Available online at http://www.ijims.com

ISSN: 2348 – 0343

Development and Validation of UV Spectrophotometric Area Under Curve (AUC) method for estimation of Pyrantel Pamoate in Bulk and Tablet Dosage Form

Rahul S. Kommawar and Madhuri A. Nagras

STES's Sinhgad College of Pharmacy, Vadgaon (Bk.), Off. Sinhgad Road, Pune - 411041, Maharashtra, India.

Corresponding author : Rahul S. Kommawar

Abstract

The aim of present work was to develop an accurate, precise, reproducible and economical UV spectrophotometric method for estimation of Pyrantel Pamoate. This method was based on Area Under Curve (AUC) of UV spectrum between 231 to 241 nm and validated as per ICH guideline Q2 (R1). The method is linear in the range of 1.5-3.5 µg/ml. The value of correlation coefficient is 0.999. Values of % relative standard deviation (%RSD) for the intra-day and inter-day precision indicated that method is precise. Results of the recovery studies (99.94 %) showed accuracy of the method. LOD and LOQ were calculated as 0.017µg/ml and 0.0541µg/ml respectively. The developed method can be used for routine estimation of Pyrantel Pamoate in bulk and tablet dosage forms.

Keywords: Pyrantel Pamoate, Estimation, UV Spectrophotometry, Area Under Curve (AUC), Validation.

Introduction

Pyrantel Pamoate is an orally administered veterinary anthelmintic that is effective against a variety of round worms and hook worms in dogs, cats, horses, birds and rabbits. It is also used to treat pin worms in humans.[1] Mechanism of action is drug exerts its action as a depolarizing blocking agent that particularly affords Spastic paralysis in susceptible helminths.[2] Pyrantel Pamoate is chemically known as [E]-1,4,5,6-Tetrahydro -1 – methyl – 2 –[2-(2-thienyl)vinyl]pyrimidine 4,4'- methyl enebis [3- hydroxy 2 – napthoate]. This drug is official in United State Pharmacopoeia (USP).[3] Literature survey revealed some HPLC methods have been reported for estimation of this drug[4,5,6,7.]. Only few papers have been available in the literature using spectrometry for estimation of Pyrantel Pamoate as combined dosage forms.[8] In this context, we wish to further explore UV spectrophotometry using Area Under Curve (AUC) for estimation of Pyrantel Pamoate in bulk & tablet dosage form.[9]

Materials and Methods

APPARATUS AND INSRTUMENTATION

Shimadzu UV 1800 with matched quartz cells and equiped with UV Prob Software, was used for this work. Single pan electronic balance [Shimadzu, AX 200, (Japan)] was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra Lab. UCB 40, India). Calibrated volumetric glasswares (Borosil) were used in this study.

MATERIALS

Active pharmaceutical ingredient (API) Pyrantel Pamoate was supplied as a gift sample by Concept Pharmaceuticals Ltd., Aurangabad, (Maharashtra, India). Commercially available tablets (Nemocid®) containing 250 mg of Pyrantel Pamoate were obtained from local pharmacy. AR-grade Methanol (as a solvent) was purchased from Merck India Ltd., Mumbai.

METHOD DEVELOPMENT

PREPARATION OF STANDARD SOLUTION

The standard stock solution of Pyrantel Pamoate was prepared by transferring, accurately weighed, 10 mg of API to 100 ml of volumetric flask. The drug was dissolved with sonication in 50 ml of methanol and volume was made up to the mark by using methanol. The standard stock solution (100 µg/ml) was further diluted with methanol to get the concentration of 10 µg/ml.

SELECTION OF WAVELENGTH RANGE

The standard solution of 10µg/ml was scanned between 400 nm to 200 nm in UV spectrophotometer against methanol as blank after baseline correction. Wavelength range was selected around wavelength maxima (236 nm). Different working standards were prepared between 1.5-3.5 µg/ml. Various wavelength range were tried and final range between 231-241 nm was selected on the basis of linear relationship between area and corresponding concentration (Figure 1).

AREA UNDER CURVE (AREA CALCULATION)

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing item calculates the area covered by the curve and horizontal axis. Here horizontal axis represents baseline.

$$\text{Area calculation } (\alpha + \beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$$

Whereas, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelengths representing start and end point of curve region. In this study area was integrated between wavelength ranges from 231 to 241 nm.

PREPARATION OF CALIBRATION CURVE

Working solutions were prepared from standard stock solution by further dilution with methanol to obtain the concentration of 1.5, 2.0, 2.5, 3.0 and 3.5 µg/ml, respectively. These solutions were scanned from 400 to 200 nm and Area Under Curve (AUC) was integrated in the range of 231 to 241 nm. The calibration curve was plotted between Area Under Curve (AUC) against concentration (Figure 2).

ASSAY OF TABLET FORMULATION

Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to 10 mg of Pyrantel Pamoate was accurately weighed and transferred to a 100 ml

of volumetric flask and diluted up to mark with methanol. The solution was filtered with Whatmann filter paper No. 41 and the first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10µg/ml solution with same solvent and subjected for UV analysis. This procedure was repeated in triplicate (Table 1).

METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline .[10]

LINEARITY AND RANGE

The linearity was determined by using working standard solutions between 1.5-3.5 µg/ml. The spectrums of these solutions were recorded and Area Under Curve (AUC) was integrated in wavelength range 231-241 nm. Calibration curve of Area Under Curve (AUC) vs. Concentration was plotted after suitable calculation and simple linear regression was performed (Figure 2). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation.

METHOD PRECISION

REPEATABILITY

The precision of the method was checked by repeatedly injecting (n = 6) standard solutions of Pyrantel Pamoate (10 µg/ml). Area Under Curve (AUC) of each of these solutions was measured in the range of 231-241 nm % relative standard deviation (%RSD) was calculated (Table 2).

INTERMEDIATE PRECISION (REPRODUCIBILITY)

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 same concentrations of standard solutions of Pyrantel Pamoate (2.5µg/ml). The results were reported in terms of % relative standard deviation (%RSD). The results were tabulated. (Table 2).

ACCURACY

The accuracy for the analytical procedure was determined at 80 %, 100 % and 120 % levels of standard solution. Area Under Curve (AUC) was measured in the range of 231-241 nm and results were expressed in terms of % recoveries. Three determinations at each level were performed and % RSD was calculated. The results were tabulated. (Table 3).

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

Five sets of known concentrations (1.5-3.5 µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae as

$$\textbf{LOD} = 3.3 \times \frac{SD}{S} \qquad \textbf{LOQ} = 10 \times \frac{SD}{S}$$

Where,

SD is standard deviation of y-intercept of the calibration curves.

S is mean slope of five calibration curves.

Results and Discussion

An attempt was made to develop a simple and specific UV spectrophotometric AUC method for the determination of Pyrantel Pamoate in bulk and tablet dosage form. The generated regression equation was ${}_{241}\int^{231}$ Ad = 0.393X + 0.006(R^2 = 0.999) Where, ${}_{241}\int^{231}$ Is Area Under Curve (AUC) between 231 to 241 nm, R^2 is correlation coefficient. The R^2 value as 0.999 indicates that developed method was linear. The proposed method was found to be precise as % R.S.D values for intraday as well interday precision were satisfactory. The drug at each of the 80 %, 100 % and 120 % levels showed good recoveries 99.94 %. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 0.017 µg/ml and 0.054 µg/ml, respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, reproducible and reliable. The method can be used for the routine analysis of Pyrantel Pamoate in bulk and tablet dosage form. The validation parameters are summarized. (Table 4).

Conclusion

It can be concluded from the results that the proposed method was linear, accurate, precise, simple and reproducible for the determination of Pyrantel Pamoate in bulk and tablet dosage form. This method was validated as per ICH guidelines. Results suggest that this method can be used for routine estimation of Pyrantel Pamoate in bulk and tablet dosage form.

Acknowledgement

The authors are grateful to Concept Pharmaceuticals Ltd., Aurangabad (Maharashtra, India) for providing API of Pyrantel Pamoate as gift sample and Dr. K.N. Gujar, Principal, Sinhgad College of Pharmacy, Vadgaon (Bk.), Pune for providing necessary facilities for this project.

References

1. Davidson G, Plumb CD. Veterinary drug hand book, Client Information Edition: Wiley, John and Sons; 2003.
2. Kar A. Medicinal Chemistry. New Delhi: New Age International Publishers; 2007.
3. United States of Pharmacopoeia-28/ National Formulary-23. Asian Ed. United States Pharmacopoeial Convention, Inc., Rockville MD; 2005; 1678.
4. Oltean EG. Development and validation of an HPLC method for the quantitation studies of Praziquantel and Pyrantel Pamoate., Veterinary Drug, 2011; vol:5(1).
5. Argekar AP, Raj SV, Kapadia SU. Simultaneos determination of mebendazole and Pyrantel Pamoate from tablets by high performance liquid chromatography-reverse phase (RP-HPLC), Elsevier, 1997; 44(11):1959-65.
6. Morovjan G, Sokan P, Makranszki L, et al. Determination of fenbendazole and Pyrantel Pamoate in dog plasma by HPLC.J Chromatogr A.,1998; 797(1-2):237-44.

7. Allender VVJ, High performance liquid chromatographic determination of Oxantel and Pyrantel Pamoate.J Chromatogr Sci., 1988; 26(9):470-2.
8. Piantavini MS, Potents FL, Uber CP, et al. Chemometric quality inspection control of Pyrantel Pamoate,febantel and praziquantel in veterinary tablets by mid infrared spectroscopy. Spectrochim Acta A Mol Biomol spectro SC., 2014; 125:396- 403.
9. Shimadzu Corporation-Kyoto Japan, Analytical & Measuring Instruments Division, Instruction Manual Operation Guide-UV 1800, 2008; 13.21-13.25.
10. ICH Harmonized-Tripartite Guidelines. Validation of Analytical Procedure: Text and Methoology Q2 (R1), Geneva; November, 2005.

