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Research Paper

Extraction-Free Spectrophotometric Determination of Pyrantel Pamoate in Pharmaceuticals

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The objective of this work was to develop and validate two direct, simple, highly sensitive, rapid and extraction-free spectrophotometric methods for the determination of Pyrantel pamoate (PYP) in commercial dosage forms. The methods are based on the formation of ion-pair complex between the base form of Pyrantel pamoate (PYL) and two sulphonthalein dyes, namely, phenol red (method A) and thymol blue (method B), followed by the measurement of absorbance at 430 nm and 420 nm respectively. Conformity to Beer's law enabled the assay of drug in the range 0.02-0.5 and 0.05-0.8 μg ml^{-1} with apparent molar absorptivities of 5.11×10^5 and 2.55×10^5 l mol^{-1} cm^{-1} for method A and method B, respectively. The Sandell sensitivity values, limits of detection (LOD) and quantification (LOQ) values have also been reported for both the methods. The stoichiometry of the ion-pair complexes as evaluated by Job's continuous variations method was 1:2 and the conditional stability constants (log K_f) were calculated to be 6.92 and 8.19 for method A and method B, respectively. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis; the relative error (%RE) was ≤ 2.17% and the relative standard deviation (RSD) was < 1.77%. The methods were successfully applied to the determination of drug in tablets without interference by the common co-formulated substances. Statistical comparison of the results with the reference method showed good agreement and indicated that no significant difference in accuracy and precision.

Key Words: Pyrantel Pamoate; Spectrophotometry; Phenol Red; Thymol Blue; Ion-Pair; Dosage Forms

Introduction

Pyrantel pamoate [PYP], chemically known as 1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)ethenyl] pyrimidine has been shown to be very effective against *A. lumricoides,* hookworms, *T. orientalis* and *E. vermicularis* (Rim and Lim, 1972; Rim and Lim, 1973). PYP could be effectively used to control serum mineral levels in children with intestinal parasitic infection (Olivares *et al*., 2003).

The determination of small amounts of PYP in pure form as well as in pharmaceutical preparations is very important for medical and pharmaceutical needs. PYP is the subject of monograph in the USP (The US Pharmacopoeia, 2005) which describes chromatographic methods for bulk drug and its formulations. Literature survey reveals that PYP in swine liver has been assayed by gas chromatography-mass spectrometry (Susan *et al*., 1990). Liquid chromatography [HPLC] has been applied to determine the drug in animal feeds (Thorpe, 1988), swine feeds and supplements (Lowie *et al*., 1983; Goras, 1981), dog plasma (Morovjan *et al*., 1988) and in pharmaceuticals (Oltean, 2011; Allender, 1988; Lucie *et al*., 2012; Guo *et al*., 2004; Raman and Shinde, 1999; Argekar *et al*., 1997; Halkar *et al*., 1997; Allender, 1988). Several other methods such as HPTLC (Anon, 1998; Zarapkar *et al*., 1997), spectrofluorimetry (Refaat *et al*., 1987), UV-spectrophotometry (George *et al*., 1971; Langade *et*

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al., 2010; Prasad *et al*., 1999; Hancu and Gyeresi, 1999), voltammetry and polarography (Rajeev *et al*., 2006; Gupta *et al*., 2010; Tiwari *et al*., 2009) and ISE-potentiometry (Aubeck and Hampp, 1992) are also reported in the literature for the determination of PYP in pharmaceuticals. Many of the above methods would require expensive and sophisticated instruments which are not always available in many laboratories.

Visible spectrophotometry is regarded as the most convenient analytical technique in pharmaceutical analysis due to its inherent simplicity, sensitivity, fair accuracy and precision; and its availability in most quality control and clinical laboratories. The literature on the visible spectrophotometric methods for PYP is, however, scanty. The blue coloured species, formed due to the redox reaction (Basavaiah and Prameela, 2003) between PYP and Folin-Ciocalteau (FC) reagent in alkaline medium, has served as the basis for the assay of drug. Charge-transfer complex (Shingbal and Rao, 1987) resulting from the action of chloranil on PYP in dioxan was used by Shingbal and Rao for the quantification of the drug in pure form as well as in tablets. Condensation of malonic acid with acetic anhydride in the presence of PYP resulted in a coloured product (Refaat *et al*., 1987), allowing the spectrophotometric determination of PYP. Lakshmi and Reddy (Lakshmi and Reddy, 1998a) have proposed four methods based on the formation of C-T complex between PYP and chloranilic acid measurable at 540 nm (method A). Method B involved the reduction of F-C reagent by the drug in alkaline medium followed by the measurement of the blue species at 760 nm. In the other two methods (C&D) PYP was reacted with a known excess of NBS (method C) or $KMnO_4$ (method D) and the residual oxidant was reacted with celestein blue or cresyl fast violet acetate and the resultant unbleached dye colour was measured at 545 or 600 nm. The same authors (Lakshmi and Reddy, 1998b) have also determined the drug by three other methods based on the formation of chloroform-soluble ion-pair between PYP and three dyes, Wool Fast Blue, Supracen Violet 3B and Azocarmine G followed by the measurement of the extracted ion pairs at 600, 590 and 540 nm, respectively. Some of these methods suffer from one or the other disadvantage such as poor sensitivity (Shingbal and Rao, 1987), use of less stable oxidants and multi-reagent systems (Lakshmi and Reddy, 1998a) or measurement at shorter wavelength of 333 nm (Refaat *et al*., 1987). The extractive spectrophotometric procedures (Lakshmi and Reddy, 1998b) too are not free from limitations. These are tedious, labour-intensive, time-consuming and highly prone to loss of analyte. These also require large amounts of high purity solvents, which are often hazardous and result in the production of toxic laboratory waste.

