Research Article

Extractive Spectrophotometric Methods for Determination of Pramipexole Dihydrochloride in Pharmaceutical Preparations through Ion-Pair Technique

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ABSTRACT

Simple, sensitive, precise, reproducible and validated visible spectrophotometric methods have been developed for the determination of antiparkinson agent, pramipexole dihydrochloride monohydrate (PRM) in pure form and pharmaceutical preparations. The proposed spectrophotometric methods are based on the formation of yellow colored ion-pair complexes between PRM, and three dyes, bromocresol purple (BCP), bromophenol blue (BPB and) methyl orange (MO) with absorption maxima at 410, 416 and 421 nm, respectively. Several parameters such as pH, buffer type, reagent volume, sequence of addition and effect of extracting solvent were optimized to achieve high sensitivity, stability, low blank reading and reproducible results. Under the optimum reaction conditions, linear relationships were found between the absorbance's over the concentration ranges of 2.0–12, 1.0-10 and 2.0–16 µg ml-1 with good correlation coefficients ≥ 0.9992 and LOD of 0.58, 0.28 and 0.60 µg ml-1 and the calculated molar absorptivity values are 1.1737 × 104, 2.3043 × 104 and 1.2847 × 104 l mol-1 cm-1 using BCP, BPB and MO methods, respectively. The stoichiometric ratio of the formed ion-pair complexes was found to be 1:1 (drug: reagent) for all methods as deduced by Job's method of continuous variation. Various analytical parameters have been evaluated and the results have been validated by statistical data. The proposed methods were validated in accordance with ICH guidelines and successfully applied to the analysis of PRM in pharmaceutical preparations (tablets). Statistical comparison of the results obtained by applying the proposed methods with those of the official method revealed good agreement and proved that there was no significant difference in the accuracy and precision between the results. The reliability of the methods was further ascertained by performing recovery studies using the standard addition method.

Keywords: Pramipexole dihydrochloride monohydrate, Ion-pair complex, Dyes, Spectrophotometry, Tablets.

INTRODUCTION

Pramipexole dihydrochloride monohydrate (PRM) is chemically designated as (6S)-6-N-propyl-4,5,6,7tetrahydro-1,3-benzothiazole-2,6-diamine dihydrochloride monohydrate (Figure 1). PRM is a non-ergot dopamine agonist recently approved for the treatment of early and advanced Parkinson's disease. It is also used in restless legs syndrome [1].

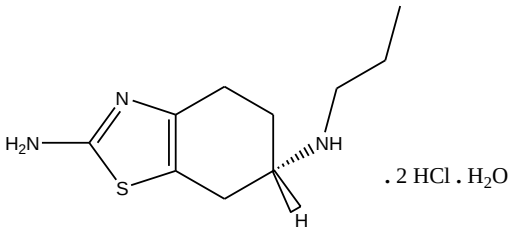


Figure 1: The chemical structure of pramipexole dihydrochloride monohydrate (PRM).

The literature revealed that numerous methods have been reported for the determination of PRM in pure form and pharmaceutical preparations such as spectrophotometry [2-12], spectrofluorimetry [7], electrochemical methods [13, 14], high-performance liquid chromatography (HPLC) [15-20], LC-MS/MS [21-23] and capillary zone electrophoresis [24]. Most of the reported methods (except spectrophotometric methods) are either not appropriately sensitive or tedious and utilized expensive instruments that are not available in most quality control laboratories and the procedures are not simple to perform.

Visible spectrophotometric methods represent the most convenient analytical technique in most quality control laboratories because of their selectivity. In addition, they are easier, less expensive and less time consuming compared with many other methods. The previously spectrophotometric methods for determination of PRM suffer from one or other disadvantage such as poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid–liquid extraction step and use of expensive reagent or large amounts of organic solvents. For these reasons, it was worthwhile to develop a new, simple, cost effective, selective and sensitive spectrophotometric method for the determination of PRM in pure form and pharmaceutical formulations. The analytically important functional groups of PRM were not properly exploited for designing suitable spectrophotometric methods for the determination of PRM. Hence a new sensitive and flexible visible spectrophotometric method was developed based on the reactivity of PRM with acid dye reagents such as BCP, BPB

and MO because of the presence of the amino group (the basic group) in PRM.

