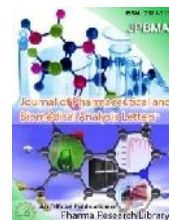




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## Research Article

## Open Access

### Analytical Method Development and Validation for the Simultaneous Estimation of Telmisartan and Hydrochlorothiazide by UV-Spectrophotometric Method in Bulk and Tablet Dosage Form

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#### ABSTRACT

The Spectroscopic method was successfully developed for the separation of Hydrochlorothiazide and Telmisartan by using as solvent methanol, detection wavelength were at 271nm and 296 nm. The Spectroscopic method was done in solvent using methanol and the instrument lab india 3000+ with uv win software. The linearity study of Hydrochlorothiazide and Telmisartan was found in concentration range of 2µg/ml-12µg/ml and 5µg/ml-30µg/ml and correlation coefficient ( $r^2$ ) was found to be 0.999 and 0.997, % recovery was found to be 98.56% and 99.96%, %RSD for repeatability was 1.2, % RSD for intermediate precision was 1.9. The precision study was precision, robustness and repeatability. LOD value was 3.72 and 0.0242 and LOQ value was 7.40 and 0.0202 respectively. The Proposed method is validated and it is useful for the determination and estimation of hydrochlorothiazide and Telmisartan in its bulk and pharmaceutical dosage form.

**Keywords:** Telmisartan, UV visible spectrophotometer, Hydrochlorothiazide, LOD, LOQ.

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## 1. Introduction

Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension. The usually effective dose telmisartan is 40–80 mg once daily. Some patients may already benefit at a daily dose of 20 mg. In cases where the target blood pressure is not achieved, telmisartan dose can be increased to a maximum of 80 mg once daily. Telmisartan is contraindicated during pregnancy. Like other drugs affecting therenin-angiotensin system (RAS), telmisartan can cause birth defects, stillbirths, and neonatal deaths. It is not known whether the drug passes into the breast milk. Also it is contraindicated in bilateral renal artery stenosis in which it can cause renal failure.

Hydrochlorothiazide is a diuretic medication often used to treat high blood pressure and swelling due to fluid buildup. Other uses include diabetes insipidus, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine. For high blood pressure it is often recommended as a first line treatment. HCTZ is taken by mouth and may be combined with other blood pressure medications as a single pill to increase the effectiveness. Potential side effects include poor kidney function, electrolyte imbalances especially low blood potassium and less commonly low blood sodium, gout, high blood sugar, and feeling faint initially upon standing up.[2] While allergies to HCTZ are reported to occur more often in those with allergies to sulfa drugs this association is not well supported. It may be used during pregnancy but is not a first line medication in this group.

It is in the thiazide medication class and acts by decreasing the kidneys' ability to retain water. This initially reduces blood volume, decreasing blood return to the heart and thus cardiac output. Long term, however, it is believed to lower peripheral vascular resistance

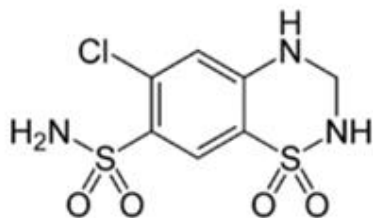


Figure 1: Structure of Telmisartan

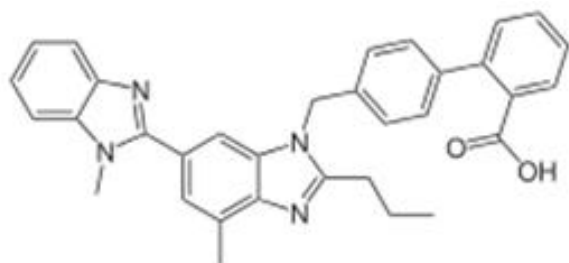


Figure 2: Structure of Hydrochlor Thiazide

### Analytical methods

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or

structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results.

The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte (s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report.

The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many standard analytical methods have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own in-house methods or adapt existing ones for specific purposes.

## 2. Materials and Methods

**2. Selection of wavelength:** 10 mg of Telmisartan and Hydrochlorothiazide was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Telmisartan and Hydrochlorothiazide. The isobestic point was taken as detection wavelength.

### Preparation of the individual Hydrochlorothiazide standard preparation

10 mg of Hydrochlorothiazide working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of methanol and sonicate to dissolve it completely and make volume up to the mark with the methanol (Stock solution). Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with methanol.