In recent years, as a highly potent alternative to ion-pair extractive spectrophotometry, extraction-free spectrophotometry based on ion-pair formation between basic drugs and dyes has emerged in the field of pharmaceutical analysis (Al-Ghanam, 2006; Manjunatha *et al*., 2008; Abdine *et al*., 2002; Kovacs-Hadady and Fabian, 1998; Ramesh *et al*., 2010; El-Kerdawy *et al*., 1993; Abdine, 2000; Safwan *et al*., 2006; Rajendraprasad *et al*., 2010). These papers describe two simple, rapid, inexpensive and validated spectrophotometric methods for the determination of PYP based on the formation of dichloromethane soluble ion-pairs, formed between PYP and two dyes, thymol blue and phenol red which were measured without involving extraction step. The reaction conditions were thoroughly studied; and under optimal conditions, these methods produced a highly sensitive and selective tool for the determination of drug in bulk form and in commercial dosage form.

Experimental

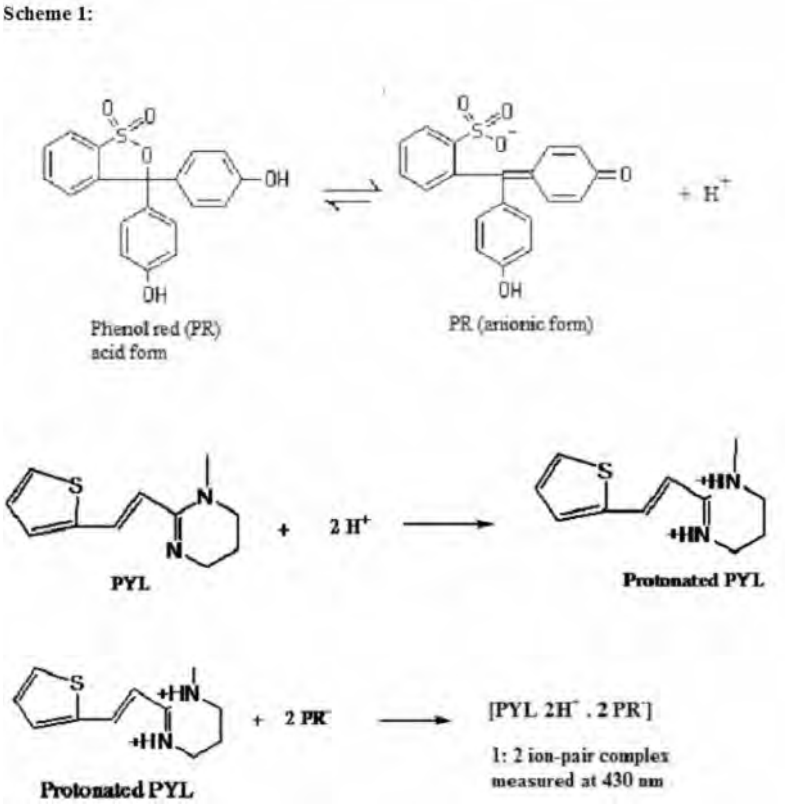
Apparatus

All absorbance measurements and spectral runs were made on a Systronics model 166 (Ahmedabad, India) digital spectrophotometer with 1 cm path length quartz cells.

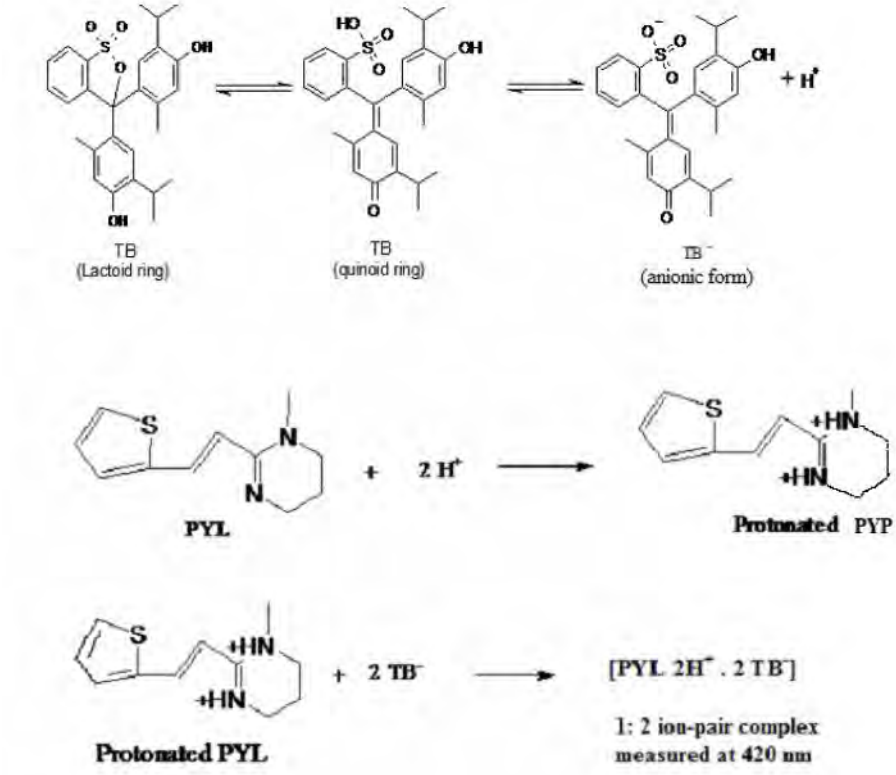
Materials and Methods

All reagents used were of analytical reagent grade while HPLC grade organic solvents were used. Chloroform and acetone were (spectroscopic grade,

Scheme 1:



Scheme 2:



Sp. gr. 1.32) purchased from Merck Mumbai, India.

