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Development of analytical method for pyrantel embonate using spectrofluorometry

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ABSTRACT

A simple, robust, selective and sensitive spectrofluorometric method has been developed for the determination of pyrantel embonate in bulk and pharmaceutical formulations. The method was based on the scanning of aqueous solution of the drug and formulation. The fluorescence was measured at the excitation of 235 nm and the emission was determined at 520 nm. The method showed high sensitivity with linearity range from 4 to 24 µg/ml. The lower limit of detection (LOD) was found to be 0.66 µg/ml and the limit of quantization (LOQ) was determined as the lowest concentration was found to be 2.2 µg/ml. The variables that affected the reaction were carefully studied and optimized. The proposed method was applied successfully for the determination of pyrantel embonate in bulk and pharmaceutical formulations. The mean percentage recovery is was found to be 101.16±1.057.

Keywords: Pyrantel embonate, Spectrofluorometry, Estimation.

INTRODUCTION

Pyrantel embonate is a yellow colored, crystalline power which is slightly soluble in water. Its chemical name 1, 4, 5, 6-tetrahydro-1-methyl-2-(trans-2-(2-thienyl)-vinyl)-pyrimidine embonic acid. The structure of the compound pyrantel embonate is presented in figure 1. It is broad spectrum anthelmintic. Pyrantel embonate is indicated for use in the horse for the control and treatment of adult infections of large and small strongyles, Pinworms, Roundworms, Tapeworms. There are many pharmaceutical preparations available for pyrantel embonate from different manufacturers. Hence it is needed that an accurate and simple method for its quantitative determination. Białecka, W and Kulik, A (2010) have established a method to estimate the pyrantel embonate HPLC method [1]. Other than HPLC method we were unable to find out any method that has been published so far. Hence the aim of present work was to develop a simple, sensitive, and selective spectrophotometric method using material on solvent for the determination of pyrantel embonate in bulk as well as pharmaceutical formulation.

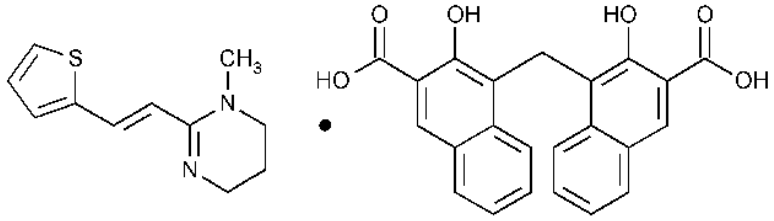

Figure 1: Structure of pyrantel embonate

MATERIALS AND METHODS

2.1. Experimental:

2.1.1. Instrumentation:

The instruments used for the development process are UV-visible spectrophotometer (Systronics 2202), spectrofluorimeter (PerkinElmer LS55), sonicator (Branson 2510) and electronic balance (Mettler Toledo).

2.1.2. Chemicals and Reagents:

Pure pyrantel embonate has been obtained as gift sample from Indthu Remedies, Chennai and was used as such for further analysis. Formulations were purchased from the local pharmacies and used for analysis. Water (double distilled water) and all other chemicals used in the analysis were AR grade.

2.2. Procedure:

2.2.1. Determination of absorption and fluorescence spectra:

Absorption maxima and fluorescence maxima were carried out for the standard solution of 8 µg/ml concentration by scanning from 400-200 nm in UV-Vis spectrophotometer and based on the absorption maxima the emission maxima was determined.

2.2.2. Preparation of stock solution:

100 mg of pure drug pyrantel embonate was weighed and transferred to a 100 ml volumetric flask. 50 ml of distilled water was added to the above flask, mixed well, ensured the complete solubility and the volume was made up with the distilled water.

2.2.3. Preparation of sample solution:

The average weight of the tablets was determined by weighing 20 tablets and these were powdered. Tablet powder equivalent to 10 mg of pyrantel embonate was weighed and transferred to a 100 ml volumetric flask. About 25 ml of distilled water was added and sonicated for 5min for complete dissolution of drug. The volume was made up with distilled water and filtered through Whatman filter paper. Dilutions were made with distilled water to attain a concentration of 8 µg/ml. Six replicate analysis were carried out with sample weighed individually and the average weight of tablet was found to be 0.6248 g.