### Preparation of the individual Telmisartan standard preparation

10 mg of Telmisartan working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the

methanol (Stock solution). Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with methanol.

### Preparation of the Hydrochlorothiazide and Telmisartan standard and sample solution

#### Sample solution preparation:

10 mg of Hydrochlorothiazide and 2 mg Telmisartan tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

#### Analytical Method Validation:

##### Validation parameters

##### 1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

##### 2. Linearity

##### Preparation of stock solution

10 mg of Hydrochlorothiazide and 2 mg of Telmisartan working standard were accurately weighed and were transferred separately into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. From the above stock solution prepared series concentrations within the range of 2ppm to 12 ppm for hydrochlorothiazide and 5 ppm to 30 ppm for Telmisartan.

##### Procedure

Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

##### Acceptance criteria

Correlation coefficient should be not less than 0.999.

##### Accuracy

##### Preparation of standard stock solution

10mg of Hydrochlorothiazide and 2 mg of Telmisartan working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

##### Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

##### Preparation of stock solution

10 mg of Hydrochlorothiazide and 2mg of Telmisartan working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further

pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

**Procedure** The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

##### Acceptance criteria

The % RSD for the area of five sample injections results should not be more than 2%.

##### Selection of solvent

Solutions of Telmisartan and Hydrochlorothiazide were prepared in different solvents like methanol, ethanol, acetonitrile and UV spectrum of each were recorded by scanning between 200-400 nm.

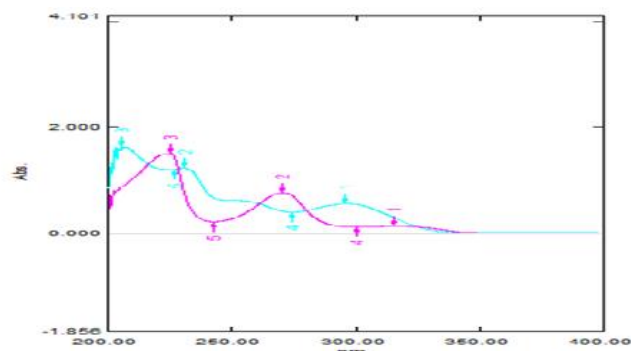


Figure 1: Overlay Normal spectra of Telmisartan and Hydrochlorothiazide in Methanol

## 3. Results and Discussion

### Validation of the Method

#### Linearity

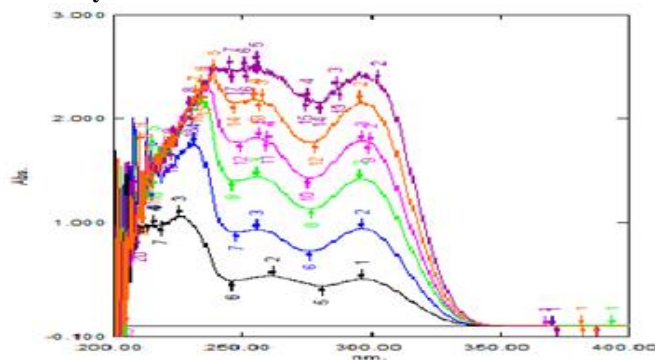


Figure 2: Linearity curve of Telmisartan

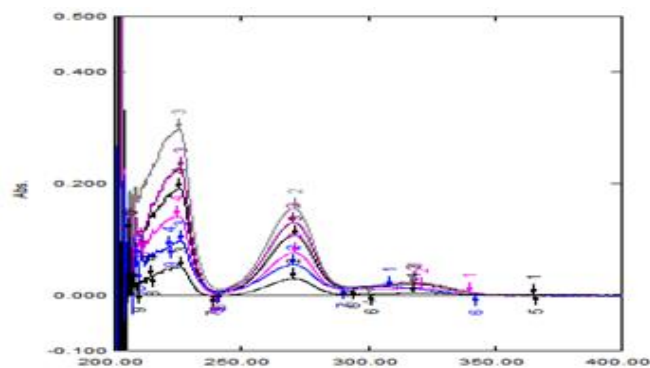


Figure 3: Linearity of Hydrochlorothiazide

Telmisartan was found to be linear in a concentration range of 5-30µg/ml. The absorbances of these solutions were noted at wavelengths 296 and 271 nm, respectively. Calibration curves were plotted using concentration Vs absorbance at wavelength of 296 nm and the slope, intercept and correlation coefficient values were found to be 0.008, 0.007 and 0.997 respectively. At wavelength 271nm, slope, intercept and correlation coefficient values were found to be 0.007, 0.015 and 0.997, respectively.