Pharmaceutical grade PYP (99% purity) was received as a gift from IPCA, Pharmaceutical Company, Ratlam, India. Nemocid tablets and Nemocid oral suspension (IPCA Laboratories Ltd., Ratlam, India) were purchased from local commercial sources.

Phenol red (PR) and Thymol blue (TB) [both 0.01%]- was prepared by dissolving 0.01 g each of Phenol red (B.D.H. Ltd. Poole England) and thymol blue (Thomas Baker, Mumbai, India) in 100 ml acetone.

Standard Drug Solution

A stock standard solution containing 100 µg ml^{-1} PYL was prepared as follows: pure Pyrantel pamoate (28.86 mg) was first dissolved in 10 ml Di-methylformamide (DMF) in a 125 ml separating funnel, 5 ml of 1 M NaOH was then added followed by 20×4 ml of chloroform. The resulting content was shaken for 15 minutes. The lower organic layer was collected in a beaker containing anhydrous Sodium sulphate. The water-free organic layer was transferred into a 100 ml calibrated flask and diluted with the same solvent to get 100 µg ml^{-1} with respect to PYL (pamoate free pyrantel). This solution was further diluted stepwise with chloroform to get working concentration of 1 µg ml^{-1} for both the methods.

Recommended Procedures

Method A

Into a series of 5 ml calibration flasks, aliquots (0.1-2.5 ml) of 1.0 µg ml^{-1} PYL standard solution equivalent to 0.02-0.5 µg ml^{-1} PYL were accurately transferred. To each flask 1.0 ml of 0.01% phenol red (PR) was added and the mixture was diluted to 5 ml with chloroform. After 5 minutes, the absorbance of the yellow colored ion-pair complex was measured at 430 nm against the reference blank similarly prepared.

Method B

Different aliquots (0.25-4.0 ml) of a standard PYL solution (1.0 µg ml^{-1}) were transferred into a series of 5 ml calibrated flasks using a microburette and the total volume was adjusted to 4.0 ml with chloroform followed by the addition of 1.0 ml of 0.01% TB solution. After 5 min, the absorbance of the resulting yellow colored chromogen was measured against the reagent blank at 420 nm.

Standard graph was prepared by plotting the absorbance *vs* drug concentration, and the concentration of the unknown was computed from the respective regression equation derived using Beer's law data.

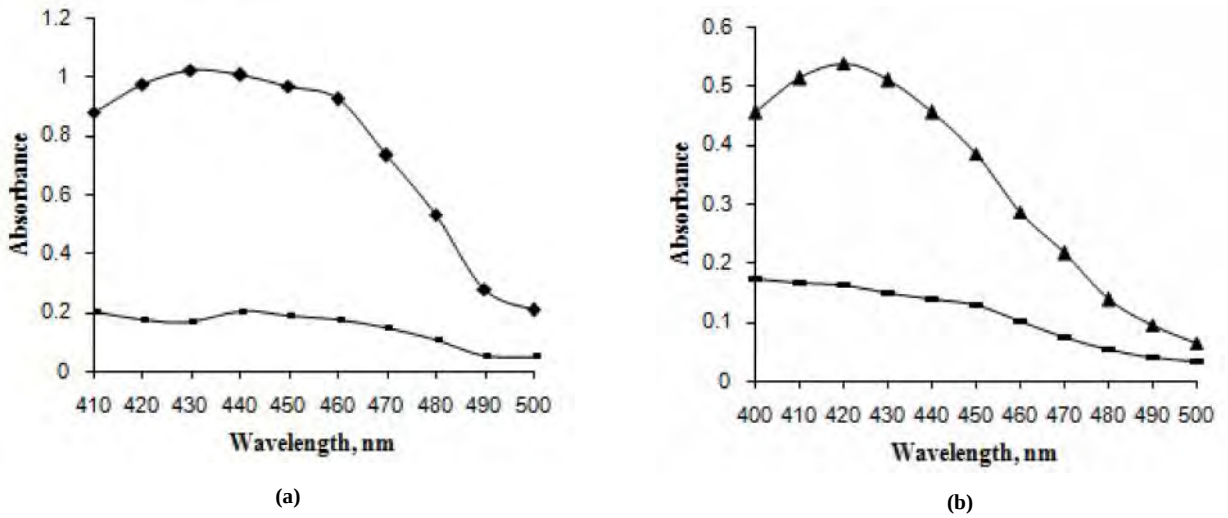


Fig. 1: Absorption spectra of: PYL-PR ion-pair complex (-■-), PYL-TB ion-pair complex (-▲-), (0.6 µg ml^{-1} PYL each), and respective blanks (-o- and -Δ-)

Procedure for Commercial Dosage Forms

Tablets : Twenty tablets were weighed and ground into a fine powder. A portion of the powder equivalent to 28.86 mg of PYP was accurately weighed and transferred into 125 ml separating funnel. The procedure of conversion of PYP to PYL base was followed as done for pure PYL. The resulting solution of 100 µg ml^{-1} PYL was diluted stepwise to get 1.0 µg ml^{-1}, and an appropriate aliquot was subjected to analysis by using the procedures described above.

