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SPECTROPHOTOMETRIC DETERMINATION OF OXOLAMINE PHOSPHATE IN BULK AND ORAL SYRUP DOSAGE FORM BY USING ACIDIC DYES

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ABSTRACT

Two simple, sensitive, precise, accurate and cost effective spectrophotometric methods have been developed for the estimation of Oxolamine phosphate (OMP) in bulk and formulations. These methods were based on the yellow colored ion pair complex formation with Tropeolineooo (TPooo) (M-1) and Bromothymol blue (BTB) (M-2) with exhibiting the maximum absorbance at 430nm and 485nm respectively. In these both methods the calibration curves are in the range of 10-60 µg/ml of the drug. The molar absorpitivities were found to be 1.219 x 10^3, 3.485 x 10^3 L mol^{-1} cm^{-1}; Sandell's sensitivities were 0.1557, 0.2234 µg cm^{-2}; LOD values were 1.1231, 0.1026 and the LOQ values were 3.4035, 0.3111 for both methods M-1 and M-2 respectively. The developed methods were sensitive, precise, accurate, robust, rapid and convenient. The proposed methods were successfully applied for the estimation of OMP in syrup dosage form without interference from excipients.

INTRODUCTION

Oxolamine phosphate (Fig.1) is an analgesic-anti-inflammatory, local anesthetic and antispasmodic drug. Chemically it is defined as 3-phenyl-5β-diethylaminoethyl-1,2,4-oxadiazole: phosphoric acid (B. Silvestrini, C.Pozzatti., 1961). Oxolamine phosphate is not official in pharmacopoeias. (Rele Rajan V, Sawant Swapnil., 2016).

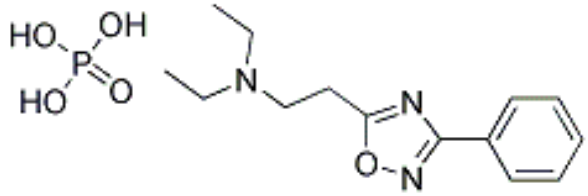


Figure 1: Structure of Oxolamine phosphate

The therapeutic importance of the OMP has encouraged several researchers to develop analytical methods for its determination in bulk and pharmaceutical dosage forms. They include potentiometric titration method (Rajan VR, Sachin SP., 2013), UV spectrophotometric method (D.Murali , C.Rambabu.,2014), (Rele RV, Sawant SA., 2012), first-order derivative spectrophotometric method , for the area under curve technique (Rele RV, Sawant SA *et al.*, 2013), second order derivative UV spectrophotometric method (Rele RV., 2014). The above described UV spectrophotometric methods were simple but, they suffered from lack of selectivity as it involves measurements at shorter wavelength. Visible spectrophotometric methods (Kumar VP, Kishore CHV., 2013, Srinivas B, Yadagiriswamy P *et al.*, 2015). The above reported spectrophotometric methods suffered from one or more disadvantages like use of costly reagent, less sensitivity, lack of precision and accuracy. Stability indicating HPLC

method (D.Murali, C.Rambabu., 2016, Sekhar Reddy BRC, Bhaskar Rao NV., 2013, Rajan VR., 2014). These HPLC methods are time-consuming, complex, expensive and requires a skilled person to operate the instrument. Hence, it was necessity to develop certain sensitive, precise, accurate and economical visible spectrophotometric methods, which prompted the author to choose OMP in the present investigation by exploiting functional groups present in its structure. The present investigation describes two visible spectrophotometric methods (M-1&M-2) for the determination of OMP in bulk and pharmaceutical dosage forms. The developed methods were based on ion pair complexation of OMP with TPooo and BTB (method M-1 & method M-2).

MATERIALS AND METHOD:

Instrumentation: ELICO (Hyderabad, India) double beam model SL 244 digital spectrophotometer, ELICO (Hyderabad, India) LI 120 model pH meter and Schimadzu electronic weighing balance (Kyoto, Japan) TW223L model were used for the present investigation.

Materials and reagents: All chemicals were of analytical reagent grade. Double distilled water was used to prepare all the solutions. All the solutions were prepared a fresh daily.