The aim of the present work is to develop simple, sensitive, accurate, precise, low-cost and validated spectrophotometric methods for the determination of PRM in pure form and pharmaceutical preparations with no need for any expensive or sophisticated instruments. The proposed methods are based on the ability of PRM to form stable ion-pair complexes with BCP, BPB and MO.

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with a 5.0 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm. The pH values of different buffer solutions were checked using Adwa AD1000 pH-meter (Romania) combined with a glass-electrode was used to measure the pH-values.

Materials and reagents

All reagents, solvents and chemicals used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the work.

Materials

Pure sample of pramipexol dihydrochloride monohydrate (PRM) was kindly supplied by EVA Pharma S.A.E., Cairo, Egypt, with a purity of 99.30 ± 0.61% by applying the offical method [1]. All pharmaceutical preparations were obtained from commercial sources in the local markets. Ramixol® tablets, labeled to contain 0.25 and 1.0 mg PRM per tablet, product of EVA Pharma S.A.E., Cairo, Egypt.

Preparation of stock standard solution

A stock standard solution (100 µg ml^{-1}) and (1.0×10^{-3} mol l^{-1}) of PRM were prepared by dissolving 10 and 30 mg of pure PRM in 20 ml of bidistilled in a 100-ml volumetric flask and completed to 100 ml with bidistilled water. This solution was stable for at least one week when kept in the refrigerator. Serial dilution with the same solvent was performed to obtain the appropriate concentration range

Reagents

Bromocresol purple (BCP), bromophenol blue (BPB) and methyl orange (MO) (BDH Chemicals LTD, Poole, England) and used without further purification. Stock solutions (0.1% w/v) and (1.0×10^{-3} mol l^{-1}) of reagents were prepared by dissolving the appropriate weight of each dye in 10 ml of ethanol (96%) and diluted to 100 ml in a calibrated flask with the same solvent. These solutions were kept in the refrigerator.

Series of buffer solutions of KCl–HCl (pH=1.0-2.2), NaOAc–HCl (pH=1.99-4.92), NaOAc–AcOH (pH=3.4-5.6) and potassium hydrogen phthalate–HCl (pH=2.0-7.0) were prepared by following the standard methods [25]. The pH of each solution was adjusted to an appropriate value by the addition of 0.2 mol l^{-1} hydrochloric acid or sodium hydroxide with the help of the pH meter. Freshly prepared solutions were always employed. Chloroform, methylene chloride and ethanol (BDH), anhydrous sodium sulfate (Prolabo).

General recommended procedure

Accurately measured aliquots (0.1–1.6 ml) the of PRM (100 µg ml^{-1}) were transferred into 10 ml measuring flasks. Volumes of 2.0 and 1.5 ml of (0.1% w/v) (BCP or BPB) and MO, respectively were added. Then, 3.0 ml acetate buffers at the optimum pH 3.0 and 3.5 using BCP and (BPB or MO), respectively and the volume was completed to 10 ml with distilled water. The ion-pairs were extracted with 10 ml of dichloromethane by shaking for 2.0 min, and then the combined dichloromethane extracts were dried over anhydrous sodium sulfate. The absorbance of the yellow colored ion-pair complexes was measured at 410, 416 and 421 nm, using BCP, BPB and MO, respectively, within 10 min of extraction against the reagent blank similarly prepared in the same manner except an addition of drugs. All measurements were made at room temperature (25 ±2°C). In both the methods, a standard curve was prepared by plotting the increasing absorbance values versus concentrations of drug. A linear equation for the standard curve was calculated by linear regression.

