Journal of Reports in Pharmaceutical Sciences 2015, 4(2), 143-154

Assay of Pyrantel Pamoate Using Iron (III) Chloride and Three Complexing Agents by Spectrophotometry

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ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2014-12-23
Revised: 2015-02-02
Accepted: 2015-02-10
ePublished:2015-03-02

***Keywords*:**
Assay
Ferricyanide
Pyrantel pamoate
Spectrophotometry
1, 10-phenanthroline
2, 2'-bipyridyl

A B S T R A C T

Three rapid, simple, moderately sensitive and selective spectrophotometric methods are described for the determination of pyrantel pamoate (PYP) in bulk drug and in its pharmaceutical formulations. The methods are based on the reduction of ferric chloride by PYP in neutral medium and subsequent complexation of iron (II) with ferricyanide (method A), 1, 10-phenanthroline (method B) and 2, 2'-bipyridyl (method C). The absorbances of resulting colored products were measured at 750, 520 and 530 nm, respectively. Under the optimum conditions, Beer's law enabled the determination of the drug in the concentration ranges 3.0-35, 1.0-30 and 2.0-35 μg mL^{-1} with apparent molar absorptivities of 1.38×10^4, 2.06×10^4 and 1.23×10^4 L mol^{-1} cm^{-1} for method A, method B and method C, respectively. The Sandell sensitivity values, limits of detection (LOD) and quantification (LOQ) values have also been reported for all methods. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis; the relative error (%RE) was ≤ 3.0 % and the relative standard deviation (RSD) was ≤ 1.98 %. The developed methods were successfully applied to the determination of drug in tablets and suspension without interference by the common excipients.

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Introduction

Pyrantel pamoate (PYP), chemically known as [1, 4,5,6-tetrahydro-1-methyl- 2-[2-(2 thienyl) ethenyl] pyrimidine], is an anthelmintic drug [1] and has been effectively used to kill hookworms and control serum mineral levels in children with intestinal parasitic infection [2,3]. PYP is listed in official United States Pharmacopoeia (USP) monograph [4], which describes a chromatographic technique for the assay of PYP in bulk and formulations.

Literature survey reveals that several techniques have been reported for the assay of pyrantel pamoate in pharmaceutical formulations, and include liquid chromatography (LC) [5], high performance liquid chromatography (HPLC)[6-16], high performance thin layer chromatography (HPTLC) [17,18], gas chromatography with mass spectrometry (GC/MS) [19], voltammetry [20-22] and ion-selective electrode potentiometry[23]. However, the above techniques are complex; require sophisticated and expensive instruments and skilled operator which are not always found in many laboratories of developing and under developed countries. Hence, there is a need for simple, selective and low cost method, especially for routine quality control analysis of pharmaceuticals containing PYP.

Spetcrophotometry has been sparsely employed for the assay of PYP. UV-spectrophotometric methods, one for the drug when present alone [24] and two in combination with mebendazole[25, 26] are found in the literature. Few methods based on condensation [27], redox [28], ion-pair and charge-transfer complexation reactions [29-31] have been reported for PYP. The condensation product of PYP with malonic acid and acetic anhydride was employed by Refaat et al. [27]. The blue colored species [28] produced on reacting drug with F-C reagent in alkaline medium served as the basis for the assay of drug in 2.5-25 μg mL^{-1} concentration range. Lakshmi and Reddy [29] described the use of three dyes, wool fast blue BL, supracen violet 3B and azocarmine G for the extractive spectrophotometric assay of PYP. The drug when reacted with chloranil in dioxane medium formed the colored charge-transfer complex which was measured at 560 nm [30]. Four methods [31] based on diverse chemical reactions were described by Lakshmi and Reddy. The above methods suffer from one or the other disadvantages such as heating step [27], and poor sensitivity [28,30]. The extraction methods [29], besides involving tedious and time-consuming liquid-liquid extraction steps, require careful pH control and preconversion of the salt into base by an additional extraction step. The methods are also prone to cause incomplete recovery of the analyte from the aqueous phase leading to erroneous results. Two procedures reported by Lakshmi and Reddy [31] are indirect and involve multi-step reactions.