Method M-1: 0.2% (w/v) Tropeoline ooo (TPooo): Prepared by dissolving 200 mg of Tpooo (Merck specialties pvt. Ltd; Mumbai, India) in 100 ml of distilled water. 0.1M HCl (v/v): Prepared by diluting 8.6 ml of concentrated HCl (Sdfine-Chem limited, Mumbai, India) to 1000 ml with distilled water in 1000 ml volumetric flask. Chloroform (Sdfine-Chem limited, Mumbai, India) for extraction of the ion pair complex.

Method M- 2: 0.2% (w/v) Bromothymol blue (BTB): Prepared by dissolving 200 mg of BTB (Merck specialties pvt. Ltd; Mumbai, India) in 100 ml of distilled water. 0.1M HCl (v/v): Prepared by diluting 8.6 ml of concentrated HCl (Sdfine-Chem limited, Mumbai, India) to 1000 ml with distilled water in 1000 ml volumetric flask. Chloroform (Sdfine-Chem limited, Mumbai, India) for extraction of the ion pair complex. Pure and tablet dosage forms of oxolamine phosphate: Oxolamine phosphate was obtained as gift sample from Orbit Pharma Laboratories (Ahmedabad, India) and used as received. Perebron syrup (labeled to contain 50 mg of oxolamine phosphate/5 ml) manufactured by Elmor Laboratorios was used in the present study.

Preparation of stock and working standard solutions: Stock solution of OMP was prepared by dissolving 100 mg of OMP in 50 ml of distilled water in a 100 ml volumetric flask and then make up to the mark with distilled water (1.0 mg/ml). This stock solution was diluted stepwise with the same solvent to obtain working standard solutions of concentration 200 µg/ml for methods M-1 and M-2 respectively.

General assay procedure for Methods M-1& M-2: After systematic and detailed study of the various experimental parameters involved as described under results and discussions the following procedures were recommended for the determination of OMP in bulk and ORAL syrup dosage form. Aliquots of (0.5–3.0 ml) standard drug solution (200 µg/ml) of OMP were transferred into a series of 125 ml separating funnels. The volume in each separating funnel was adjusted to 3.0 ml with distilled water. 6 ml of 0.1 M HCl and 2 ml of 0.2% each of TPooo and BTB were added to each separating funnel separately. The funnels were shaken vigorously with 10 ml of chloroform for 20 min, and then allowed for clear separation of the two phases. The separated organic phase was transferred to a 10 ml volumetric flask. The extract was diluted to the mark with chloroform and mixed well. The absorbance of the yellow colored ion pair complex was measured at 430nm and 485nm against the similar reagent blank respectively. The amount of oxolamine phosphate deduced from calibration curves (Fig. 2 & Fig. 3).

Assay of OMP in Perebron syrup samples: The viscous Perebron syrup (labeled to contain 50 mg of OMP/5 ml) was shaken thoroughly to make homogenous mixture. A volume of the syrup equivalent to 100 mg of OMP was transferred accurately into a 100 ml calibrated volumetric flask containing 20 ml of distilled water. The flask was shaken well for the complete solubility of OMP in distilled water. The resulting solution was filtered through Whatmann No.1 filter paper. The filtrate was transferred to a 100 ml standard flask and diluted to 100 ml with distilled water. The

filtered solution was suitably diluted with distilled water. Convenient aliquots were subjected to analysis by the procedures described in the methods M-1 & M-2. The percentage recovery of the OMP was calculated from the corresponding calibration curve or corresponding regression equation.

RESULTS AND DISCUSSION:

Optimization of experimental conditions: The optimum conditions for the color development in present spectrophotometric methods (M-1 & M-2) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in general assay procedures.

Methods M-1 and M-2: In these methods formation of ion-pair complex between the OMP, TPooo and BTB takes place. The experimental conditions were established by studying the effect of various parameters like volume and concentration of acid, TPooo and BTB for the maximum color development and organic solvent used for extraction of the ion-pair complex. The results are incorporated in Tables 1 & 2.

Determination of absorption maxima (λmax): In order to ascertain the optimum wavelength the absorption spectra of the colored chromogens formed in the proposed methods (M-1 & M-2) were scanned in the wavelength region of 400-700 nm against a corresponding reagent blank. The results are graphically presented in the Figures 2 & 3.