Procedure for commercial tablets

Twenty tablets were finely pulverized and weighed. A weighed quantity of the powdered tablets equivalent to 1.0 mg of PRM was dissolved in about 20 ml of bidistilled water and the mixture was transferred into a 100-ml volumetric flask, and the flask was sonicated for 30 min. The volume was completed to the mark with bidistilled water, mixed well. Aliquots' containing the drug in the final concentration ranges BCP, BPB and MO methods were analyzed as described under "Construction of the Calibration Graph". The concentration of the drug was determined either from the calibration curve or using the corresponding regression equation.

Stoichiometric relationship

The stoichiometric ratios of the ion-pairs formed between PRM and the reagents were determined by applying the continuous variation method [26] at the optimum wavelengths. In continuous variation method, equimolar solutions were employed: a 1.0×10^{-3} mol l^{-1} standard solution of drug and 1.0×10^{-3} mol l^{-1} solution of dye was used. A series of solutions was prepared in which the total volume of the studied drugs and the dye was kept at 2.0 ml. The drug and reagent were mixed in various complementary proportions (0.2:1.8, 0.4:1.6, 0.6:1.4, 0.8:1.2, 1.0:1.0, 1.2:0.8, 1.4:0.6, 1.6:0.4, 1.8:0.2) and

completed to volume in a 10 ml calibrated flask with the appropriate solvent for extraction following the above-mentioned procedure. In the molar ratio method [27], the concentration of PRM was kept constant 1.0 ml of (1.0 x10 $^{-3}$ mol l^{-1}) while that of dyes (1.0 x10 $^{-3}$ mol l^{-1}) is regularly varied (0.2 – 2.4 ml). The absorbance of the prepared solutions measured at optimum condition and at the optimum wavelength for each complex.

RESULTS AND DISCUSSION

Absorption spectra

The proposed methods are based on the reactivity of amine group of PRM with three dyes (BCP, BPB and MO). The nitrogenous drugs are present in positively charged protonated forms and anionic dyes present mainly in anionic form at a pH ≥ 3.0. So, when PRM treated with dye at pH range (2.8-5.0) of acidic buffer solutions, a yellow ion-pair complex which is extracted with organic solvent is formed. The absorption spectra of the yellow ion-pair complexes formed between PRM and BCP, BPB or MO reagents and show maximum absorbances at 410, 416 and 421 nm, respectively against the blank solution (Figure 2).

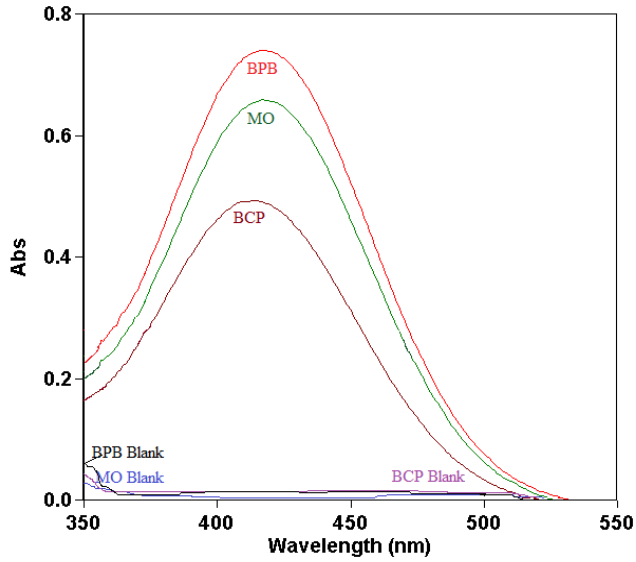


Figure 2: Absorption spectra of ion-pair complexes of 12, 10 and 16 µg ml^{-1} PRM using (0.1% w/v) BCP, BPB and MO reagents, respectively against reagent blank.

Optimization of the reaction Conditions

Many preliminary experiments established optimum conditions necessary for rapid and quantitative formation of colored ion-paired complexes achieve the maximum stability and sensitivity. Optimum condition was fixed by varying one parameter at a time while keeping other parameter constant and observing its effect on the absorbance.

