WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 9, 1178-1186. Research Article ISSN 2277–7105

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AREA UNDER CURVE APPROACHES FOR TRICLABENDAZOLE ESTIMATION IN BULK AND TABLET DOSAGE FORM

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Article Received on 05 April 2026,
Article Revised on 25 April 2026,
Article Published on 01 May 2026,

https://doi.org/10.5281/zenodo.20023787

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How to cite this Article: Dr. Sowmya H. G.[1], Karthik M. L.[*2], Dr. Naveen Kumar G. S.[3] (2026). Analytical Method Development And Validation Of Area Under Curve Approaches For Triclabendazole Estimation In Bulk And Tablet Dosage Form. World Journal of Pharmaceutical Research, 15(9), 1178–1186.

ABSTRACT

Triclabendazole in bulk and pharmaceutical dose form can be estimated using a straightforward, accurate, and precise zero-order derivative UV spectroscopic approach that has been developed and validated. The absorbance of Triclabendazole in methanol reaches its maximum at 306 nm, and the Area under curve in absorption spectra measured between wavelength range in 301nm and 311nm, and its concentration falls between 2 and 12 µg/ml according to Beer's Law. After conducting a linearity investigation, the regression coefficient (R2) was shown to be 0.995, indicating good linearity and precision across this concentration range. The limits of detection (LOD) and quantitation (LOQ) were determined to be 1.55 µg/ml and 4.71 µg/ml respectively, while the percentage recovery was determined to be 99.5%, 99%, and 103.3%. Additionally, the method's relative standard deviation (%RSD) values were less than 2%, indicating exceptional precision. Following ICH criteria, all validation parameters—linearity, accuracy,

precision, robustness, ruggedness, LOD, and LOQ—were evaluated. Triclabendazole in bulk and pharmaceutical dosage forms can be routinely estimated using the designed and tested approach.

KEYWORDS: Triclabendazole; Area Under curve; Validation; RP-HPLC; HPTLC, Anthelmintic.

INTRODUCTION

Triclabendazole is a benzimidazole derivative that is mostly used as an anthelmintic medication. It works particularly well against liver flukes, or Fasciola hepatica. It functions by interfering with parasites' ability to use microtubules. Reliable analytical techniques for its quantification and quality control are essential because of its clinical significance, particularly in parasite infections in humans and animals. In contrast to other trematodicidal medications, triclabendazole (TCBZ, 6-chloro-5(2-3 dichlorophenoxy) 2-methyl thiobenzimidazole), a halogenated benzimidazole (BZD) thiol derivative, exhibits high efficacy against both the immature and mature stages of Fasciola hepatica in sheep and cattle.[2] Intestinal nematode infections are one of the main causes of financial losses in the sheep breeding industry. As the prevalence of parasite resistance continues to rise, veterinarians are now using a range of anthelmintic medications in combination to treat animals quickly.[3] Triclabendazole dissolves readily in organic solvents like acetonitrile and methanol but is essentially insoluble in water. Methanol is frequently chosen as a solvent for UV spectrophotometric measurement because of its solubility properties. Achieving clear spectra, a well-shaped peak, and repeatable absorbance values depends heavily on the choice of solvent.

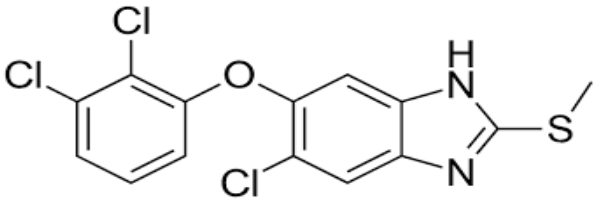

Figure 1: Chemical Structure of Triclabendazole.

Few analytical methods using UV, HPLC, RP-HPLC, and HPTLC have been described for determining Triclabendazole in pure medication and pharmaceutical dosage forms, according to review of the literature. The current endeavor attempts to create and validate a Area under curve UV Spectrophotometric method for estimating Triclabendazole in tablet and bulk dose form.

MATERIALS AND METHODS

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals: Triclabendazole pure drug gift sample was given by Ce-Chem Pharmaceuticals Pvt Ltd and its pharmaceutical dosage Triclabendazole 20 tablets obtained from Medsuvac Lifesciences.

Solvent: Methanol is used as a solvent.

Selection of analytical wavelength

Appropriate dilutions of Triclabendazole were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400 nm. The absorption spectra obtained and show maximum absorbance at 301 nm and 311nm, as the wavelength for detection.

Preparation of standard stock solution

100mg of Triclabendazole was weighed accurately transferred into 100 ml of volumetric flask and diluted in Methanol upto the mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml into 10 ml individual volumetric flask and diluted in Methanol up to the mark, this gives 2, 4, 6, 8, 10 and 12µg/ml concentration.

Preparation of sample solution

20 tablets of Triclabendazole marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Triclabendazole was transferred into 100ml volumetric flask then it was diluted with Methanol and make upto the mark.