Method validation: Validation was carried out by assessing the parameters like linearity range, sensitivity, stability of the colored species, precision, accuracy and robustness according to the International Conference on Harmonization guidelines for validation of analytical procedures.

Linearity: A linear correlation was found between absorbance at λmax and concentration of OMP for the proposed methods (M-1 & M-2). The graphs (Figures.4&5) showed negligible intercept and are described by the equation: Y = c + mX. The linearity was evaluated by linear regression analysis of the Beer's law data by least-square regression method, which was used to calculate the correlation coefficient, intercept and slope of the regression line and the values are presented in Table 3. The small values of the intercept of the regression equation and the regression coefficient (>0.99) values obtained, specify that there is a good correlation between absorbance values and OMP concentration in all the proposed methods (M-1 & M-2).

Sensitivity: Molar absorptivity, Sandell's sensitivity, limits of detection (LOD) and limit of quantification (LOQ) are calculated as per the current International Conference on Harmonization guidelines to assess the sensitivity of the proposed methods (M-1 & M-2). The results are summarized in Table 4. The high values of molar absorptivity & low values of Sandell's sensitivity, LOD and LOQ revealed excellent sensitivity of the proposed methods (M-1 & M-2).

Stability of colored species: The stability of the colored species formed in the proposed methods (M-1 & M-2) were monitored by keeping the solution at room temperature (25±10c) and then measuring the absorbance of the solution at their corresponding optimum wavelength at regular intervals of time. The results are presented in Table 4. The increased stability (>1 hr) of colored species formed in the proposed methods (M-1 & M-2) helped in proceeding with large batches of samples and their comfortable measurements easily.

Accuracy and Precision: The accuracy and precision of the proposed methods (M-1 & M-2) were determined by intra-day and inter-day analysis. In order to determine the intra-day accuracy and precision of the proposed methods (M-1 & M-2), solution containing fixed concentration (within the working limits) of OMP at three different concentrations levels (10, 30 & 60 μg/ml for methods M-1 & M-2) were prepared and analyzed in five replicates by the proposed methods under the optimized experimental conditions. The relative standard deviations and recoveries in the intra-day analyses for the proposed methods (M-1 & M-2) were calculated and are summarized in Table 5.The results obtained for methods M-1& M2 revealed that the mean recoveries in the range of 100.06-100.38%, 99.96-100.01%, respectively. The relative standard deviations for methods M-1 & M-2 were in the range of 0.072-0.486%, 0.048-0.186% respectively. The inter-day analysis was assessed by carrying out the analysis for three consecutive days.

Table 1: Optimization of conditions for the assay of OMP by method M-1

Parameter	Investigation conditions	Optimized condition	Remarks
λ_{max} (nm)	400-500	430	
Volume of 0.1 M HCl (ml)	2.0-10.0	6.0	The absorbance of ion-pair complex was found to increase with increasing HCl volume and became constant at 6 ml. Beyond this volume, the absorbance of ion pair complex remained constant.
Volume of 0.2% TPooo (ml)	1.0-5.0	2.0	2 ml of TPooo solution was sufficient for covering broad range of Beer's law limit.
Choice of organic solvent for extraction	Chloroform, Benzene, Carbon Tetrachloride and Butanol	Chloroform	Among the various organic solvents tried, chloroform was found to produce the highest absorbance, extraction power and stability of the formed ion-pair complex

Table 2: Optimization of conditions for the assay of OMP by method M-2

Parameter	Investigation conditions	Optimized condition	Remarks
λmax (nm)	400-500	430	
Volume of 0.1 M HCl (ml)	2.0-10.0	6.0	The absorbance of ion-pair complex was found to increase with increasing HCl volume and became constant at 6 ml. Beyond this volume, the absorbance of ion pair complex remained constant.
Volume of 0.2% BTB (ml)	1.0-5.0	2.0	To cover the broad range of Beer's law limit 2 ml of BTB was sufficient.
Choice of organic solvent for extraction	Chloroform, Benzene, Carbon Tetrachloride and Butanol	Chloroform	Among the various organic solvents tried, chloroform was found to produce the highest absorbance, extraction power and stability of the formed ion-pair complex