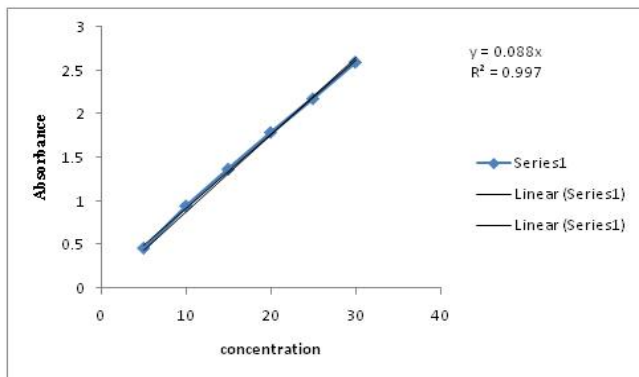


Figure 4: Calibration graph of Telmisartan at 296nm

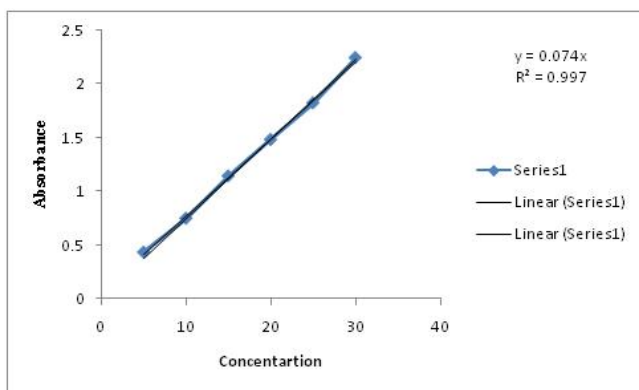


Figure 5: Calibration graph of Telmisartan at 271nm.

Table 1: Calibration data of Telmisartan at 296nm and 271 nm

S.No	Conc. (µg/ml)	Absorbance at 296nm	Absorbance at 271nm
1	5	0.452	0.537
2	10	0.942	0.852
3	15	1.364	1.168
4	20	1.788	1.583
5	25	2.179	1.923
6	30	2.598	2.343

**Hydrochlorothiazide**

Hydrochlorothiazide was found to be linear at a concentration range of 2-12µg/ml. The absorbances of these solutions were noted at 271 and 296 nm, respectively. Calibration curves were plotted using concentration Vs absorbance. At a wavelength of 271 nm, the slope, intercept and correlation coefficient values were found to be 0.080, 0.001 and 0.999, respectively, fig. 7.1.4. At wavelength 275 nm, the slope, intercept and correlation coefficient values were found to be 0.005, 0.006 and 0.999, respectively.