Effects of buffer type and pH

It was observed that the effective extraction of the complex depends on the type of the buffer used and its pH. The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as KCl–HCl (pH 1.0-2.2), NaOAc–HCl (pH 1.99-4.92) and NaOAc–AcOH (pH 3.6-5.6). It is evident that the maximum color intensity and maximum absorbance were found in NaOAc_HCl buffer. It is evident that the absorbance of the ion pair complex was maximal at pH 3.0 and 3.5 using BCP and (BPB or MO) methods, respectively (Figure 3). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-4.0 ml). The higher absorbance value and reproducible results were obtained by using 3.0 ml of buffer solutions.

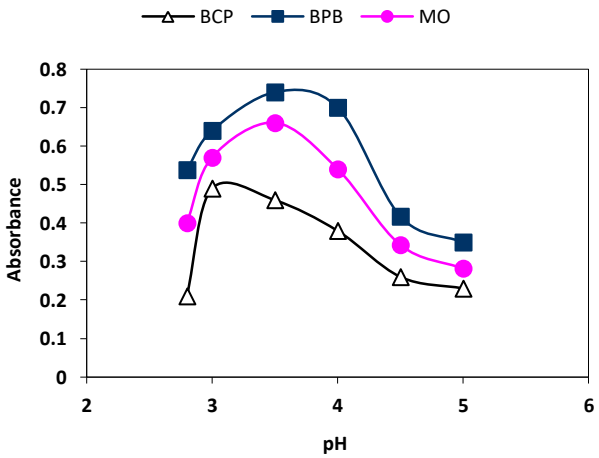


Figure 3: Effect of pH of buffer solution on ion pair complex formation between 12, 10 and 16 µg ml^{-1} PRM and (0.1% w/v) BCP, BPB and MO reagents, respectively against reagent blank.

Effect of reagent concentration

The PRM concentration was kept constant, while the concentrations of (0.1% w/v) BCP, BPB or MO reagents were varied from 0.5–4.0 ml. The results showed that the absorbance of the extracted ion-pairs increased by increasing the volume of reagent till 2.0 and 1.5 ml of (0.1% w/v) (BCP or BPB) and MO, respectively. After this volume, the absorbance remains constant by increasing the volume of the reagents. So, an excess of reagents has no effect on the determination of PRM.

Choice of extracting solvent

Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed method. Dichloromethane was preferred to other solvents for its selective and obtained the highest absorbance with dichloromethane. It was also observed that only one extraction with total volume 10 ml solvent was adequate to achieve a quantitative recovery of the complexes, maximum absorbance intensity and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

Effect of shaking time and temperature

The optimum shaking time was investigated by shaking from 0.5-5.0 min. Maximum and constant absorbance values were obtained when extracted after 1.5 min

