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Research Article

NEW SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF OXOLAMINE IN FORMULATION

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ABSTRACT

Five simple, sensitive and economical spectrophotometric methods have been developed for the determination of Oxolamine in commercial dosage forms. The methods were based on the formation of colored complex of Oxolamine with different reagents. The absorbance of the formed color complex is measured at the wavelength of maximum absorbance of the complex against the reagent blank treated similarly. All these method have different linearity ranges. Statistical analysis proves that the proposed methods are reproducible and selective for the estimation of Oxolamine in bulk drug and in its tablet dosage form.

Keywords: Oxolamine, Spectrophotometric Methods, O-phenonthrolin, ARS, PNA, CTC.

INTRODUCTION

The syntheses of 1,2,4-oxadiazole systems all rely on hydroxylamine for providing a preformed N-O linkage. The preparation of the respiratory anti-inflammatory and antitussive agent oxolamine starts by acylation of the alkoxide from the N-hydroxyamidine with 3-chloropropionyl chloride; the presence of the negative charge on oxygen results in the formation of the O-acylated product. Treatment of that intermediate with triethylamine leads to cyclization via imine formation to afford the 1,2,4-oxadiazole. Displacement of the terminal chlorine with diethylamine gives the the corresponding amine Oxolamine.

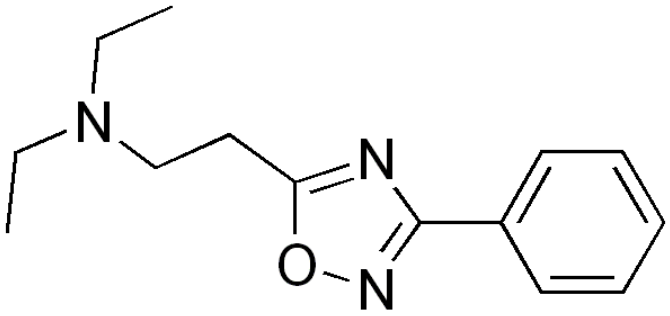

Figure 1: Structure of Oxolamine

Experimental Procedure:

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Double beam UV-Visible Spectrophotometer is used for measuring the absorbance's of the color formed during the analysis.

Preparation of reagents:

O-phenonthrolin: Weigh accurately 200 mg of O-phenonthrolin and was dissolved in 100 ml of distilled water with warming.

Fe (III) solution: Accurately 250 mg of anhydrous ferric chloride was weighed and was taken in a100 ml graduated volumetric flask. It was dissolved in little amount of distilled water and the final volume was made up to the mark with distill water.

Alizarin Red S (ARS) solution: weigh 200 mg of ARS and is dissolved in 100ml of distill water.

Woll Faster Blue Black (WFBBL) solution: weigh 200 mg of WFBBL and is dissolved in 100ml of distill water.

Para Nitro Aniline (PNA) solution: Accurately 100 mg of PNA was weighed and was taken in a 100 ml

graduated volumetric flask. It was dissolved in 0.2 M HCl solution and made up to the mark.

$NaNO_2$ solution: accurately 100 mg of $NaNO_2$ was weighed and was taken in a 100 ml graduated volumetric flask. It was dissolved in distilled water and made up to the mark.

NaOH solution (4 %, 1M): Accurately 4g of NaOH was weighed and was taken in a 100ml graduated volumetric flask. It is dissolved in distilled water and made up to the mark.

Cobalt Thiocyanate solution: The solution was prepared by dissolving 7.25g of cobalt nitrate and 3.8g of ammonium thiocyanate in 100ml of distill water.

P^H 2 Buffer solution: the solution was prepared by mixing 306ml of 0.1M tris sodium citrate with 694ml of 0.1M HCL and the P^H adjusted to 2.

HCl solution (1N): Prepared by diluting 86 ml of conc. HCl to 1000 ml with distilled water and standardized.

Preparation of working standard drug solution:

The standard Oxolamine (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg /ml (stock solution I). 20 ml of stock solution I was diluted to 100 ml with Methanol (Stock solution II, 200µg/ml) and the resulting solution was used as working standard solution.