This paper reports simple, rapid and accurate spectrophotometric methods which are based on reducing ability of PYP where iron(III) is reduced and the resulting iron(II) is either complexed with ferricyanide or chelated with 1,10-phenanthroline or 2,2'-bipyridyl. The methods were demonstrated to overcome most limitations of the previously reported methods.

Materials and Methods

Apparatus

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

Chemicals and reagents

All Chemicals and reagents used were of analytical reagent grade and double distilled water was used throughout the investigation.

Standard drug solution

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

A stock standard solution equivalent to 100 μg mL^{-1} PYP was prepared by dissolving 10 mg PYP in 20 mL dimethyl sulfoxide (DMSO) in a 100 mL

calibrated flask, after shaking for complete dissolution, diluted to the mark with water and used.

Ferric chloride ($FeCl_3$) (0.5 M): The aqueous solution of 0.5 M $FeCl_3$. $6H_2O$ (S.D. Fine Chem., Mumbai, India) was prepared by dissolving 13.5 g of the chemical in 100 mL of 0.1 M HCl. The resulting 0.5 M $FeCl_3$ used in method A and this solution was further appropriately diluted to get 3 mM $FeCl_3$ with water and used in methods B and C.

Potassium ferricyanide (PFC) (0.05%): A 0.05% was prepared by dissolving 50 mg reagent in 100 mL water.

1, 10-phenanthroline (o-phen) (0.01 M): The solution was prepared by dissolving 198 mg of the 1, 10-phenanthroline hydrate (Qualigens Fine Chemicals, Mumbai, India, assay 100%) in distilled water and diluted to 100 mL with distilled water.

2,2'-bipyridyl (bpy) (0.01 M): The solution was prepared by dissolving 156 mg of the chemical (Qualigens Fine Chemicals, Mumbai, India, assay 100%) in distilled water and diluted to volume in a 100 mL calibrated flask.

Assay procedures

Method A

Into a series of 10 mL calibration flasks, aliquots (0.3-3.5 mL) of 100 µg mL^{-1} PYP standard solution equivalent to 3.0-35 µg mL^{-1} PYP were accurately transferred, and to each flask 1.0 mL each of 0.5% $FeCl_3$ and 0.05% PFC and the mixture was diluted with water. After 5 minutes, the absorbance of the blue colored complex was measured at 750 nm against the reagent blank similarly prepared.

Method B

Varying aliquots (0.1, 0.25, 0.5, 1.0, 2.0 and 3.0 mL) of the standard PYP solution (100 µg mL^{-1}) were accurately measured into a series of 10 mL calibrated flasks by means of a micro-burette and the total volume was brought to 3.0 mL by adding water. To each flask 1.0 mL each of 3 mM $FeCl_3$ and 0.01 M o-phen were added. The content was mixed well and diluted to the mark with distilled water. The absorbance of each solution was measured at 520 nm against reagent blank after 5 min.

Method C

Different aliquots (0.2–4.0 mL) of a standard PYP solution (100 µg mL^{-1}) were transferred into a series of 10 mL volumetric flasks using a micro-burette and the total volume was adjusted to 4.0 mL with water. To each flask, 1.5 mL of 3 mM $FeCl_3$ and 1.0 mL of 0.01 M bpy were successively added and the volume was brought to 10 mL with water. The flasks were stoppered, the content mixed well and after 5 min, the absorbance of the red colored chromogen was measured against the reagent blank at 530 nm.

In all the spectrophotometric methods, standard graph was prepared by plotting the absorbance versus drug concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using the absorbance-concentration data.

Procedure for commercial dosage forms

Ten tablets were weighed and pulverized. A portion of the powder equivalent to 10 mg of PYP was accurately weighed and transferred into 100 mL volumetric flask. Added 20 mL of dimethyl sulfoxide (DMSO) and shaken well for 20 minutes, then diluted to the mark with water in a calibrated flask. The content was mixed well, and filtered through a Whatman No. 42 filter paper. First 10 mL portion of the filtrate was discarded, and a suitable aliquot of the filtrate (100 µg mL^{-1}) was used for assay by applying procedures described earlier.