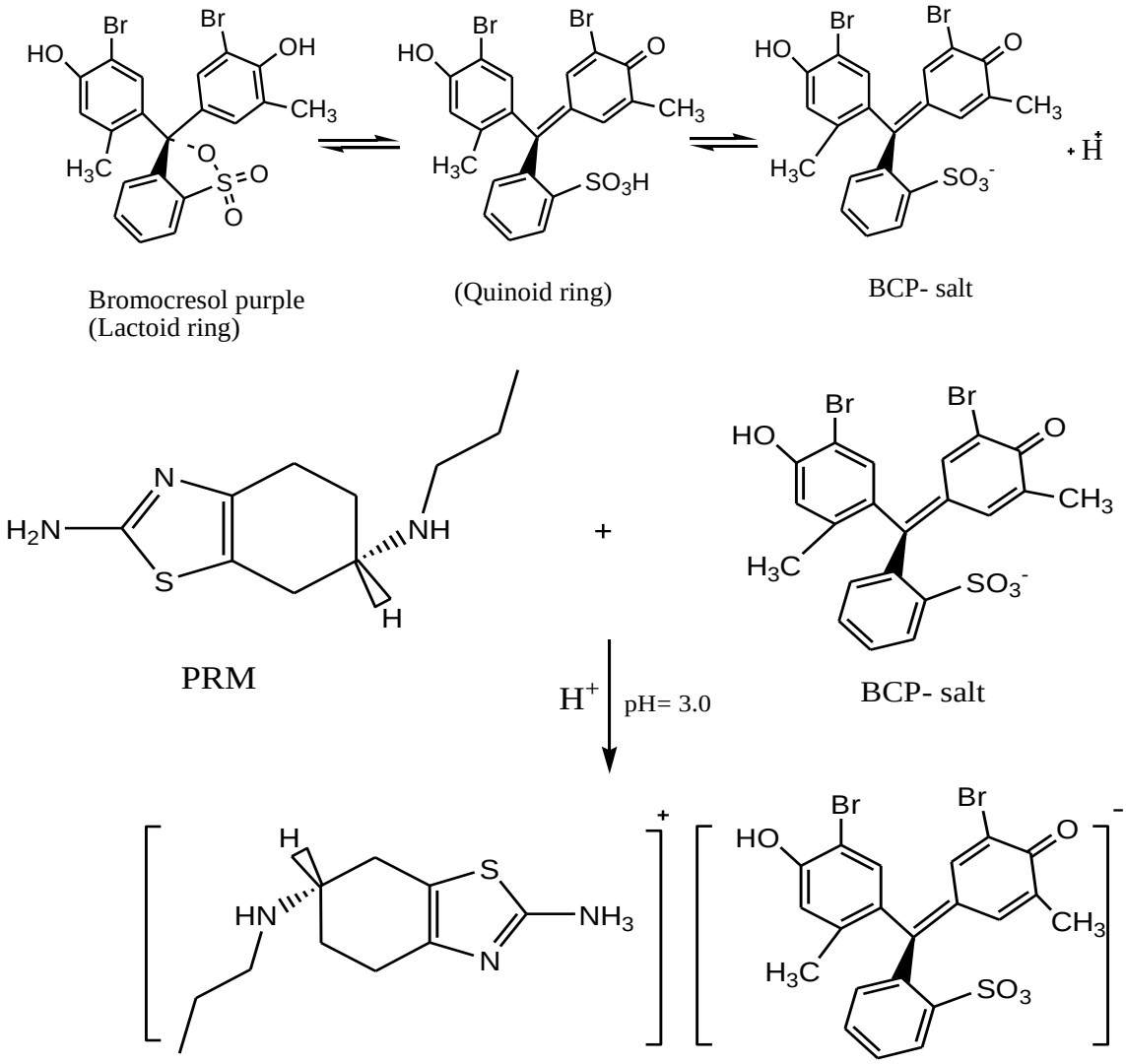
shaking. Therefore, shaking time of 2.0 min was maintained throughout the experiment. The effect of temperature on colored complexes was studied by measuring the absorbance values over the temperature range 20-35°C. It was found that the absorbance of the colored ion pair complex was constantly up to 30°C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of the dichloromethane. Therefore, the temperature chosen was room temperature (25 ± 2°C) as the best temperature for micro-determination of PRM in pure and pharmaceutical formulations. The absorbance of both complexes remains stable for at least 18 h at room temperature.

Composition of the ion-pair complexes

The molar ratio between PRM and BCP, BPB or MO in the ion-pair complexes was determined by Job's method of continuous variation. Job's method of continuous variation [26] of equimolar solutions was employed: a 1.0×10^{-3} mol l^{-1} standard solution of drug base and 1.0×10^{-3} mol l^{-1} solution of BCP, BPB or MO were used. A series solution was prepared in which the total volume of drug and reagent was kept at 2.0 ml in the total volume of 10 ml of the aqueous layer. The absorbance of extracting an ion-pair in each instance was measured at the optimum wavelength and plotted against the mole fraction of the drug. The results indicate that the molar ratio of (drug: dye) is (1:1) complex was formed through the electrostatic attraction between the positive charged PRM^+ ions and negatively charged dye, D^- ions. The extraction equilibrium can be represented as follows:

$$PRM^+_{(aq)} + D^-_{(aq)} \longleftrightarrow PRM^+D^-_{(aq)} \longleftrightarrow PRM^+D^-_{(org)}$$

Where PRM^+ and D^- represent the protonated drug and the anion of the dye (BCP^-, BPB^- or MO^-), respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively (Scheme 1).