Suspension : The contents of five 10 ml bottle syrups was pooled and appropriate quantity of suspension equivalent to 100 µg ml^{-1} PYL was measured out into a 125 ml separating funnel. The procedure for conversion of PYP to PYL base was followed as was done for pure PYL. The resulting solution of 100 µg ml^{-1} PYL was diluted stepwise to get 1.0 µg ml^{-1} and an appropriate aliquot was subjected to analysis by using the procedures described above.

Results and Discussion

Absorption Spectra

Chemically, the structure of PYL features its basic nature. This structure suggests the possibility of utilizing an anionic dye as chromogenic reagent. In chloroform, PYL is not an absorbing species in the visible region. The dye employed has insignificant absorbance (Fig. 1). In contrast, when a solution of PR/TB in acetone is added to the drug solution, an intense yellow colored product is formed immediately. This is due to an opening of lactoid ring and subsequent formation of quinoid ring (Safwan *et al*., 2006). It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally, protonated PYL forms ion-pair with the anionic dye. The possible reaction pathways are shown in scheme 1 and scheme 2. Each drug-dye ion-pair complex molecule, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction (Rajendraprasad *et al*., 2010).

Optimization Conditions

Choice of Solvent to Dissolve Dyes

Phenol red and thymol blue are incompletely soluble in chloroform hence, acetone was used to dissolve dyes to achieve complete solubility.

The experimental conditions were studied separately by measuring the absorbance of the final solution resulting from the reaction mixtures containing a fixed concentration of PYL and various amounts of the dyes. It was found that 1.0 ml of 0.01% dye solution was sufficient to produce maximum and reproducible absorbance (Fig. 2) at the respective wavelength in both the methods. The reaction time

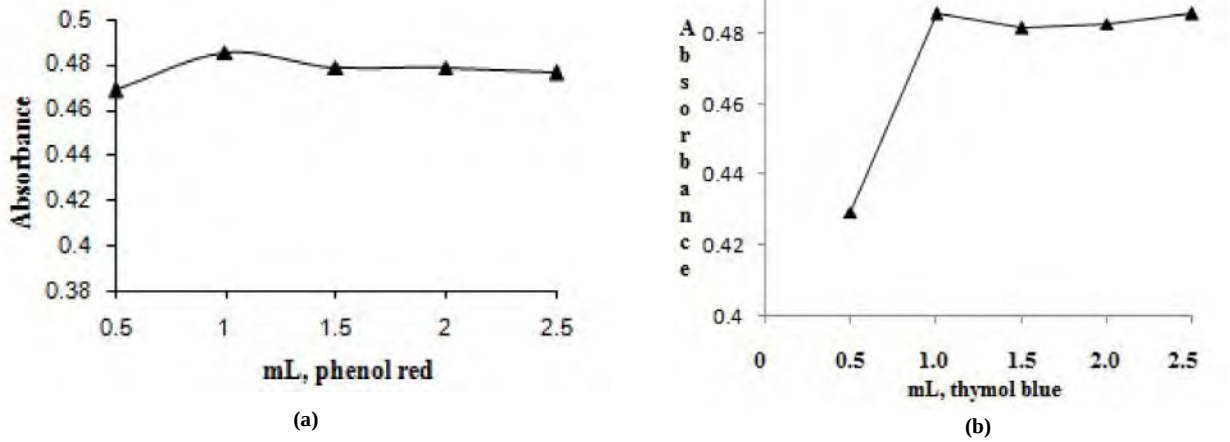


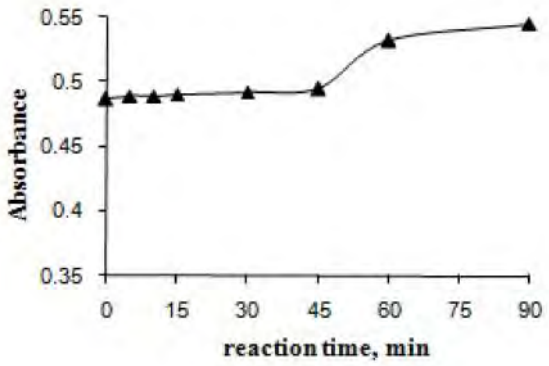
Fig. 2: Effect of reagent volume: (a) 0.01% Phenol red (0.2 µg ml^{-1} PYL) in method A and (b) 0.01% Thymol blue (0.4 µg ml^{-1} PYL) in method B

or standing time after the addition of dye was also examined; it was found that the formation of ion-pair complex was instantaneous and the complex was observed to be stable for 45 minutes in method A and 90 min in method B at room temperature as shown in Fig. 3.