METHODS

O-phenonthrolin Method: (M1)

From the standard stock solution II of Oxolamine, appropriate concentration(30 to180 ppm) is pipetted out in to a 10 ml volumetric flasks add 0.5 ml FeCl3 solution and 2 ml of 1,10 Phenonthroline were added. The tube was heated in water bath up to 30 min. after cooling the tube 2 ml of acid was added and make up to 50 ml with distilled water. Make up to 50 ml volume. The absorbance of the formed color was measured after 5min at 500 nm against a reagent blank.

ARS Method: (M2)

.In a series of 125 ml separating funnels containing aliquots of standard drug (15-90ppm) solution was taken. To this 6ml of HCl solution and 2ml of ARS solutions were added successively. The total volume of the aqous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 440nm against a similar reagent blank.

WFBBL Method: (M3)

In a series of 125 ml separating funnels containing aliquots of standard drug solution (5-30ppm) was taken. To this 6ml of HCl solution and 2ml of WFBBL solutions were added successively. The total volume of the aqous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 600nm against a similar reagent blank.

PNA method: (M4)

In a 10 ml graduated test tubes 1.0 ml of PNA solution and 1.0 ml of $NaNO_2$ solution were successively added and allowed to stand for 2 min. Later, standard drug of elected concentration (20-120ppm) is delivered into the test tube. Then 1.5 ml of NaOH solution was added and the volume in each tube was made up to 10 ml distil water. Solution attains green colour. The maximum absorbance was measured at 440nm against a reagent blank (colourless).

Cobalt Thiocyanate Method: (M5)

In to a series of 125ml separating funnels, aliquots of standard drug (4-24ppm) solution were taken. Then add 2ml of buffer solution and 8ml of Cobalt Thiocyanate solution. The volume of each aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel, 10ml of Nitrobenzene was added and the contents were shaken for 2min. the two phases were separated and organic layer was collected. The absorbance of the organic layer was measured at 680nm against a similar reagent blank.

Assay Procedure for Formulations:

An amount of finely ground tablet powder equivalent to 100 mg of Oxolamine (Jakafi - 10mg) was accurately weighed into a 100 ml calibrated flask, 60 ml of water added and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well, and filtered using a Whatman No 42 filter paper. First 10 ml portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1000 µg mL^{-1} Oxolamine) was diluted appropriately to get suitable concentrations for analysis by proposed methods.

Method Validation:

Selection of analytical concentration ranges: (linearity test)

Linearity test was evaluated by measuring the absorbance values of standard solutions. The standard stock solution of Oxolamine, appropriate aliquots were pipetted out in to a six or seven series of volumetric flasks and add the solutions required in required for each individual method. After color formation absorbance of each concentration was measured at wavelength found for the proposed method. Results were shown in Table: 1

Figure 1: Calibration curves for the proposed methods

S.NO	Parameter	M1	M2	M3	M4	M5
1	Wavelength Max	500nm	440nm	600nm	440nm	680nm
2	Concentration Range	40-100ppm	20-80 ppm	2-12ppm	40-100ppm	5-30ppm
3	Correlation coefficient	0.9991	0.9993	0.9998	0.9994	0.9997
4	Slope	0.028	0.096	0.055	0.023	0.031
5	Intercept	0.042	0.036	0.033	0.037	-0.018
6	RSD of Precision	0.39	0.00	0.34	0.42	0.79
7	Average recovery	100.55	100.21	98.96	99.33	100.55
8	Stability period	180 min	240min	100min	160min	300 min
9	LOD	0.75ppm	0.025ppm	0.075ppm	0.75ppm	0.02ppm
10	LOQ	2.5ppm	0.08ppm	0.25ppm	2.5ppm	0.066ppm
8	% Assay of Formulation	98.73	98.85	98.60	99.12	98.50

Table 1: Summery results of the proposed methods

Precision:

To evaluate the accuracy and precision of the methods, pure drug solution (Within the working limits) was analyzed and being repeated six times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision for the proposed methods (Table 2).