METHOD AND VALIDATION

The method was validated according to the ICH guidelines.

RESULT AND DISCUSSION

Method: Zero order derivative spectroscopy

Linearity

Linearity shows how well the response of the method changes in proportion to the concentration of the drug within a given range. In other words, when the concentration

increases, the absorbance should also increase in a consistent and predictable manner. The linearity was established in the range of 2-12µg/ml was measured at 301 nm and 311 nm and absorbance values are shown in table 1. The calibration curve was prepared by plotting graph against the concentration vs absorbance and therefore the graph shown in Fig-3 statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined and shown in table-2.

Precision

Precision indicates how close the results are when the same sample is tested multiple times under similar conditions. If the variation between repeated measurements is very small, the method is considered precise. Precision was established by intra-day and inter-day was determined by analysing the same concentration for six times in a same day. Inter-day precision was analysing the same concentration daily for six days shown in table-3.

Accuracy

The accuracy of an analytical method says that closeness of test results obtained by that method of the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels at 50%, 100% and 150%. In which the formulation concentration holds it constant and varied pure drug concentration. Shown in table -4.

Ruggedness

Ruggedness evaluates whether the method gives consistent results when small changes are made, such as using different analysts or performing the test on different days. A rugged method produces similar results despite these minor variations. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (Table-5).

LOD and LOQ

LOD is the smallest amount of drug that the method can detect, even if it cannot measure it accurately. LOQ is the lowest amount of drug that can be measured accurately and precisely using the developed method. LOD and LOQ were calculated by using following formula

LOD = 3.3(σ/S) and LOQ = 10 (σ / S)

LOD and LOQ value of Triclabendzole were found be 0.98 µg/mL and 2.97 µg/mL.

Table 1: Results of calibration curve at 306nm by area under curve spectroscopy.

Sl No	Concentration in µg|ml	Absorbance ± Standard deviation
1	0	0
2	2	0.137±0.00098
3	4	0.217±0.0012
4	6	0.299±0.00089
5	8	0.420±0.0012
6	10	0.532±0.0012
7	12	0.622±0.0011

** Average of six determinations*

Table 2: Regression parameters of Triclabendazole by area under curve spectroscopy.

Regression	Parameter Results
Range	2-12 µg/ml
λ_{max}	301nm & 311nm
Regression equation	y = 0.0499x + 0.0221
Slope(b)	0.0499
Intercept (a)	0.0221
Correlation coefficient	0.995
Sandell's sensitivity	0.0201
LOD(µg/ml)	0.98 µg/ml
LOQ(µg/ml)	2.97 µg/ml

Y=bx+a**

Table 3: Determination of Precision results for Triclabendazole at 306nm by area under curve spectroscopy.

Concentration (µg|ml)	Intra-day Absorbance ± Standard deviation*	% RSD	Inter-day Absorbance ± Standard deviation*	% RSD
2	0.137±0.00098	0.716	0.136±0.000753	0.55
4	0.217±0.0012	0.552	0.218±0.00055	0.252
6	0.299±0.00089	0.299	0.297±0.00089	0.301
8	0.420±0.0012	0.287	0.421±0.000753	0.179
10	0.532±0.0012	0.237	0.531±0.000516	0.0971
12	0.622±0.0011	0.187	0.621±0.0013	0.204

** Average of six determinations,** Percentage relative standard deviation.*

Table 4: Determination of accuracy results for Triclabendazole at 306nm by area under curve spectroscopy.

Spiked levels	Amount of sample (µg|ml)	Amount of standard (µg|ml)	Amount recovered	%Recovery± Standard deviation*	%RSD**
50	6	3	9.07	100.8	0.266
100	6	6	11.91	99.24	0.0766
150	6	9	15.52	103.46	0.0785

** Average of six determinations,** Percentage relative standard deviation.*

Table 5: Determination of ruggedness results of Triclabendazole at 306nm by area under curve spectroscopy.

Analysts	Day-1	Day-2
Mean absorbance	0.299	0.297
±Standard deviation*	0.000942	0.000816
%RSD**	0.315	0.275