Procedure for placebo blank and synthetic mixture analyses

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), sodium alginate (10 mg) and lactose (15 mg) was prepared by mixing and 50 mg was extracted with DMSO and solution made as described under "procedure for commercial

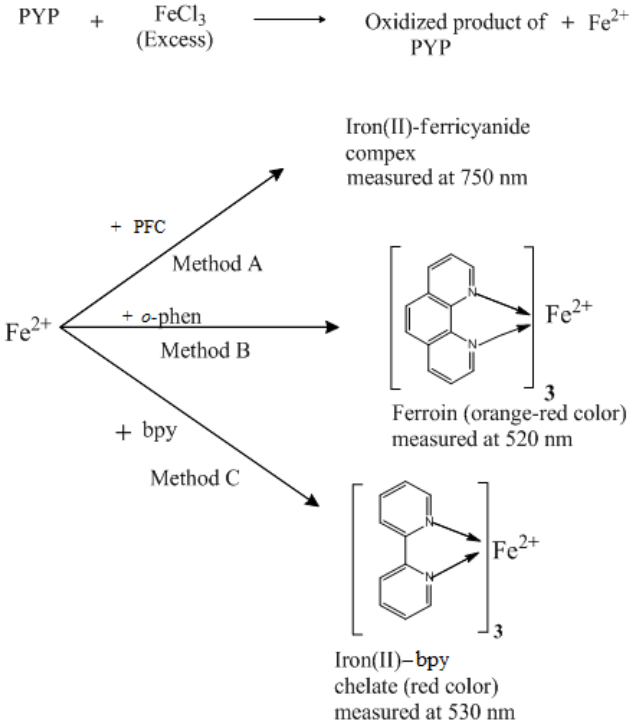
dosage forms". A convenient aliquot of solution was subjected to analysis (n=3) according to the described assay procedures.

A synthetic mixture was prepared by adding 10 mg of PYP to 10 mg of the placebo blank prepared above, homogenized and the solution was prepared as done under "procedure for commercial dosage forms". An aliquot of the extract was subjected to analysis using the procedures described above.

Results and Discussion

Absorption spectra

Iron (III) salts play a prominent role in the spectrophotometric assay of many pharmaceuticals [32-35]. Acting as an oxidant, iron (III) salt gets reduced to iron (II) salt equivalent to the amount of drug. The amount of iron (II) can be determined by using reagents such as PFC, o-phen and bpy. These properties have been utilized to develop spectrophotometric methods for the determination of PYP. The reaction proceeds through the reduction of iron (III) to iron (II) by PYP and the subsequent formation of the intensive Prussian blue color with PFC (method A), orange-red coloration of the complex with (o-phen) or tris (bpy) (method B or method C) (shown in scheme 1). The absorption spectra of the colored species show characteristic λ_{max} values as shown in Figure 1. The increasing absorbance values at 750 nm in method A, 520 nm in method B and at 530 nm in method C were plotted against the concentration of PYP to obtain the calibration graph. The experimental conditions were established by varying each parameter individually and observing its effect on the absorbance of colored species. In order to establish the favorable experimental conditions for proposed methods, PYP was allowed to react with iron (III) in the presence of PFC, o-phen or bpy.



Scheme 1. Tentative reaction scheme for the proposed methods

Optimization conditions

Preliminary experiments were performed to prepare the drug solution in a suitable solvent. It is sparingly soluble in water, completely soluble in di-methylfarmamide (DMF) and di-methylsulfoxide. Hence minimum quantity of DMSO (20 mL) was used to achieve complete solubility; the blank was colored when DMF was used.