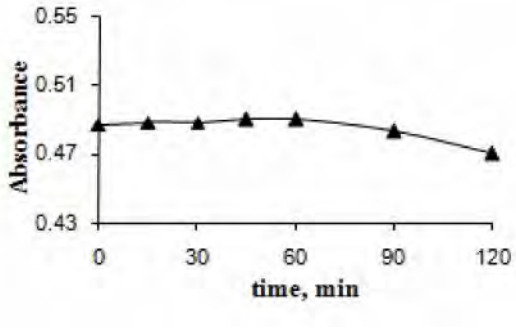
Investigation of Composition of Ion-Pair Complex

The composition of the ion-pair complex was established by Job's method of continuous variations using equimolar concentrations of the drug and the dye (9.72×10^{-5} M in method A and 7.25×10^{-5} M in method B). The results (Fig. 4) indicated that 1:2 (drug:dye) ion-pair is formed through an electrostatic attraction between the positive protonated drug and the anion of dye. Six solutions containing PYL and the dye in various molar ratios, with a total volume of 3.0 ml were prepared. The volume was made up to 5 ml using chloroform. The absorbance of all solutions was subsequently measured at respective wavelength. The resulting graph in both the methods indicate the formation of a 1:2 PYL:PR/TB complex. The conditional stability constant (K_f) of the ion-association complex was calculated from the continuous variation data using the following equation (Erk, 2003):

$$K_f = \frac{A/A_m}{[1-A/A_m]^{n+2} C_M (n)^n}$$

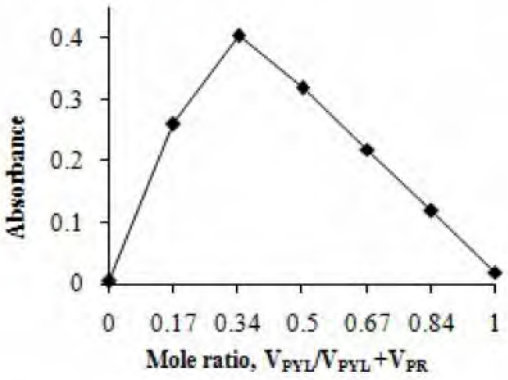


(a)

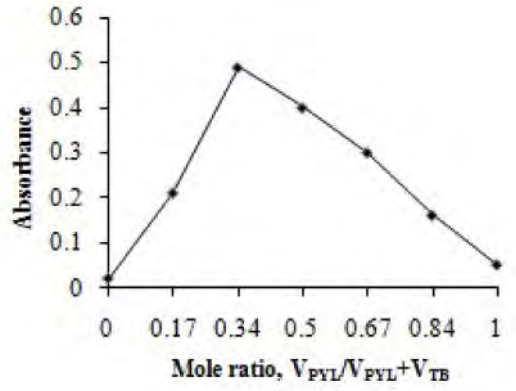


(b)

Fig. 3: Effect of reaction time, and stability of coloured species: (a) 0.2 µg ml^{-1} PYL in method A and (b) 0.4 µg ml^{-1} PYL in method B



(a)



(b)

Fig. 4: Job's continuous variations plot: (a) PYL-PR ion-pair complex (V_{PYL} and V_{PR} are volume of PYL and PR (both 9.72×10^{-5} M) respectively and (b) PYL-TB ion-pair complex (V_{PYL} and V_{TB} are volume of PYL and TB (both 7.25×10^{-5} M) respectively

where A is the observed absorbance and Am is the maximum absorbance value when all the drug present is associated, respectively. C_M is the molar concentration of drug at the maximum absorbance and n is the stoichiometry which PR/TB ion associates with drug. The log K_f values were found to be 6.32 and 7.22 for method A and method B, respectively.

Validation Protocol

Linearity, Sensitivity, Limits of Detection and Quantification

A linear correlation was found between absorbance at λ_{max} and concentration of PYL in the ranges given in Table 1. The graphs are described by the regression equation:

$$Y = a + bX$$

(where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in µg ml^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values (Zavis *et al*., 1976) of both methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines (*ICH* guidelines, 2005) using the formulae:

$$LOD = 3.3\ S/b \text{ and } LOQ = 10\ S/b,$$

(where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot) are also presented in Table 1. The high values of molar absorptivity (ε) and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

Precision and Accuracy

The assays described under "general procedures" were repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the methods. These assays were performed for three levels of analyte.

Table 1: Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	430	420
Colour stability, min	45	90
. . . . . $^{-1}$	0.02–0.5	0.05–0.80
Molar absorptivity (ε), l mol^{-1} cm^{-1}	5.11×10^5	2.55×10^5
Sandell sensitivity*, ng cm^{-2}	0.4	0.8
Limit of detection (LOD), µg ml^{-1}	0.01	0.02
Limit of quantification (LOQ), µg ml^{-1}	0.03	0.05
Regression equation, Y**		
Intercept (a)	0.0304	-0.0001
Slope (b)	2.116	1.225
Standard deviation of a (S_a)	9.98×10^{-2}	9.98×10^{-2}
Standard deviation of b (S_b)	0.2383	0.2674
Regression coefficient (r)	0.9991	0.9995

*Limit of determination as the weight in µg ml^{-1} of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.

**Y=a+bX, Where Y is the absorbance, X is concentration in µg mL^{-1}, a is intercept and b is slope.

The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤ 1.72% (intra-day) and ≤ 1.77% (inter-day) indicating high precision of the methods. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and taken concentrations for PYL. Bias {bias% = [(Concentration found - known concentration) × 100/ known concentration]} was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ≤ 2.17% demonstrate the high accuracy of the proposed methods.

Selectivity

A systematic study was performed to determine the effect of additives by analyzing the placebo blank and synthetic mixture containing PYL. A placebo blank of the composition: starch (10 mg), acacia (15

mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under the heading Procedure and sub-heading 'Tablets', and then subjected to analysis. The absorbance of the placebo solution in each case was almost equal to the absorbance of the blank which revealed no interference.