Scheme 1: Proposed reaction mechanism for the ion pair complex formation between PRM and BCP.

Method of Validation

The proposed methods have been validated for linearity, sensitivity, accuracy, precision, selectivity and recovery.

Linearity

At described experimental conditions for PRM determination, standard calibration curves with reagents were constructed by plotting absorbance vs. concentration of PRM in the ranges of 2.0-12, 1.0-10 and 2.0-16 µg ml^{-1} using BCP, BPB and MO methods, respectively. The statistical parameters calculated from the calibration graphs were given in the regression equation:

$$A = a + b\,C \qquad (1)$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg mL^{-1}, obtained by the method of least squares. The linearity of calibration graphs was proved by the high values of the correlation coefficient (*r*) and the small values of the *y*-intercepts of the regression equations. For accurate determination, Ringbom concentration range [28] was calculated by plotting log concentration of drug in µg mL^{-1} against transmittance % from which the linear portion of the curve gives an accurate range of micro determination of PRM and represented in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines [29] and illustrated in Table 2. The high molar absorptivity and lower Sandell's sensitivity values reflects the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis [30] between the results achieved from the proposed methods and that of the official method [1]. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 1), there is no significant difference between the proposed and reported method regarding accuracy and precision.

Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following equation [29, 30]:

$$LOD = 3.3\sigma/s \quad \text{and} \quad LOQ = 10\sigma/s \qquad (2)$$

Where σ is the standard deviation of reagent blank determinations, and s is the slope of the calibration curve. In accordance with the formula, the limit of detection was found to be 0.58, 0.28 and 0.60 µg ml^{-1} for BCP, BPB and MO methods, respectively. The limit of quantitation was found to be 1.93, 0.93 and 2.0 µg ml^{-1} for BCP, BPB and MO methods, respectively.

Table 1: Statistical analysis of calibration graphs and analytical data in the determination of PRM using the proposed methods.

Parameters	BCP	BPB	MO
Wavelengths λ_{max} (nm)	410	416	421
Beer's law limits µg ml^{-1}	2.0-12	1.0-10	2.0-16
Ringboom limits, µg mL^{-1}	4.0-10	2.0-8.0	4.0-14
Molar absorptivity ε, l mol^{-1} cm^{-1} x 10^4	1.1737	2.3043	1.2847
Sandell's sensitivity, ng cm^{-2}	25.75	13.12	23.53
Regression equation [a]			
Intercept (*a*)	0.0035	0.0052	-0.001
Standard deviation of intercept (*Sa*)	0.005	0.0036	0.007
Slope (b)	0.0379	0.0735	0.0423
Standard deviation of slope (*Sb*)	0.008	0.006	0.005
Correlation coefficient (*r*)	0.9996	0.9992	0.9993
LOD [b], µg ml^{-1}	0.58	0.28	0.60
LOQ [b], µg ml^{-1}	1.93	0.93	2.0
Mean ± SD	99.60 ± 0.92	99.70 ± 0.85	99.10 ± 0.70
RSD%	0.92	0.85	0.69
RE%	0.96	0.89	0.73
Calculated *t*-value [c]	0.61	0.85	0.48
Calculated *F*-value [c]	2.27	1.94	1.35

[a] A = a + bC, where C is the concentration in µg mL^{-1}, A is the absorbance units.