SNO	M1	M2	M3	M4	M5
Concentration	60PPM	40PPM	6ppm	60PPM	25PPM
1	0.47	1.253	0.507	0.63	1.24
2	0.65	1.219	0.506	0.71	1.35
3	0.46	1.20	0.503	0.56	1.46
4	0.93	1.246	0.509	0.92	1.91
5	0.44	1.289	0.508	0.58	1.26
6	0.63	1.203	0.515	0.161	1.63
RSD	0.26	0.55	0.79	0.52	0.98

Table 2: Precision results of the proposed methods

Recovery Studies:

To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug was added to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The Percentage recoveries thus obtained were given in Table 3.

Method	Recovery	% of recovery	Average Recovery
M1	50%	99.38	100.26
	100%	100.29	
	150%	101.1	
M2	50%	99.2	100.18
	100%	100.45	
	150%	100.9	
M3	50%	98.6	99.79
	100%	99.05	
	150%	98.77	
M4	50%	98.3	99.46
	100%	98.65	
	150%	101.43	
M5	50%	98.25	100.17
	100%	101.5	
	150%	100.75	

Table3: Recovery results of the proposed methods

Application to Analysis of Commercial Sample:

In order to check the validity of the proposed methods, Oxolamine was determined in commercial formulation. From the results of the determination it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. These results indicating that there was no significant difference between the proposed methods and the reference methods in respect to accuracy and precision.

S.NO	Method	Formulation	Amount prepared	Amount found	% Assay
1	M1	Cidox (50mg/ml)	60 ppm	59.23	98.71
2	M2	Cidox (50mg/ml)	40 ppm	39.88	99.7
3	M3	Cidox (50mg/ml)	6 ppm	5.99	99.83
4	M4	Cidox (50mg/ml)	60ppm	59.74	99.56
5	M5	Cidox (50mg/ml)	25 ppm	24.93	99.84

DISCUSSION

Method M1 is based on the mechanism of oxidation followed by complex formation, where in the initial reaction the anti-oxidant undergoes oxidation in the presence of ferric chloride and then the oxidized ferric chloride reacts with 1,10- phenanthroline and the drug to form a orange red colored complex which exhibits maximum absorption at wavelength of 500 nm.

In ARS and WFBBL methods drug being a base form an ion association complex with acid dyes ARS and WFBBL. The formed complex is extractable in to Chloroform from the aqueous phase. The protonated nitrogen positive charge of the drug molecule in acid medium is expected to attack the positive charge of the dye. Hence form a colored complex which is extracted with Chloroform. The obtained color chromogen show absorbance at 440nm for ARS Method and 600nm for WFBBL method.

PNA method involves the diazotization of PNA with sodium nitrate fallowed by coupling with drug in alkaline medium. The formed PNA- DRUG complex develop green color, the developed color can be estimated by using spectrophotometer at a wavelength 440 nm.

Cobalt thiocyanate is a valuable reagent for the detection and determination of Amino compound. A coordinate complex is formed when the secondary amine group of the drug is treated with Cobalt thiocyanate. The formed complex shows color. The colored complex is extractable with the Nitrobenzene from the aqueous solution. The obtained color shows absorbance at 680nm.

The linearity ranges of Oxolamine are found to be 40-100ppm, 20-80 ppm, 2-12 ppm, 40-100ppm, 5-

30ppm for M1 to M5 respectively. A linear correlation was found between absorbance and concentration of Oxolamine. The graphs showed negligible intercept and are described by the equation: Y = a + bX (where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in ug mL-1 max) .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 according to ICH guide

The accuracy of the proposed methods was further ascertained by performing Accuracy studies. The Relative standard deviations of results for the proposed were very low and the values are within the range below 2. It indicates that the high accuracy and precision for the proposed methods. The Recovery results were very close to the actual range and it revealed that co-formulated substances did not interfere in the determination.

CONCLUSION

Five useful micro methods for the determination of Oxolamine have been developed and validated. The methods are simple and rapid taking not more than 20-25 min for the assay. These spectrophotometric methods are more sensitive than the existing UV and HPLC methods, and are free from such experimental variables as heating or extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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