2.3. Validation:

Method validation [2-5] was performed in terms of linearity and range, precision and accuracy, and stability including LOD and LOQ.

2.3.1. Linearity and range:

Calibration standards of pyrantel embonate, covering the range 2-24 µg/ml were prepared with the suitable dilution made from pyrantel embonate stock solution. The calibration curve was obtained by plotting the intensity of fluorescence against of concentration of pyrantel embonate. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

2.3.2. Precision:

Method validation regarding repeatability was achieved by replicate insertion of extracted sample solution within the linearity range, where intensity of fluorescence was measured in comparison to the intensity of fluorescence of the standard. Intermediate precision study was conducted during routine operation of the system over a period of six consecutive days. Statistical evaluation revealed relative standard deviations at different values of six replicates.

2.3.3. Recovery study:

Accuracy is the percent of analyte recovered by assay from a known added amount of drug. Data from nine determinations over three concentration levels covering the specified range were obtained. The accuracy was determined with standard quality control samples prepared in triplicate at three different concentration levels (80%, 100% and 120%) covering the entire linearity range with the pre-estimated formulation by standard addition method.

2.3.4. LOD and LOQ:

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability In this study, LOD and LOQ [5] were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations.

LOD = 3.3 s/m; LOQ = 10 s/m.

Where S, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs.

2.3.5. Stability:

Problems of stability are usually encountered with these compounds, mainly at ambient temperature. The stability of pyrantel embonate was verified a median concentration which was measured for 24 h in the interval of 1 h and found that the differences are within the limit.

RESULTS AND DISCUSSION

The development of a simple, rapid, sensitive and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labour. Pyrantel embonate is a fluorescence absorbing molecule with specific fluorophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the spectrofluorometric method. It was found that two absorption maximum (λ_{max}) was observed at 235 and 288. Among that 288 nm was used as excitation wavelength, since this wavelength only shows significant fluorescence intensity at 520 nm as emission wavelength. Hence this wavelength was used for the determination of the pyrantel embonate, because at this wavelength the concentration requirement is low. The absorption and fluorescence spectra of pyrantel embonate in distilled water are presented in figure 2 and figure 3.

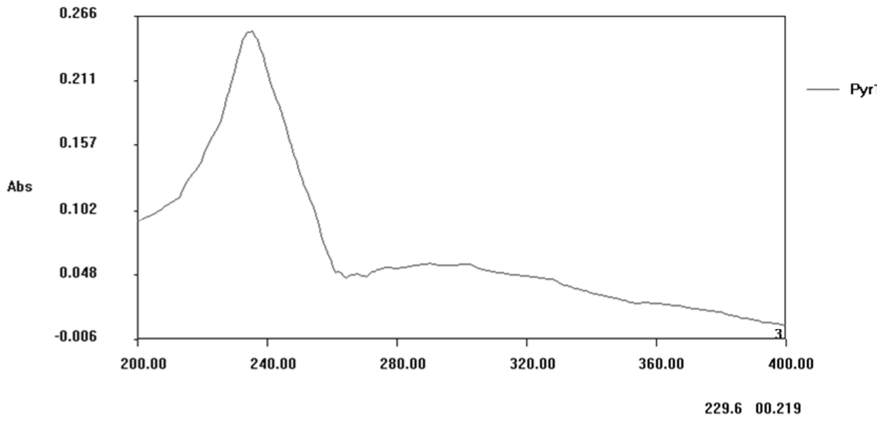

Figure 2: Absorption spectrum of pyrantel embonate

Calibration standards for pyrantel embonate covering the range of 2-24 µg/ml was prepared by the method mentioned above and the serial dilutions were made with distilled water. The calibration curve was obtained by plotting the intensity by fluorescence of the pyrantel embonate versus analyte concentration. The slope and intercept of the calibration was determined by linear regression using the least square method. The data was presented in table 1 and the calibration curve was presented in figure 4. Regression analysis of the calibration curve showed a linear relationship between the intensity of fluorescence of pyrantel embonate and the concentration with correlation co-efficient higher than in all the curves assayed in pure form.