To assess the role of the inactive ingredients on the assay of PYL, a synthetic mixture was separately prepared by adding 10 mg of PYL to the 10 mg placebo mentioned above. The drug was extracted and solution was prepared as described under the general procedure for tablets. The solutions after appropriate dilution were analyzed following the recommended procedures. The absorbance resulting from 0.3 µg ml^{-1} (in both methods) was nearly the same as those obtained for pure PYL solutions of identical concentrations. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of PYL. Further, the slopes of the calibration plots prepared from the synthetic mixture solutions were about the same as those prepared from pure drug solutions.

Robustness and Ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of dye and the effect of the changes was studied on the absorbance of the colored systems. The changes had negligible influence on the results as revealed (in Table 3) by small intermediate precision values expressed as %RSD (≤ 1.92%). Methods' ruggedness was demonstrated having the analysis done by four analysts, and also by a single analyst performing analysis on four different instruments in the same laboratory. Intermediate precision values (%RSD) in both instances were in the range 0.75-1.99% indicating acceptable ruggedness. The results are presented in Table 3.

Application

The proposed methods were applied for the quantification of PYL in commercial tablets. The results were compared with those obtained by the official method (The US Pharmacopoeia, 2005), which uses HPLC with a UV detector contains packing L3 (4.6-mm and 25-cm) column. Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and reference method with respect to accuracy and precision as revealed by the Student's *t*-value and variance ratio *F*-value. The results of assay are given in Table 4.

Recovery Study

To further assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. The recovery was

Table 2: Evaluation of intra-day and inter-day accuracy and precision

Method	PYP taken (µg ml^{-1})	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=7)		
		PYP found[a] (µg ml^{-1})	RSD[b] %	RE[c] %	PYP found (µg ml^{-1})	RSD[b] %	RE[c] %
	0.1	0.101	1.72	1.00	0.102	1.53	2.00
A	0.2	0.202	1.47	1.27	0.203	1.77	1.50
	0.3	0.297	1.39	1.11	0.296	1.29	1.16
	0.2	0.197	1.36	1.49	0.204	1.45	1.96
B	0.4	0.395	0.92	1.25	0.406	1.59	1.51
	0.6	0.588	1.15	2.00	0.587	1.32	2.17

[a]Mean value of 7 determinations; [b]Relative standard deviation (%); [c]Relative error (%)

Table 3: Method robustness and ruggedness expressed as intermediate precision

Method	Method Robustness Parameters altered			Method Ruggedness	
	PYL taken (µg ml^{-1})	*Volume of PR %RSD (n=3)	*Volume of TB %RSD (n=3)	Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
A	0.1	1.25	-	1.13	1.02
	0.2	1.92	-	1.63	0.75
	0.3	1.72	-	1.07	1.91
B	0.2	-	1.27	1.45	1.99
	0.4	-	1.74	1.97	1.17
	0.6	-	1.55	1.06	1.35

*In both methods, the volumes of reagent were 1±0.1 ml

Table 4: Results of analysis of formulations by the proposed methods

Formulation analyzed	Found* (Percent of label claim±SD)			
	Label claim	Official method	Proposed methods Method A	Method B
Nemocid tablets	250 mg/ tablet	101.5±1.07	102.5±1.45 *t* = 1.24 *F* = 1.84	99.2±1.63 *t* = 2.64 *F* = 2.32
Nemocid Suspension	250 mg/ 5 mL	102.3±1.21	102.9±1.36 *t* = 0.74 *F* = 1.26	101.3±1.25 *t* = 1.29 *F* = 1.07

*Mean value of five determinations; Tabulated *t*-value at the 95% confidence level is 2.77; Tabulated *F*-value at the 95% confidence level is 6.39

assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure PYL at three different levels (50, 100 and 150%) of the content present in the tablet powder (taken) and the total was found by the proposed methods. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 98.7 and 102.6%

Table 5: Results of recovery study *via* standard addition method with tablet

Method	Formulation studied	PYP in tablet µg ml^{-1}	Pure PYP added µg ml^{-1}	Total found µg ml^{-1}	Pure PYP recovered* percent ±SD
A	Nemocid tablets	0.102	0.05	0.154	101.5±1.12
		0.102	0.10	0.201	99.2±1.29
		0.102	0.15	0.254	100.9±1.05
	Nemocid suspension	0.099	0.05	0.153	102.4±1.05
		0.099	0.10	0.201	100.9±1.29
		0.099	0.15	0.253	101.7±1.02
B	Nemocid tablets	0.206	0.1	0.303	99.1±1.05
		0.206	0.2	0.401	98.7±1.49
		0.206	0.3	0.513	101.3±1.07
	Nemocid suspension	0.203	0.1	0.309	101.9±1.15
		0.203	0.2	0.414	102.6±0.97
		0.203	0.3	0.511	101.5±1.27

*Mean value of three determinations

with standard deviation in the range 0.97-1.49%. Closeness of the results to 100% showed the fairly good accuracy of the methods. The results are shown in Table 5.