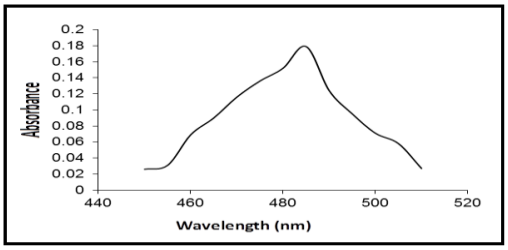


Figure 2: Absorption spectrum of OMP-TPooo

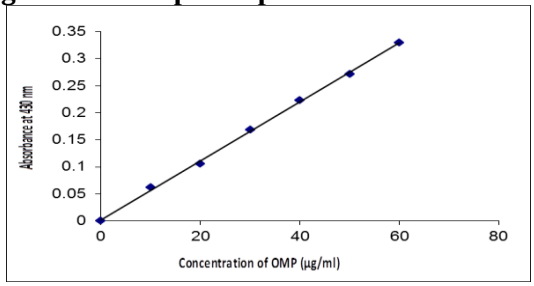


Figure 3: Absorption spectrum of OMP-BTB

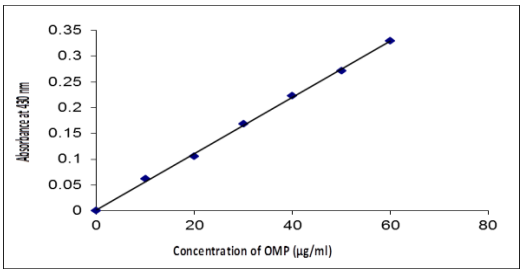


Figure 4: Beer's law plot of OMP-TPooo

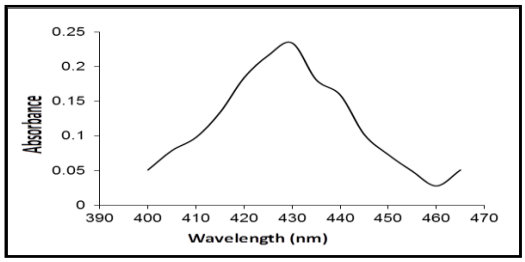


Figure 5: Beer's law plot of OMP-BTB

Table 3: Linearity and regression equation of the proposed spectrophotometric Methods

Parameter Method	Linearity range (µg/ml)	Regression equation (Y=mx+c)*	Regression coefficient (R^2)	Slope (m)	Intercept (c)
M-1	10-60	Y = 0.0043x + 0.0006	0.9989	0.0043	0.0006
M-2	10-60	Y = 0.0055x + 0.0023	0.9990	0.0055	0.0023

***Y** = Absorbance; x = Concentration of drug in µg/ml

Table 4: Sensitivity and stability of the proposed spectrophotometric methods

Parameter Method	Molar Absorbtivity (L/mole/cm)	Sandell's sensitivity (µg cm^{-2})	LOD (µg/ml)	LOQ (µg/ml)	Stability of colored species (hr)
M-1	1.219 x 10^3	0.1557	1.1231	3.4035	1.15
M-2	3.485 x 10^3	0.2234	0.1026	0.3111	1.45

Table 5: Accuracy and precision of the proposed spectrophotometric methods: intra-day analysis

Method	Concentration of OMP (µg/ml)		SD	Recovery (%)	RSD (%)	Confidence limit	
	Taken	Found*				0.05	0.01
M-1	10	10.038	0.04886	100.38	0.486	0.04085	0.06044
	30	30.048	0.05355	100.16	0.178	0.04477	0.06624
	60	60.036	0.04368	100.06	0.0727	0.03652	0.05403
M-2	10	10.001	0.01863	100.01	0.186	0.01557	0.02304
	30	30.003	0.02921	100.01	0.097	0.02442	0.03613
	60	59.980	0.02918	99.96	0.048	0.02439	0.03609

* Average five determinations

Table 6: Accuracy and precision of the proposed spectrophotometric methods: inter-day analysis