[b] LOD, limit of detection; LOQ, limit of quantification; ε , molar absorptivity.[c] The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

Accuracy and precision

To evaluate the precision of the proposed methods, solutions containing three different concentrations of PRM were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 3. Lower values of the relative standard deviation (R.S.D %) and percentage relative error (R.E %) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation:

$$\% R.E. = \left[\frac{found - taken}{taken} \right] x\ 100 \quad (3)$$

The assay procedure was repeated six times, and percentage relative standard deviation (R.S.D %) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

For the same concentrations of PRM inter- and intra-day accuracy of the methods was also evaluated. The percentage recovery values with respect to found concentrations of PRM were evaluated to ascertain the accuracy of the methods. The recovery values close to 100% as compiled in Tables 2 shows that the proposed methods are very accurate.

Table 3: Intra-day and Inter-day precision and accuracy data for PRM obtained by the proposed methods.

Method	Taken (µg ml^{-1})	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
	Intra-day				
BCP	3.0	99.20	0.70	-0.80	2.976 ± 0.022
	6.0	99.60	0.90	-0.40	5.976 ± 0.056
	9.0	99.10	1.30	-0.90	8.919 ± 0.122
BPB	4.0	99.00	0.60	-1.0	3.96 ± 0.025
	8.0	99.50	1.10	-0.50	7.96 ± 0.092
	12	98.60	1.60	-1.40	11.832 ± 0.199
MO	5.0	98.80	0.50	-1.20	4.94 ± 0.026
	10	99.30	0.90	-0.70	9.93 ± 0.094
	15	100.80	1.80	0.80	15.12 ± 0.286
BCP	Inter-day				
	3.0	100.30	0.50	0.30	3.009 ± 0.016
	6.0	99.50	0.80	-0.50	5.97 ± 0.05
	9.0	99.40	1.0	-0.60	8.946 ± 0.094
BPB	4.0	100.60	0.90	0.60	4.024 ± 0.038
	8.0	99.10	1.20	-0.90	7.928 ± 0.10
	12	100.40	1.90	0.40	12.048 ± 0.24
MO	5.0	99.70	0.60	-0.30	4.985 ± 0.031
	10	99.20	1.10	-0.80	9.92 ± 0.115
	15	99.00	1.50	-1.0	14.85 ± 0.234

[a] Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.

[b] Mean ± standard error, [b] Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).

Robustness and Ruggedness

Robustness was examined by evaluating the influence of small variation of method variables, including volume of buffer at optimum pH, dye volume and shaking time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. The analysis was performed with altered conditions by taking three different concentrations of PRM and it was found that small variation of method variables did not significantly affect the procedures as shown by the RSD values in the

range of 0.70-2.40%. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of PRM and so the proposed spectrophotometric methods are considered robust. Ruggedness was expressed as the RSD and was also tested by applying the proposed methods to the assay of PRM using the same operational conditions but using three different instruments as well as three different anaysts. The inter-analysts RSD were in the ranges 1.1-2.70%, whereas the inter-instruments RSD ranged from 0.90-2.30% suggesting that the developed methods were rugged. The results are shown in Table 3.

Table 3: Method robustness and ruggedness expressed as intermediate precision (RSD %) for PRM-dye ion pair complexes.

Method	PRM taken (µg ml^{-1})	Robustness			Ruggedness	
		Parameters altered				
		Volume of dye [a]	Volume of buffer [b]	Reaction time [c]	Inter-analysts (N=3)	Inter-instruments (N=3)
BCP	3.0	1.10	0.80	1.0	1.30	0.90
	6.0	1.70	1.20	1.40	1.10	1.50
	9.0	1.95	1.80	1.70	2.0	1.65
BPB	4.0	1.10	0.90	1.30	1.20	1.10
	8.0	1.60	1.40	1.50	1.80	1.60
	12	1.90	1.80	2.30	2.50	2.30
MO	5.0	1.40	0.70	1.70	1.30	1.30
	10	1.90	1.20	2.0	1.70	1.80
	15	2.20	2.0	2.40	2.70	2.10

[a] The volumes of dye used were 2.0 ± 0.5 ml.

[b] The volumes of buffer used were 3.0 ± 0.2 ml.