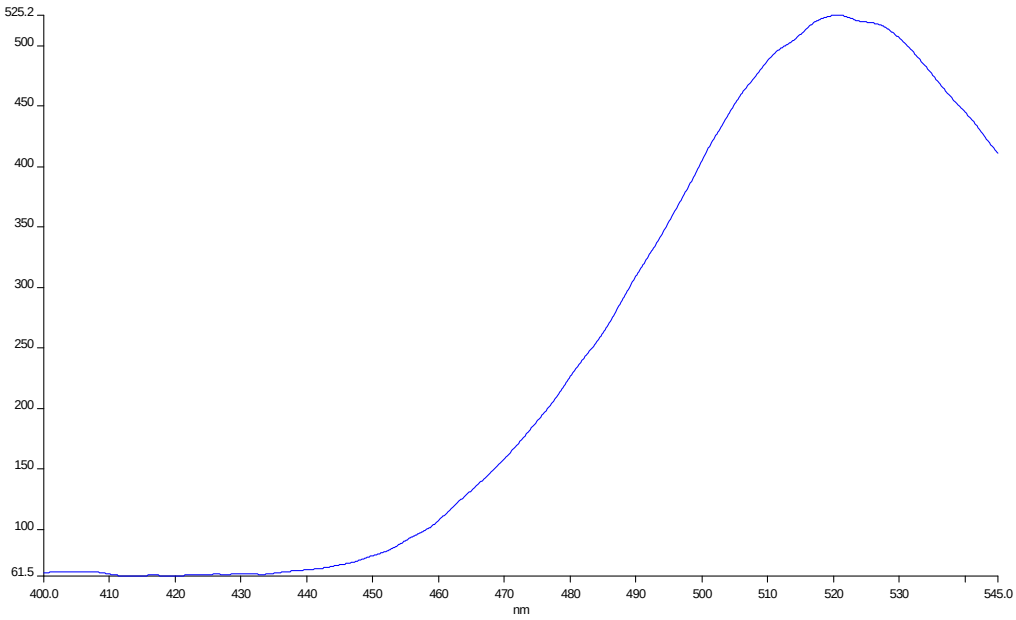

Figure 3: Fluorescence spectrum of pyrantel embonate

Calibration curve data were constructed in the range of the expected concentrations from 2 to 24 µg/ml. Beer's law was obeyed over this concentration range. The regression equation was found to be y = 29.55x. The correlation coefficient (r^2) of the standard curve was found to be greater than 0.99.

Table 1: Linearity profile of pyrantel embonate

Pyrantel embonate concentration (µg/ml)	Intensity of fluorescence
2	69.98
4	127.86
8	250.21
16	475.02
24	700.74