STRUCTURE

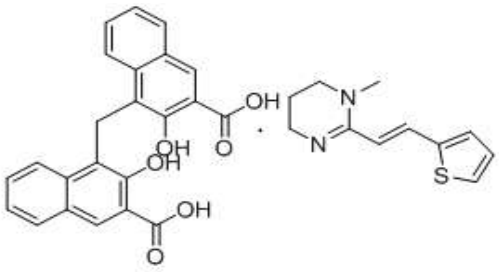

Figure 1. Pyrantel Pamoate

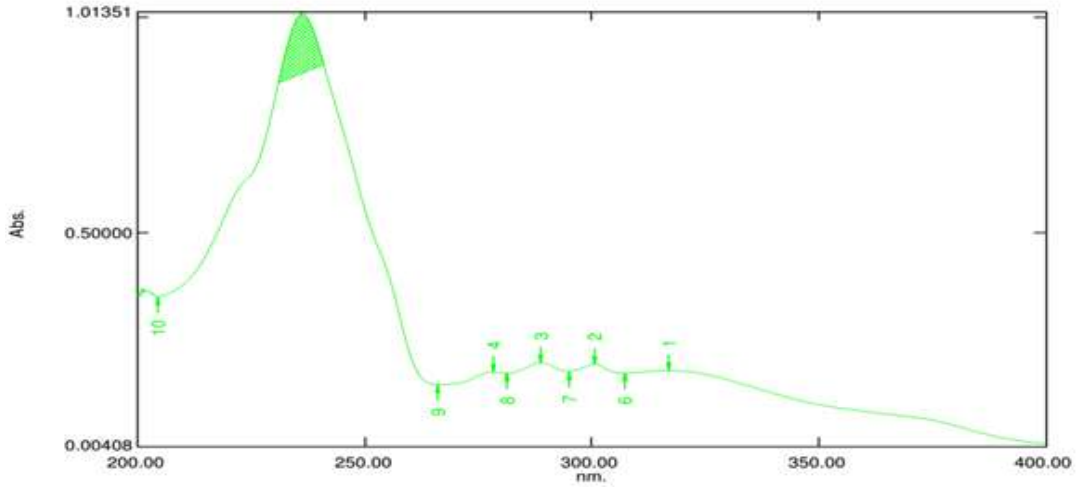

Figure 2. Area Under Curve (AUC)graph of Pyrantel Pamoate

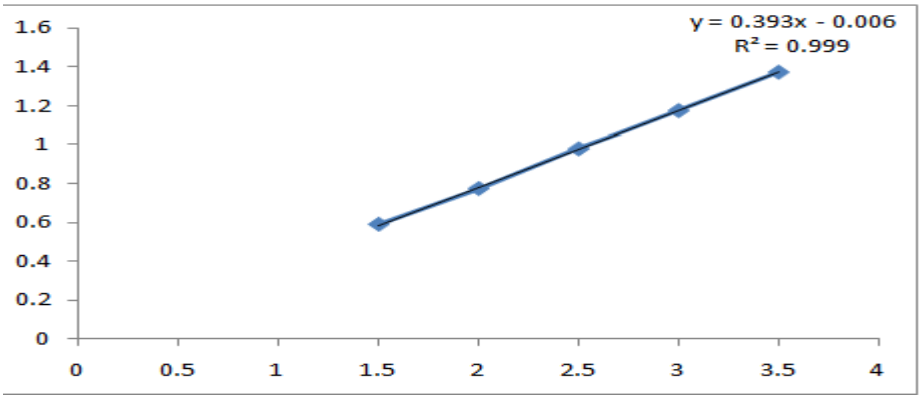

Figure 3.Calibration Curve of Pyrantel Pamoate (1.5-3.5μg/ml)

Table 1. Assay of Tablet Dosage Form.

Sr. No.	Sample solution concentration (µg/ml)	Amount Found (%)	Mean Amount Found (%)	% RSD*
1.	2.5	99.12		
2.	2.5	99.99	99.43±0.4833	0.486
3.	2.5	99.19		

*n=3, % RSD = % Relative Standard Deviation

Table 2. Precision Results for Pyrantel Pamoate.

Drug	Concentration of drug (µg/ml)	% RSD*
Pyrantel Pamoate	2.5	0.5107
Intraday	2.5	0.4841
Interday	2.5	0.6869

n=3*

Table 3. Accuracy Results for Pyrantel Pamoate

Accuracy Level	Amount added (µg/ml)	% Recovery	Mean % Recovery	% RSD*
I (80%)	18	100.01 ± 0.77826		
II (100%)	20	99.76 ± 1.18020	99.94	0.8763
III (120%)	22	100.05 ± 0.66905		

n=3*

Table 4. Summary of Validation Parameters

Parameter	Results
λ max	236
Linearity range	1.5-3.5µg/ml
Regression Equation(y=mx+c)	y= 0.393x-0.006
Correlation Coefficient (R^2)	0.999
Precision (% R.S.D) Repeatability Intraday Interday	 0.5107 0.4841 0.6869
Accuracy (Mean % Recovery)	99.94
LOD	0.017µg/ml
LOQ	0.054 µg/ml