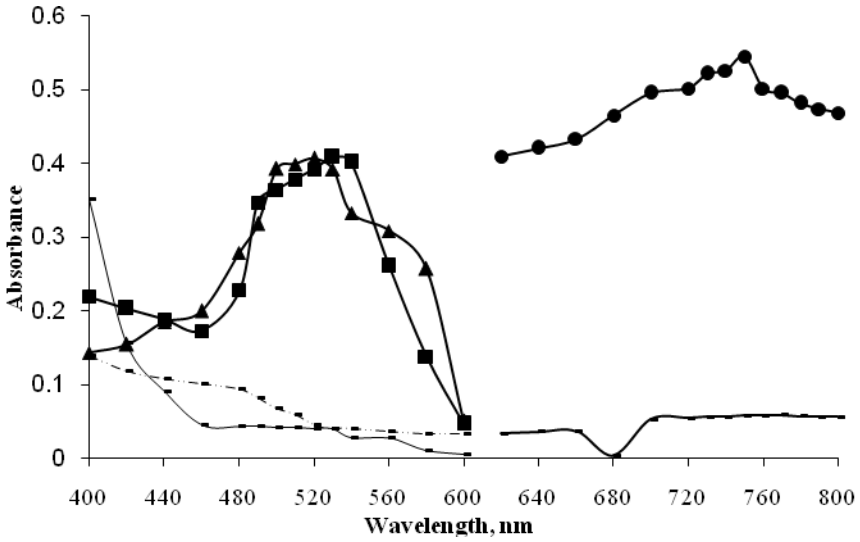


Fig. 1. Absorption spectra for method A (a) [PYP-Fe (II)-FC complex (-●-)] (20 µg mL^{-1} PYP), for method B (b) PYP-o-phen complex (-▲-) (10 µg mL^{-1} PYP) and PYP-bpy complex (-■-) (20 µg mL^{-1} PYP), and blanks (- - -)

Effect of reagent concentration

The Effect of reagent volume studied in the range from 0.5-2.5 mL, it was found that addition of 1.0 mL of 0.5 M $FeCl_3$ and 1.0 mL of 0.05% PFC (method A), 1.0 mL each of 3 mM $FeCl_3$ and 0.01 M o-phen (method B) and 1.5 mL of 3 mM $FeCl_3$ and 0.01 M bpy (method C) solutions were sufficient to obtain the maximum and reproducible absorbance for 20 µg mL^{-1} of PYP as shown in Figure 2. Smaller amounts resulted in incomplete complex formation; increased concentration of reagents gave constant absorbances.

Reaction time and stability of complexes

The reaction between drug and iron (III) was instantaneous in all methods and gave light blue color with poor sensitivity. The complex formed was instantaneous in all the methods and stable for at least 2 hours in method A and method C, and stable up to one day in method B.

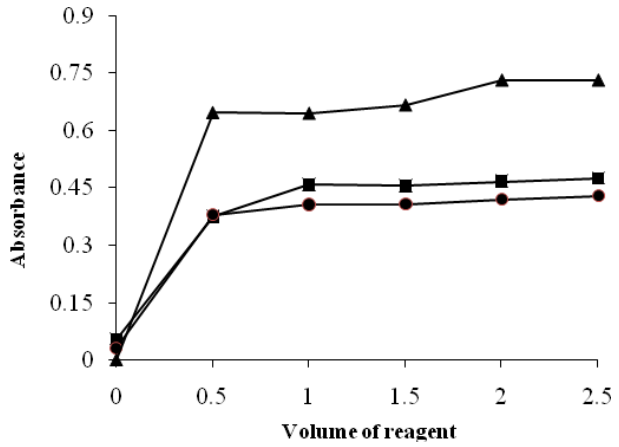


Fig. 2. Effect of reagent; (-▲-) mL iron(III) in method A, (-■-) mL o-phen in method B and (-●-) mL bpy in method C [20 µg mL^{-1} PYP each in method A & method C and 10 µg mL^{-1} PYP in method B]

Analytical performance

Linearity, sensitivity, limits of detection and quantification Under the optimum conditions described, Beer's law holds over the concentration ranges 3.0-35, 1.0-30 and 2.0-35 µg mL^{-1} for methods A, B and C respectively. A linear correlation was found between absorbance at λ_{max} and concentration of PYP 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[36] of all the three methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines for sensitivity of the proposed methods [36] using the formulae:

LOD = 3.3 S/b 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.