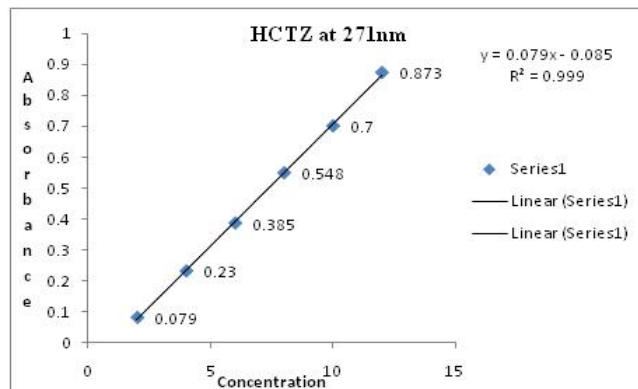


Fig 6: Calibration graph of Hydrochlorothiazide at 271nm

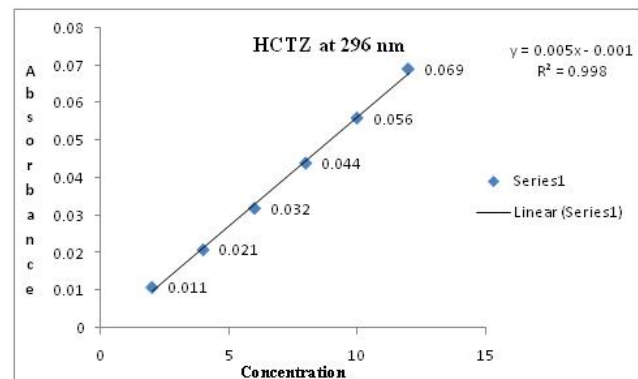


Fig 7: Calibration graph of Hydrochlorothiazide at 296nm

Table 2: Calibration data of Hydrochlorothiazide Calcium at 271 and 296nm

S. No	Concentration (~g/ml)	Absorbance at 271 nm	Absorbance at 296 nm
1	2	0.079	0.011
2	4	0.230	0.021
3	6	0.385	0.032
4	8	0.548	0.044
5	10	0.700	0.056
6	12	0.873	0.069

**Precision:**

Precision studies were performed by preparing the standards three times and measuring the absorbances of drugs at 296 nm and 271 nm. Low RSD values indicate that the method is precise.

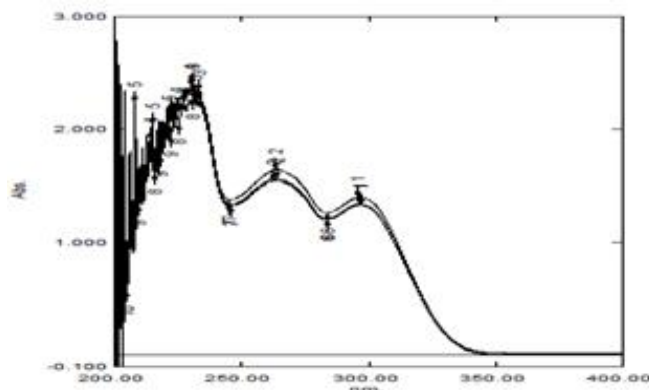


Figure 8: Precision Chromatogram of Telmisartan

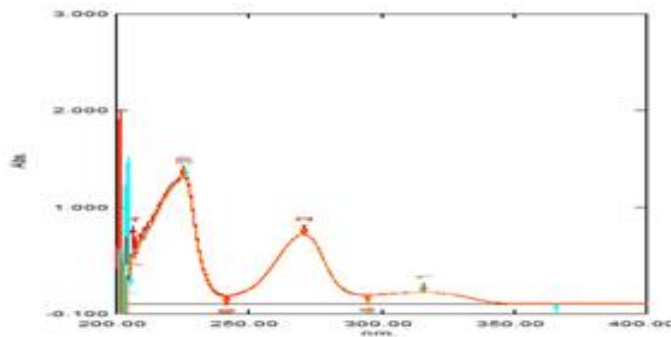


Figure 9: Precision Chromatogram of Hydrochlorothiazide

**Recovery studies**

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder (10mg), a known quantity of standard Telmisartan and Hydrochlorothiazide were added at 80%, 100% and 120% level and the contents were re-analyzed by the proposed method.