[c] The reaction times were 2.0 ± 0.5 min.

Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure drugs (50, 100 and 150% of the level present in the tablet) to a fixed amount of PRM in tablet powder (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\% \text{ Recovery} = \frac{[C_F - C_T]}{C_p} \text{x } 100 \qquad (4)$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drug) added to tablets preparations. The results of this study presented in Table 4 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Applications to pharmaceutical formulations

The proposed methods have been successfully applied to the determination of PRM in dosage forms (Ramixol® tablets 0.25 mg and 1.0 mg). The results in Table 5 showed that the excipients in the dosage forms do not interfere and the methods are successful for the determination of PRM. A statistical comparison of the results obtained for the determination of PRM from the same batch of material by the proposed and official method [1] is shown in Table 4. The results agreed well with the label claim and agree with the results obtained by the official method [1]. When the results were statistically compared with those of the reported methods by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [30]. Hence, no significant difference between the proposed methods and the reported methods at the 95 % confidence level with respect to accuracy and precision (Table 5).

Table 4: Results of recovery experiments by standard addition method for the determination of PRM in tablets using the proposed methods.

Samples	Taken drug in tablet (µg ml^{-1})	Pure drug Added (µg ml^{-1})	BCP		BPB		MO	
			Total found (µg ml^{-1})	Recovery [a] (%) ± SD	Total found (µg ml^{-1})	Recovery [a] (%) ± SD	Total found (µg ml^{-1})	Recovery [a] (%) ± SD
Ramixol® tablets (0.25 mg)	4.0	2.0	5.946	99.10±0.70	5.976	99.60±1.0	5.958	99.30±0.80
	4.0	4.0	7.96	99.50±1.20	7.92	99.00±0.80	7.92	99.00±0.90
	4.0	6.0	10.05	100.50±1.50	9.97	99.70±1.30	10.12	101.20±1.60
Ramixol® tablets (1.0 mg)	4.0	2.0	5.958	99.30±0.90	5.91	98.50±0.50	5.958	99.30±0.50
	4.0	4.0	8.032	100.40±1.40	8.0	100.0±1.0	7.888	98.60±0.80
	4.0	6.0	9.95	99.50±1.60	9.90	99.0±1.70	10.08	100.80±1.20

[a] *Average of six determinations.*

Table 5: Results of analysis of tablets by the proposed methods for the determination of PRM and statistical comparison with the official method [1].

Samples	Recovery [a] (%) ± SD			Official method[1]
	BCP	BPB	MO	
Ramixol® tablets (0.25 mg)	99.70 ± 1.0	99.20 ± 0.50	99.80 ± 0.80	99.50 ± 0.70
t-value [b]	0.37	0.48	0.63	
F-value [b]	2.04	1.96	1.31	
Ramixol® tablets (1.0 mg)	99.10 ± 0.40	99.55 ± 0.72	99.00 ± 0.38	99.30 ± 0.50
t-value [b]	0.70	0.64	1.07	
F-value [b]	1.56	2.07	1.73	

[a] *Average of six determinations.*

[b] *The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).*

CONCLUSION

A new, simple, rapid and cost-effective spectrophotometric method have been developed for determination of PRM in bulk drug and tablets through the application of extractive ion–pair complexation reaction with dyes and validated as per the current ICH guidelines. Compared with the existing visible spectrophotometric methods, the proposed methods have the advantages of relatively simple, rapid, cost-effective, free from auxiliary reagents and more sensitive for determining PRM in pure form and pharmaceutical formulations. Moreover, the proposed methods are free from tedious experimental steps such as heating unlike the previously reported spectrophotometric methods cited earlier. The most attractive feature of these methods is its relative freedom from interference by the usual diluents and excipients in amounts far more than their normal occurrence in pharmaceutical preparations. The statistical parameters and the recovery data reveal high precision and accuracy of the methods besides being robust and rugged. Therefore, the validated method could be useful for routine quality control assay of PRM in pure form and pharmaceutical preparations.

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