Table 1. Sensitivity and regression parameters

Parameter	Method A	Method B	Method C
λ_{max}, nm	750	520	530
Color stability, h	> 2	> 30	> 2
Linear range, µg mL^{-1}	3.0–35	1.0–30	2.0-35
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.38×10^4	2.06×10^4	1.23×10^4
Sandell sensitivity*, µg cm^{-2}	0.0431	0.0289	0.0482
Limit of detection (LOD), µg mL^{-1}	0.72	0.55	0.85
Limit of quantification (LOQ), µg mL^{-1}	2.17	1.67	2.59
Regression equation, Y**			
Intercept (a)	-0.0153	0.0186	0.0288
Slope (b)	0.0254	0.0322	0.0185
Standard deviation of a (S_a)	7.36×10^{-3}	9.98×10^{-3}	$2..72 \times 10^{-3}$
Standard deviation of b (S_b)	4.06×10^{-3}	4.07×10^{-3}	5.4×10^{-3}
Regression coefficient (r)	0.9987	0.9979	0.9956

*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.

Precision and accuracy

The procedures described under "Assay 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. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤ 1.98 % (intra-day) and ≤ 1.94 % (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 PYP. 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 ≤ 3.0 % demonstrate the high accuracy of the proposed methods.

Effects of interference

The results obtained from placebo blank and synthetic mixture analyses revealed that the inactive ingredients used in the tablet preparation did not interfere in the assay of active ingredient. The absorbance values obtained from the placebo blank solution were almost equal to the absorbance of the blank which revealed no interference from the common additives. To study

the role of additives added to the synthetic sample, the analysis of synthetic mixture solution prepared as described earlier yielded percent recoveries of 98.2±2.01, 98.9±1.86 and 102.1±1.17 (n=5) for method A, method B, and method C, respectively, demonstrated the accuracy as well as the precision of the proposed method and complement the findings of the placebo blank analysis with respect to selectivity.

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] %
A	10.0	10.20	1.05	2.00	10.3	1.36	3.00
	20.0	20.30	1.63	1.50	20.4	1.23	2.00
	30.0	29.75	1.98	0.83	30.7	1.14	2.33
B	15.0	14.74	1.06	1.73	15.24	1.35	1.60
	20.0	20.19	0.92	0.95	20.21	0.89	1.05
	25.0	24.72	1.45	1.12	25.25	1.92	1.00
C	10.0	9.90	1.56	1.00	10.2	1.89	2.00
	20.0	20.30	0.92	1.50	20.5	0.89	2.50
	30.0	29.65	1.41	1.17	30.4	1.94	1.33

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

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of reagent and reaction time, 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 by small intermediate precision values expressed as % RSD (≤ 2.01 %). Method 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 where ≤ 2.13 % indicating acceptable ruggedness. The results are presented in Table 3.

Table 3. Method robustness and ruggedness expressed as intermediate precision (%RSD)

Method	Nominal concentration	Robustness: Parameters altered		Ruggedness	
		Reaction time* (n=3)	Reagent volume# (n=3)	Inter-analysts (n=3)	Inter- instruments (n=3)
A	10.0	2.01	1.52	0.89	1.52
	20.0	0.95	1.62	1.27	0.81
	30.0	1.02	0.99	1.96	1.17
B	15.0	1.94	1.12	0.78	2.13
	20.0	0.75	1.74	1.51	1.72
	25.0	1.24	0.95	1.25	2.05
C	10.0	1.64	1.02	1.78	2.02
	20.0	0.69	1.24	0.85	1.27
	30.0	1.05	1.45	1.92	0.92

* Reaction time was 5.0 ± 1 min, # Volume of reagent 1.0 ± 0.1 mL

Application to pharmaceutical formulations

The proposed methods were successfully applied to the quantification of PYP in pharmaceutical formulations. The results were compared with these obtained using an official method [4]. Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and official 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.

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

Formulation analyzed	Label claim (mg/tablet)	Found* (Percent of label claim±SD)			
		Official method	Proposed methods		
			Method A	Method B	Method C
Nemocid tablets	250 mg	99.2±1.29	100.5±1.05 t = 1.74 F = 1.51	101.3±1.85 t = 2.09 F = 2.06	100.8±1.63 t = 1.72 F = 1.60
Nemocid Suspension	250 mg	101.6±0.87	102.4±1.26 t = 1.17 F = 2.10	100.9±0.95 t = 1.22 F = 1.19	102.1±1.15 t = 0.78 F = 1.75

*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.