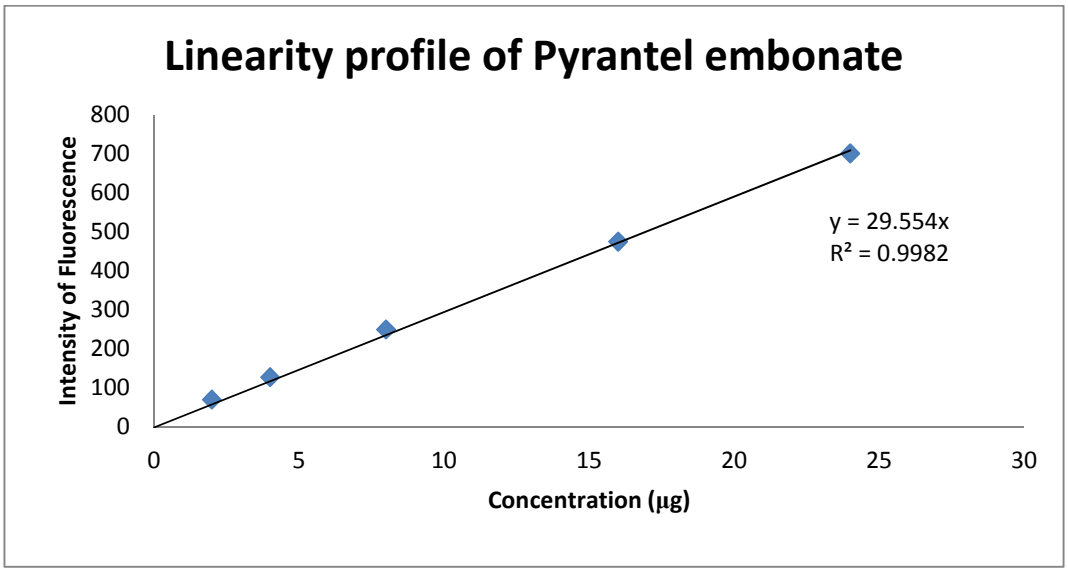

Figure 4: Calibration curve of pyrantel embonate

The precision was carried out as described in method and the results were presented in table 2. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision values hence; the developed method can be used to analyze the pyrantel embonate in tablet formulation. The mean of the precision value is 100.013 %. This value was obtained from 98.675 – 101.263%.

Table 2: Analysis of tablet formulation

S. No	Weight of the sample (g)	Intensity of fluorescence	Drug Content (mg)	Percentage found (%)
1	0.044	101.38	143.256	99.483
2	0.0452	104.95	144.349	100.242
3	0.0451	103.88	143.193	99.439
4	0.0439	100.34	142.093	98.675
5	0.0434	101.51	145.405	100.976
6	0.0437	102.49	144.818	101.263
		Mean	144.019	100.013
		S.D	1.432	0.994

The mean of the three different recovery studies were presented in table 3. In that overall mean of the recovery studies were 101.56 ± 1.057%. The drug pyrantel embonate in formulation was well identified under this condition. There is no interference is observed in different blank samples of pyrantel embonate.

Table 3: Recovery study of pyrantel embonate

S.No	Level added (%)	Pure or drug added (mg)	Mean as recovery
1	80	8	101.38 ±1.44
2	100	10	101.57 ± 1.26
3	120	12	100.49 ± 0.481

The stability study of the solution was carried out as described in method and the values were presented in table 4. From the data it inferred that there is no significant difference while the final sample solution was kept for 24 hrs at ambient temperature

Table 4: Stability of the solution

Time (hrs)	Intensity of fluorescence
0 min	102.28
30 min	102.21
60 min	102.27
90 min	102.28
After 24 hrs	102.20

Figure 4 show that the regression analysis of calibration curve for pyrantel embonate in pure form showed a linear relationship between the intensity of fluorescence and the concentration with correlation. Correlation coefficient was found to be higher than 0.99 in all the curves assayed.

The LOD determined as the amount drug was found to be 0.66 µg/ml and the LOQ was determined as the lowest concentration was found to be 2.2 µg/ml in formulation. The result of the interferences study showed that no interference of any component with the drug has been proved and was found from the recovery of pyrantel embonate was 101.56 ± 1.057. This indicates that the absence of interferences of any component with drug in the analysis.

Ruggedness was performed as described in method by two different analysts, the method could not be repeated in a different laboratory or using different equipment and their results were prescribed in table 5. Robustness was performed as described in method and the results were presented in table 6. From the robustness data different solvents has been proved that there are no significant changes when the drug is analyzed in different wavelength.

Table 5: Ruggedness study

S. No.	Analyst – 2	Analyst – 2
1	118	120
2	113	115
3	128	130

Table 6: Robustness study

S.No.	Solvent	Intensity of fluorescence
1	Qualigens	113
2	Fischer's	114

CONCLUSION

A spectrofluorometric method for quantifying pyrantel embonate in formulation has been developed and validated. The linear range of the proposed spectrofluorometry method was 2- 24 µg/ml. The assay is selective, precise, accurate and linear over the concentration range from 2- 24 µg/ml, the concentration of pyrantel embonate used for the precision study is 8 µg/ml in formulations could be precisely quantified and detected was approximated 2.2 µg/ml and 0.66 µg/ml respectively. Also, the proposed method involved spectrofluorometry measurements with comparable analytical performance devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. Therefore, these methods can be recommended for the routine analysis of pyrantel embonate in quality control and clinical laboratories.

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