Conclusions

Two spectrophotometric methods for the determination of Pyrantel in bulk drug and in pharmaceutical dosage forms were developed and validated for accuracy, precision, linearity, robustness and ruggedness. The methods employ normal conditions and rely on well-characterized ion-pair formation reactions. Besides, the methods have the advantages of simplicity without involving heating or extraction step and high sensitivity (Table 6). The proposed methods are less time consuming, simple and in addition, offer advantages in determining PYL, (in pharmaceutical preparations), when extraction difficulties arise with other spectrophotometric methods. Hence, the proposed methods could be adopted for quality control in pharmaceutical industries.

Table 6: Comparison of the performance characteristics of the present methods with the published methods

S.No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (µg ml^{-1}) ε (l mol^{-1}cm^{-1})	Remarks	Ref
1	Malonic acid-acetic anhydride	Condensation product measured	415 455	-	Requires heating	19
2	FC reagent/ Na_2CO_3	Molybdenum/Tungsten blue chromogen measured	760	2.5-25 1.45×10^4	Reaction slow	28
3	Chloranil	Charge-transfer complex radical anion measured in dioxane	560	25-400	Less sensitive	29
4	a) *p-CAA	Charge transfer complex radical anion measured	540	12.5-62.5 0.43×10^4	Less sensitive	30
	b) FC reagent	Blue colored chromogen measured	760	2.0-10.0 3.55×10^4	Slow reaction	
	c) NBS - celestein blue	Absorbance of residual dye measured	540	1.0-10.0 4.91×10^4	Use of unstable oxidants, multi-reagents & multi-step reactions	
	d) $KMnO_4$- cresyl fast violet acetate	Absorbance of residual dye measured	600	1.0-10.0 4.91×10^4		
5	Wool fast blue BL, Supracen violet 3B, Azocarmine G	Measurement of absorbance of ion-pair complex	600 590 540	-	Involves tedious extraction step, rigid pH control and use of large quantity of organic solvent	31
6	a) Phenol red	Measurement of absorbance of ion-pair complex	430	0.02-0.5	Extraction-free, highly sensitive, wide linear dynamic range, no pH control	Present work
	b) Thymol blue		420	5.11×10^5 0.05-0.8 2.55×10^5		

*FC- Folin-Ciocalteau reagent, *p*-CAA- chloranilic acid, NBS-N-bromosuccinide, $KMnO_4$-potassium permanganate

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References

Abdine H H (2000) Spectrophotometric determination of cisapride using some sulfonphthalein dyes *Alex J Pharm Sci* **14** 75-78

Abdine H, Belal F and Zoman N (2002) Simple spectrophotometric determination of cinnarizine in its dosage forms *IL Farmaco* **57** 267-271

Al-Ghanam S M (2006) A simple spectrophotometric method for the determination of β-blockers in dosage forms *J Pharm Biomed Anal* **40** 151-156

Allender W J (1988) High-performance liquid chromatographic determination of oxantel and pyrantel pamoate *Oxford Journals* **26** 470-472

Allender W J (1988) High-performance liquid chromatographic determination of oxantel and pyrantel pamoate *J Chrom Sci* **26** 470-472

Anon (1998) Simultaneous determination of pyrantel pamoate and mebendazole in tablets by high performance thin layer chromatography *Indian Drugs* **35** 49-51

Argekar A P, Raj S V and Kapadia S U (1997) Simultaneous determination of mebendazole and pyrantel pamoate in tablets by high-performance liquid chromatography-reverse phase (RP-HPLC) *Talanta* **44** 1959-1965

Aubeck R and Hampp N (1992) Ion-selective membrane electrodes for the determination of pyrantel with low protein interference *Anal Chim Acta* **256** 257-262

Basavaiah K and Prameela H C (2003) Assay of pyrantel in pharmaceutical formulations by extraction spectrophotometry *Chem Anal (Warsaw)* **48** 327-334

El-Kerdawy M M, Moustafa M A, El-Ashry S M and El-Waseef D R (1993) Spectrophotometric determination of certain phenothiazines and their dosage forms using bromophenol blue *Anal Lett* **26** 1669-1680

Erk N (2003) Extractive spectrophotometric methods for the novel antidepressant drug in bulk and pharmaceutical dosage forms by using bromothymol blue and bromocresol green *Anal Lett* **36** 1183-1196

George A F, Robert F M and Richard L W (1971) Spectrophotometric determination of pyrantel in pyrantel pamoate bulk samples and pharmaceutical formulations *J Pharm Sci* **60** 111-113

Goras J T (1981) High performance liquid chromatographic method for pyrantel tartrate in swine feeds and supplements *J Ass Off Anal Chem* **64** 1291-1296

Guo Z, Xu Y and Ying Y (2004) Determination of compound albendazole tablet by HPLC *Lei Huaxi Yaoxue Zazhi* **19** 462-463

Gupta V K, Jain R, Jadon N and Radhapyari K (2010) Adsorption of pyrantel pamoate on mercury from aqueous solution studies by stripping voltammetry *J Coll and Inter Sci* **350** 330-335

Halkar U P, Rane S H and Bhandari N P (1997) Reverse phase high-performance liquid chromatographic determination of pyrantel pamoate and mebendazole in tablets *Indian Drugs* **34** 194-196

Hancu G and Gyeresi A (1999) Chromatographic and UV-spectrophotometric assay of some anthelmintic drugs *Farmacia (Bucherest)*, **47** 11-19

International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London

Kovacs-Hadady K and Fabian I (1998) The determination of benzalkonium chloride in eye-drops by difference spectrophotometry *J Pharm Biomed Anal* **16** 733-740