Method	Concentration of OMP (µg/ml)		SD	Recovery (%)	RSD (%)	Confidence limit	
	Taken	Found*				0.05	0.01
M-1	10	10.015	0.03078	100.15	0.307	0.02573	0.03807
	30	30.045	0.05295	100.15	0.176	0.04427	0.06550
	60	60.015	0.06115	100.08	0.101	0.05113	0.07564
M-2	10	10.020	0.04137	100.20	0.412	0.03459	0.05117
	30	30.033	0.04103	100.10	0.136	0.03430	0.05075
	60	60.021	0.05023	100.03	0.083	0.04200	0.06213

***** Average five determinations

Table 7: Recovery data of the proposed spectrophotometric methods

Method	Spiked level	Concentration of OMP (µg/ml)			SD	RSD (%)	Recovery (%)
		Taken	Spiked	Found*			
M-1	50	10	5	15.046	0.01527	0.101	100.31
	100	10	10	20.053	0.00251	0.012	100.26
	150	10	15	25.039	0.03005	0.120	100.15
M-2	50	10	5	15.006	0.02702	0.180	100.03
	100	10	10	20.045	0.04186	0.208	100.22
	150	10	15	24.984	0.01665	0.066	99.86

*****Average five determinations

Table 8: Robustness of the proposed spectrophotometric methods

Method	Parameter	Concentration of OMP (µg/ml)		SD	Recovery (%)	RSD (%)
		Taken	Found*			
M-1	Volume of 0.1M HCl (6.0 ± 0.2 ml)	10	10.02	0.0374	100.20	0.373
		60	59.98	0.0141	99.96	0.023
	Volume of 0.2% TPooo (2.0 ± 0.2 ml)	10	9.98	0.0122	99.80	0.122
		60	60.01	0.0259	100.01	0.043
M-2	Volume of 0.1M HCl (6.0 ± 0.2 ml)	10	9.985	0.02258	99.85	0.226
		60	59.98	0.02727	99.96	0.045
	Volume of 0.2% BTB (2.0 ± 0.2 ml)	10	10.01	0.01876	100.10	0.187
		60	59.97	0.03527	99.95	0.058

*****Average three determinations

Table 9: Assay of OMP in syrup by the proposed spectrophotometric methods

Method	Labeled claim (mg)*	Found**	SD	% RSD	% Recovery	*t*-Value[$]	F-Value[$$]
M-1	50	50.010	0.0364	0.7290	100.12	0.6712	1.0332
M-2	50	50.001	0.03025	0.6049	100.02	0.1349	1.5150

* Average of five determinations

***** Labeled claim: mg/5ml of syrup sample, ** Average of three determinations

[$] tabulated *t* value - 2.306, [$$] tabulated F value - 6.39

The relative standard deviations and recoveries in the inter-day analyses for the proposed methods (M-1 & M-2) were calculated and are summarized in Table 6. From the results given in the Table 6, it was observed that the mean recoveries for methods M-1 & M-2 were in the range of 100.08-100.15%, 100.03-100.20%, With relative standard deviations in the range of 0.101-0.307%, 0.083-0.412% respectively.

The relative standard deviation indicated the high intra- and inter-day precision of the methods. The percent recovery indicated good accuracy and an agreement between the theoretical value and the real value of concentration. Thus, ensuring the proposed

methods (M-1 & M-2) are effective for the determination of the OMP.

Recovery study: The accuracy of the proposed methods (M-1 & M-2) was further evaluated by recovery study using standard addition technique. For this study, fixed concentration of the pure OMP was spiked to the preanalyzed dosage form. The resulting solutions were once again analyzed by proposed methods (M-1 & M-2). The percent recovery of OMP was then calculated using the corresponding calibration curve or regression equation. The percent recovery of the OMP was in the range of 100.15-100.31% and 99.86-100.22% for the methods M-1 & M-2, respectively. Good recoveries were obtained, which indicated the high accuracy of the and moreover, the absence of interference from the tablet excipients also indicated the selectivity of the proposed methods (M-1 & M-2). The results were summarized in Table 7.

Method robustness: In order to ascertain the robustness of the proposed methods (M-1 & M-2), minute deliberated changes were made in the following experimental conditions at two different concentration levels (M-1 & M-2 – 10 & 60 µg/ml).

Method M-1: Volume of 0.1M HCl solution varied from 5.8 ml to 6.2 ml at constant volume of TPooo reagent and volume of 0.2% TPooo changed from 1.8 ml to 2.2 ml at constant volume of 0.1M HCl solution.

