | 15.8 | 98.75 |
| | | 10 | 0.441 | 19.7 | 98.50 |
| | | 14 | 0.512 | 23.9 | 99.58 |

Table 4: Assay of rizatriptan

| Label claim (mg) | Amount obtained (mg) | % Assay |
|---|---|---|
| 10 | 9.94 | 99.40 |
| 10 | 9.95 | 99.50 |
| 10 | 9.97 | 99.70 |
| Average | 9.95 | 99.53 |
| SD | | 0.1528 |
| % RSD | | 0.1535 |

## CONCLUSION:

This novel multivariate technique is more accurate, precise, reproducible, cost-effective, and sensitive than classical UV-Visible Spectrophotometry for Rizatriptan assay. This multilinear regression analysis is proven desirable for the testing standard drug and other dosage forms of RIZ. 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 RIZ formulations 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.

## FUNDING SOURCES

There is no funding to report.

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