Lakshmi C S R and Reddy M N (1998) Assay of pyrantel in pharmaceutical formulations by extraction spectrophotometry *East Pharm* **41** 127-128

Lakshmi C S R and Reddy M N (1998) Spectrophotometric estimation of pyrantel in pharmaceutical formulations *Ind J Pharm Sci* **60** 302-304

Langade A S, Charde R M, Charde M S and Tajne M R (2010) Spectrophotometric estimation of pyrantel pamoate by 2 wavelength method *J Pharm Res* **3** 212-215

Lowie D M, Teague T R, Quick E F and Foster C L (1983) High-pressure liquid chromatographic determination of carbadox and pyrantel tartrate in swine feed and supplements *J Ass Off Anal Chem* **66** 602-605

Lucie H, Ivana B, Dalibor S, Ludmila M, Alena L, Zdenek O and Petr S (2012) Optimization of an HPLC method for the simultaneous determination of pyrantel pamoate, praziquantel, fenbendazole, oxfendazole and butylhydroxyanisole using a phenyl stationary phase *Anal Methods* **4** 1592-1597

Manjunatha D H, Shaikh S M T, Harikrishna K, Sudhirkumar R, Kandagal P B and Seetharamappa J (2008) Simple and sensitive spectrophotometric methods for the determination of acebutolol hydrochloride in bulk sample and pharmaceutical preparations *Eclet Quim* **33** 37-40

Morovjan G, Csokan P, Makranszki L, Abdellah-Nagy E and Toth K (1988) Determination of fenbendazole, praziquantel and pyrantel pamoate in dog plasma by high-performance liquid chromatography *J Chrom A* **797** 237-244

Olivares J L, Fernandez R, Heta J, Rodrignez G and Clavel A (2003) Serum mineral levels in children with intestinal parasitic infection *Dig Dis* **21** 258-261

Oltean E G (2011) Development and validation of a RP- HPLC method for the quantitation studies of praziquantel and pyrantel pamoate *Medicament Veter/Veter Drug* **5** 64-68

Prasad P B N, Rao A C S, Mathur S C, Kumar Y and Talwar S K (1999) Simultaneous spectrophotometric determination of mebendazole and pyrantel pamoate in pharmaceutical dosage forms *Indian Drugs* **36** 403-407

Rajeev J, Nimisha J and Radhapyari K (2006) Determination of antihelminthic drug pyrantel pamoate in bulk and pharmaceutical formulations using electro-analytical methods *Talanta* **70** 383-386

Rajendraprasad N, Basavaiah K and Vinay K B (2010) Sensitive and selective extraction-free spectrophotometric determination of quetiapine fumarate in pharmaceuticals using two sulfonphthalein dyes *J Preclin Clin Res* **4** 24-31

Raman R and Shinde V M (1999) Simultaneous determination of pyrantel pamoate and mebendazole in tablets by reverse phase HPLC *Indian Drugs* **36** 167-172

Ramesh P J, Basavaiah K, Devi O Z and Vinay K B (2010) Simple and sensitive spectrophotometric assay of ofloxacin in pharmaceuticals based on ion-pair reaction *Chem Ind Chem Eng Quart* **16** 353-366

Refaat I H, El-Kommos M E, Farag H H and El-Rabat N A (1987) Spectrofluorometric and spectrophotometric determination of some tertiary amine drugs *Bull Pharm Sci* **10** 85-102

Rim H J and Lim J K (1972) Treatment of enterobiasis and ascariasis with combantrin (pyrantel pamoate) *Trans Roy Soc Trop Med Hyg* **66** 170-175

Rim H J and Lim J K (1973) Anthelmintic effect of oxantel pamoate and pyrantel pamoate suspension *Asian J Med* **9** 393-396

Safwan A, Fawaz C M and Roula B (2006) Spectrophotometric determination of alfuzosin HCl in pharmaceutical formulations with some sulfonphthalein dyes *Intl J Biomed Sci* **2** 273-278

Shingbal D M and Rao V R (1987) A simple colorimetric method for the determination of pyrantel pamoate in pharmaceutical preparations *Indian Drugs* **25** 22-24

Susan T S C, Nancy C, Charlie B J and Philip K (1990) Determination of pyrantel in swine liver by flame ionization gas chromatography and confirmation by gas chromatography/mass spectrometry *J Ass Off Anal Chem* **73** 883-886

The US Pharmacopoeia (2005) The National Formularly, USP 24. NF 19, USP Convention Inc. 12601, Twinbrook Parkway, Rockville, MD, p.1678

Thorpe V A (1988) Collaborative study: high-pressure liquid chromatographic determination of carbadox and pyrantel tartrate in animal feeds *J Chrom Sci* **26** 545-550

Tiwari D C, Jain R and Sahu G (2009) Voltammetric behaviour of pyrantel pamoate at a composite polymer membrane electrode *J Ind Chem Soc* **10** 1047-1050

Zarapkar S S, Kolte S S and Rane S H (1997) Simultaneous determination of pyrantel pamoate and mebendazole in tablets by high performance thin layer chromatography *Indian Drugs* **34** 707-709

Zavis H, Ludvik D, Milan K, Ladislaw S and Frantisck V (1976) Handbook of Organic Reagents in Inorganic Analysis, Translated by Stanislav, K, Dr. Chalmers The Series and Translation Editor: University of Aberdem, Ellis Horwood Limited, Chichester, A Division of John Wiley & Sons IC, New York, London, Sydney, Toronto, p.364.