Method M-2: Variation of volume of 0.1M HCl solution is 5.8 ml to 6.2 ml at constant volume of BTB reagent and volume of 0.2% BTB varied from 1.8 ml to 2.2 ml at constant volume of 0.1M HCl solution.

In the above-changed conditions, percentage relative standard deviation was in the range of 0.058-0.226%, 0.042-0.599% for the methods M-1 & M-2 respectively. The low (≤1.64%) relative standard deviation for all the parameters of robustness indicated that the proposed methods (M-1 & M-2) are robust for the quantification of the OMP (Table 8).

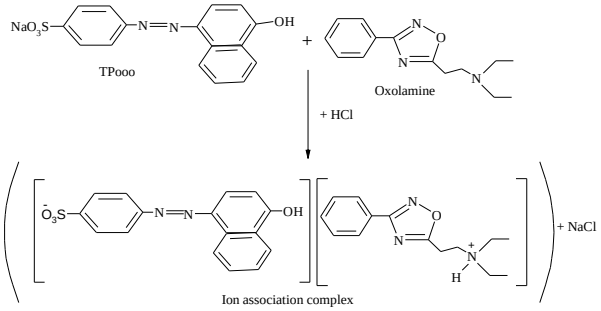
Application of the proposed methods:

To ascertain the suitability of the proposed methods (M-1 & M-2) for the assay of pharmaceutical formulation (oral syrup) containing OMP was analyzed. The results are summarized in Table 8. The excellent recovery with low relative standard deviation (≤1.39%) value confirmed the appropriateness of the proposed methods (M-1 & M-2) for the routine analysis of OMP in oral syrup dosage forms. The results obtained were further compared statistically by applying the Student's t-test and F-test for accuracy, precision respectively that are shown in Table 9. The calculated t-value and F-value at 95% confidence level did not exceed the tabulated values of 2.306 and 6.39, respectively, which indicated that there is no difference in the proposed methods with respect to accuracy and precision.

CHEMICAL BASIS OF THE COLORED PRODUCTS:

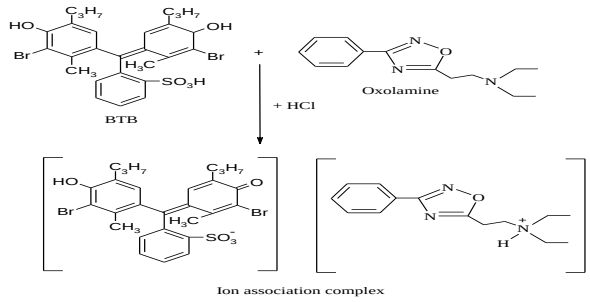
Method M-1:

The results obtained in this method were based on extractive spectrophotometry. The OMP exhibits basic character essentially due to the presence of amino group. Under acidic condition, the amino group of OMP was protonated and suphonic group of TPooo disassociates to form negative charge. The positively charged OMP and negatively charged TPooo form a yellow colored ion association complex that is extractable with chloroform from the aqueous phase. The ion association complex exhibits maximum absorption at 485 nm.



Method M-2:

The results obtained in the method M1 are based on extractive spectrophotometry. In acidic medium, the tertiary nitrogen atom of OMP is protonated to give positive charge and sulphonic group of BTB dissociates to give negative charge. The positively charged OMP forms chloroform extractable yellow colored ion-pair complex with negatively charged BTB. The complex showed absorption maxima at 430 nm against the reagent blank. Based on analogy the structure of ion association complex is shown in scheme.



CONCLUSION:

Oxolamine phosphate was determined in either bulk and in oral syrup dosage form by employing two visible spectrophotometric methods (M-1& M-2). The proposed methods are sensitive, precise, accurate, robust, rapid, convenient and cost effective since they do not require any special working conditions. The proposed methods are based on the characteristic properties of different functional groups such as secondary amine and tertiary amine group of OMP. Statistical analyses of the results have shown that the proposed methods (M-1 & M-2) have good precision and accuracy. Results of the analysis of OMP in the oral syrup dosage form revealed that the proposed methods (M-1& M-2) are suitable for the determination of OMP in syrup dosage form without interference from excipients.

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