** Average of six determinations,** Percentage relative standard deviation.*

FIGURES

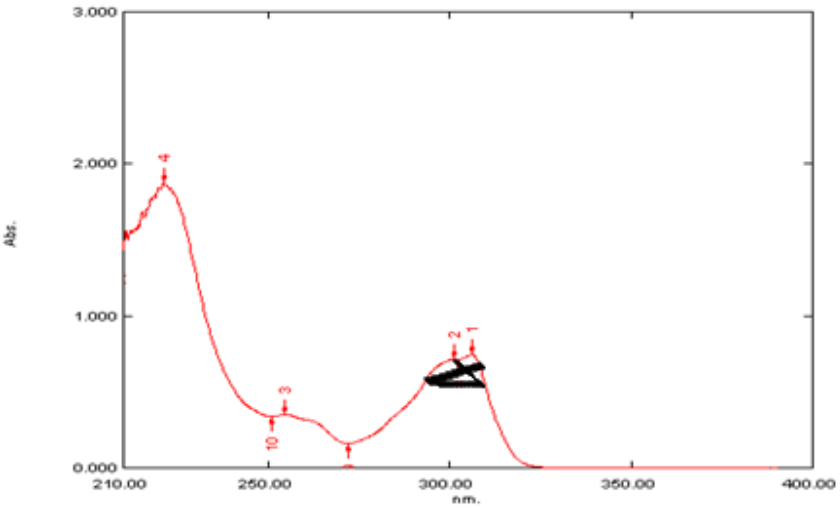

Fig. 2: Area under curve spectrum of Triclabendazole at 301 nm to 311 nm.

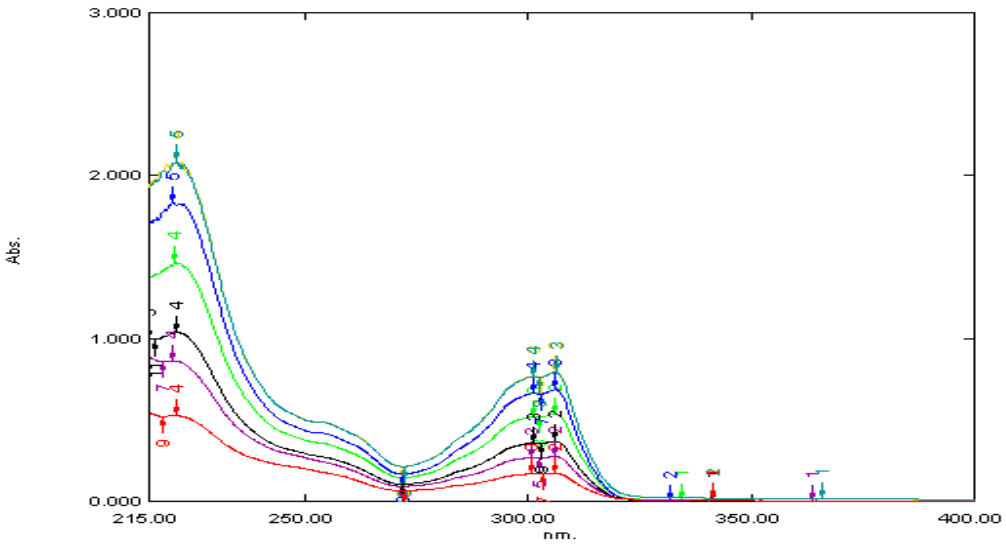

Figure 3: Area under curve overlain spectra of Triclabendazole showing absorbance at 301 nm to 311 nm.

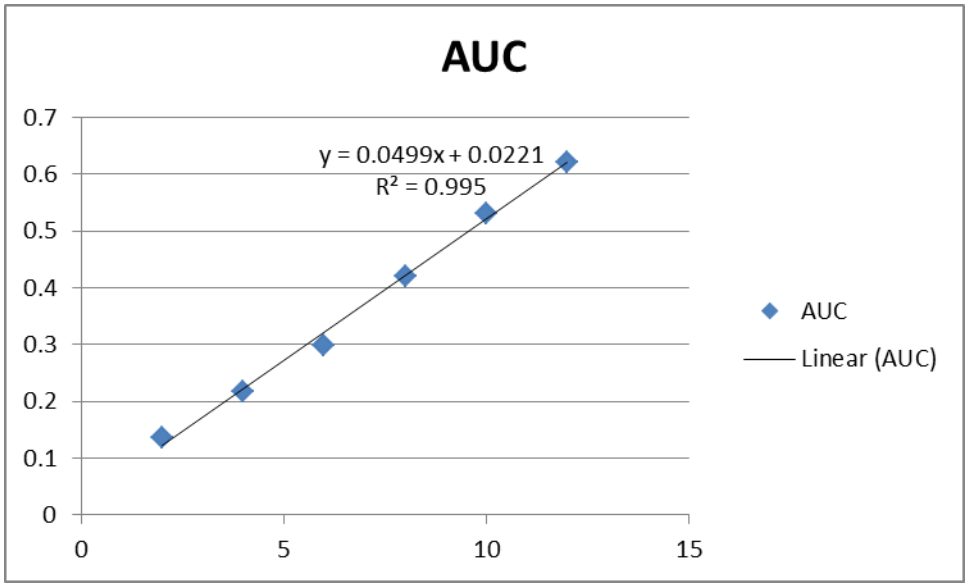

Figure 4: Calibration curve of Triclabendazole by Area under curve.

CONCLUSION

The analytical method developed for Triclabendazole was validated as per ICH guidelines demonstrating simplicity, specificity, accuracy, economy, and sensitivity. This method is suitable for regular analysis of Triclabendazole in both bulk form and pharmaceutical preparations.

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