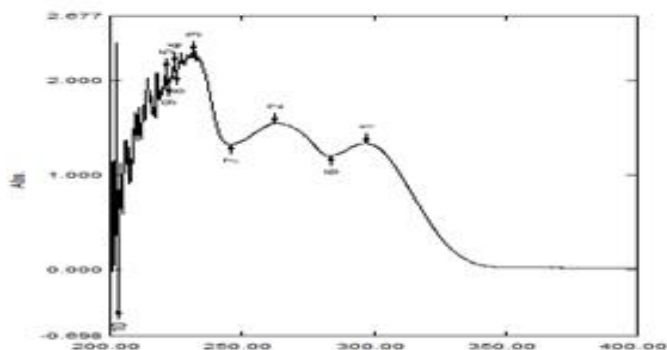


Figure 9: Accuracy 80%

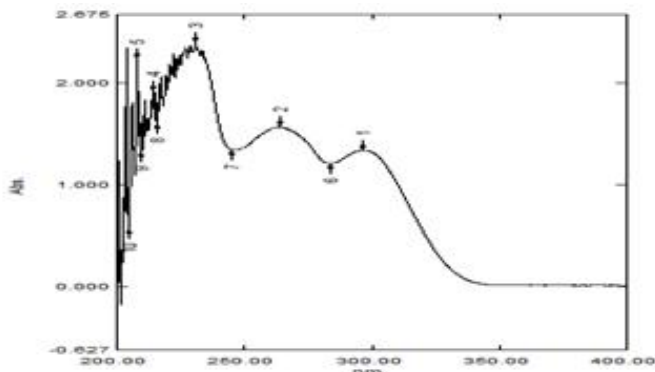


Figure 10: Accuracy 100%

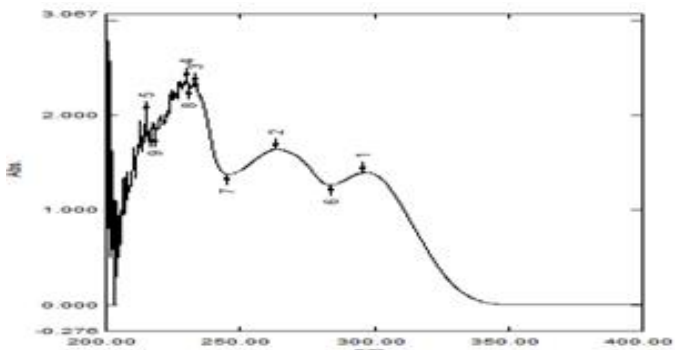


Figure 11: Accuracy 120%

**Analysis of Formulation**

**Preparation of standard solutions**

Standard stock solution of Telmisartan was prepared by dissolving 10 mg of the drug in methanol and the volume was made up to 10ml in a standard flask. From the stock solution, concentrations ranging from 5-30 µg/ml was prepared for Telmisartan and 10mg of drug is dissolved in methanol and volume was made upto 10ml from the stock solution concentration ranging from 2-12µg/ml was prepared for Hydrochlorothiazide and scanned in the UV region.

**Preparation of sample solution**

Two tablets are powdered and the average weight was calculated. A quantity equivalent to 40 mg of drug was dissolved in Methanol. Finally the volume was made up to get a working concentration of 10µg/ml each of Telmisartan and Hydrochlorothiazide and absorbances were noted at 296nm and 271m. The amounts of Telmisartan and Hydrochlorothiazide were calculated using the simultaneous equation given below

At  $\lambda_1$   $A_1 = ax_1bc_x + ay_1bc_y$

$\lambda_2$   $A_2 = ax_2bc_x + ay_2bc_y$

$$C_{fvx} = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2}$$

$$C_{ofx} = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

$A_1$  = absorbance of formulation at 296nm.

$A_2$  = absorbance of formulation at 271nm.

$ax_1$  = Absorptivity of Telmisartan at 296nm.

$ax_2$  = Absorptivity of Hydrochlorothiazide at 271nm.

$ay_1$  = Absorptivity of Telmisartan at 296nm.

$ay_2$  = Absorptivity of Hydrochlorothiazide at 271nm.

$C_{fvx}$  = Concentration of Telmisartan.

$C_{ofx}$  = Concentration of Hydrochlorothiazide

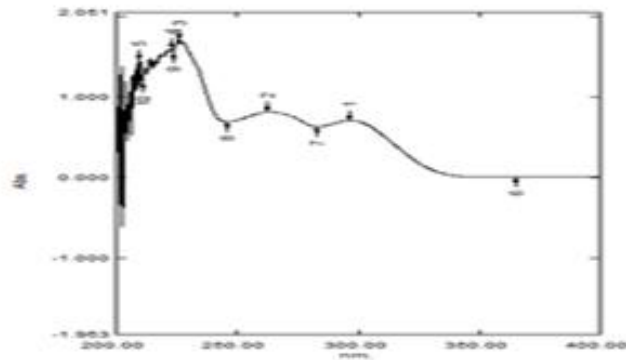


Figure 12: UV spectra of formulation

**4. Conclusion**

A new method was established for simultaneous estimation of Hydrochlorothiazide and Telmisartan by UV visible spectroscopy. The Linearity was performed in UV in the range of 5 µg/ml to 30 µg/ml for Telmisartan in two wavelengths like 296 and 271 nm. for hydrochlorothiazide in the range of 2 µg/ml to 20 µg/ml in 271nm and 296 nm. The co efficient of correlation was found to be less than 0.99 in both wavelengths. Precision and recovery studies also found to be with the range, % recovery was found to be

98.56% and 99.96%, %RSD for repeatability was 1.2, % RSD for intermediate precision was 1.9. The precision study was precision, robustness and repeatability. LOD value was 3.72 and 0.0242 and LOQ value was 7.40 and 0.0202 respectively.

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