Recovery study

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. The recovery was 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 PYP 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 values ranged between 98.5 and 102.6 % with standard deviation in the range 0.63-1.57 %. Closeness of the results to 100 % showed the fairly good accuracy of the methods. The results are shown in Table 5. Comparison of the performance characteristics of the present methods with the published methods are shown in Table 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	10.05	5.0	14.91	99.1±1.17
		10.05	10.0	20.15	100.5±0.79
		10.05	15.0	24.72	98.7±1.09
	Nemocid suspension	10.24	5.0	15.36	100.8±1.02
		10.24	10.0	19.94	98.5±0.63
		10.24	15.0	25.57	101.3±1.27
B	Nemocid tablets	10.13	5.0	14.93	98.7±1.20
		10.13	10.0	19.99	99.3±1.49
		10.13	15.0	25.71	102.3±1.35
	Nemocid suspension	10.09	5.0	15..15	100.4 ± 1.41
		10.09	10.0	20.61	102.6±1.57
		10.09	15.0	25.44	101.4±1.54
C	Nemocid tablets	10.08	5.0	14.94	99.1±1.05
		10.08	10.0	19.98	99.5±1.29
		10.08	15.0	25.26	100.7±1.12
	Nemocid suspension	10.21	5.0	15.47	101.7±1.15
		10.21	10.0	20.63	102.1±1.24
		10.21	15.0	25.59	101.5±1.02

Conclusions

Three visible spectrophotometric methods for the determination of PYP in bulk drug and in pharmaceutical dosage forms were developed and validated for accuracy, precision, linearity, robustness and ruggedness. The methods employ normal conditions compared to those previously reported, and rely on well-characterized redox-complexation reactions. Besides, these methods have the advantages of speed and simplicity without involving heating or extraction step; use aqueous solutions of eco friendly reagents. When extraction difficulties arise with other published methods, with these methods, one can do the analysis at low cost without losing accuracy. The methods can be used as alternative methods to reported ones for the routine determination of PYP in the pure form and in pharmaceutical formulations.

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

Sl. No	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (μg mL^{-1}) ε (Lmol^{-1}cm^{-1})	Remarks	Ref
1	Malonic acid-acetic anhydride	Condensation product was measured	415 455	-	Requires heating	**27**
2	*FC reagent/ Na_2CO_3	Measurement of absorbance of molybdenum blue chromogen	760	2.5-25 1.45×10^4	Reaction slow	**28**
3	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	**29**
4	Chloranil	Formed CT complex was measured in dioxane	560	25-400	Less sensitive	**30**
5	*p-CAA	Measurement of absorbance of charge transfer complex radical anion	540	12.5-62.5 0.43×10^4	Use of organic solvent	**31**
	*FC reagent	Absorbance of blue colored chromogen	760	2.0-10.0 3.55×10^4	Slow reaction	
	NBS - celestein blue	Absorbance of reduced product was measured	540	1.0-10.0 4.91×10^4	Unstable oxidants, multi- reagents & multi-step reactions	
	$KMnO_4$ - cresyl fast violet acetate	Measurement of absorbance of reduced product	600	1.0-10.0 4.91×10^4		
6	*PFC	Absorbance of blue colored chromogen measured	750	3.0-35 1.38×10^4	Sensitive, wide linear dynamic range, use of ecofriendly chemicals, uses aqueous solution	**Present work**
	o-phen	Measurement of absorbance of formed chelate	520	1.0-30 2.06×10^4		
	bpy		530	2.0-35 1.23×10^4		

*FC- Folin-Ciocalteu reagent, p-CAA- chloranilic acid, NBS-N-bromosuccinide, $KMnO_4$-potassium permanganate, PFC-potassium ferricyanide, o-phen-1,10-phenanthroline, bpy-2,2'-bipyridyl.

Conflict of interest

Authors do not have any conflict of interest with the commercial identities mentioned in this article.

Acknowledgements

Authors thank the quality control manager IPCA pharmaceutical company, Ratlam, India Ltd., for gifting pure pyrantel pamoate and the authorities of the University of Mysore, Mysore, for permission and facilities. Prof. K. Basavaiah thanks UGC, New Delhi for the award of UGC-BSR faculty fellowship. One of the authors (NS), thank the authorities of the University of Mysore, Mysore, for providing research facilities and for awarding a UGC NON-NET PhD fellowship.

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