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Research Article

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Spectrophotometric estimation of oxolamine citrate in bulk and pharmaceutical dosage form by first order derivative and area under curve methods

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ABSTRACT

A simple and precise first order derivative and area under curve [AUC] UV- spectrophotometric methods have been developed and validated for the estimation of oxolamine citrate in bulk and its tablet formulation. The standard and sample solutions of oxolamine citrate were prepared in distilled water. Oxolamine citrate was estimated at 229.2 nm for the first order derivative UV-spectrophotometric method (A), while in area under curve (AUC) method (B) the zero order spectrum of oxolamine citrate was measured in between 228.6 nm to 246.4 nm. Beer's law was obeyed in the concentration range of 1 to 14 µg / ml with coefficient of correlation value 0.9991 for first order derivative method. Similarly in AUC method, Beer's law was obeyed in the concentration range of 1 to 14 µg / ml with coefficient of correlation value 0.9999. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation, which was within the range of 1.06915 % to 2.2714 % for the above two methods. The proposed methods were successfully applied for the determination of oxolamine citrate in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Oxolamine citrate, Derivative spectroscopy, Area under curve method.

INTRODUCTION

In this communication the present work proposes UV spectrophotometric methods for assay of oxolamine citrate from bulk drug and pharmaceutical formulation. It's chemical name is 5- (2 -[diethyl amino] ethyl) 3-phenyl-1,2,4 oxadiazole citrate. Oxolamine is an anti-inflammatory drug. This drug is in Chemical Abstracts Service Registry Number [1]. Drug is not official in any pharmacopeia. Literature survey reveals liquid chromatography methods [2-3] for assay of this drug. Rapid, simple and reliable UV spectrophotometric methods are developed for the determination of oxolamine citrate. These methods can be used for the routine analysis and research organization. In the proposed work optimization and validation of these methods are reported. The structure of oxolamine citrate is shown in Fig.1.

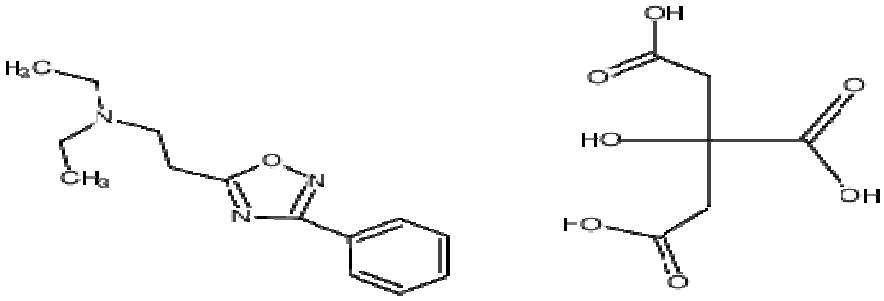
EXPERIMENTAL SECTION

Instrument and reagents

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral

measurements were done by using UV-Probe 2.42 software. Reference standard of oxolamine citrate was obtained from reputed firm with certificate analysis.

Fig. 1: Chemical structure of oxolamine citrate



Preparation of standard drug solution

100 mg standard oxolamine citrate was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of distilled water for 15 minutes. The volume was made up to the mark with distilled water to give a stock solution of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with distilled water to give a working standard solution of concentration 100 µg/ml.

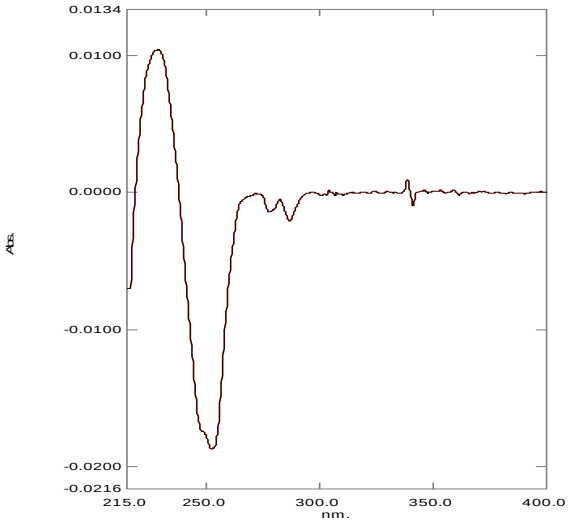
Estimation from tablets

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of oxolamine citrate was weighed and transferred in 100 ml of volumetric flask. A 30 ml of distilled water was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with distilled water to give concentration as 100 µg /ml. Such solution was used for analysis.

Method A: First order derivative method

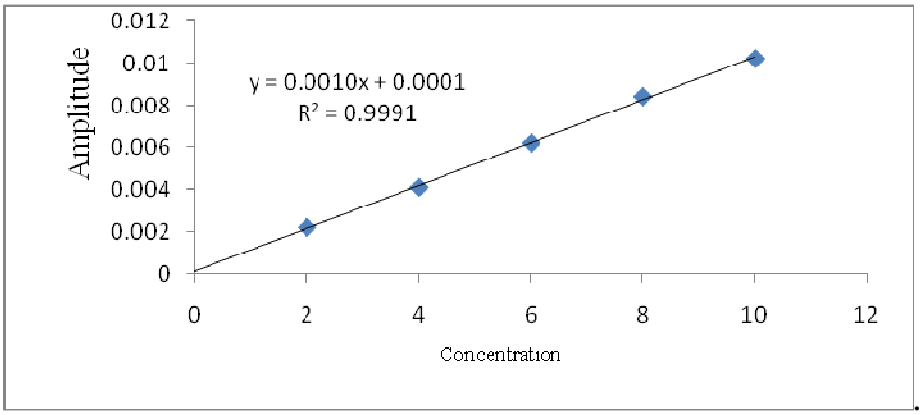
For the selection of analytical wavelength, 10 µg/ml solution of oxolamine citrate was scanned in the spectrum mode from 400 nm to 190 nm by using distilled water as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured between 230 nm to 228 nm (Fig. 2).

Fig. 2. First order derivative spectrum of oxolamine citrate (10 µg/ml) showing absorbance at 229.2 nm



Into series of 10 ml graduated flask, varying amount of sample solutions of oxolamine citrate were pipetted out and volume was adjusted with distilled water. Solutions were scanned between 400 nm to 190 nm in spectrum mode. The first order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at between 230 nm to 228 nm by using distilled water as blank. The calibration curve was prepared in the concentration range of 1 to 14 µg/ml (Fig. 3).

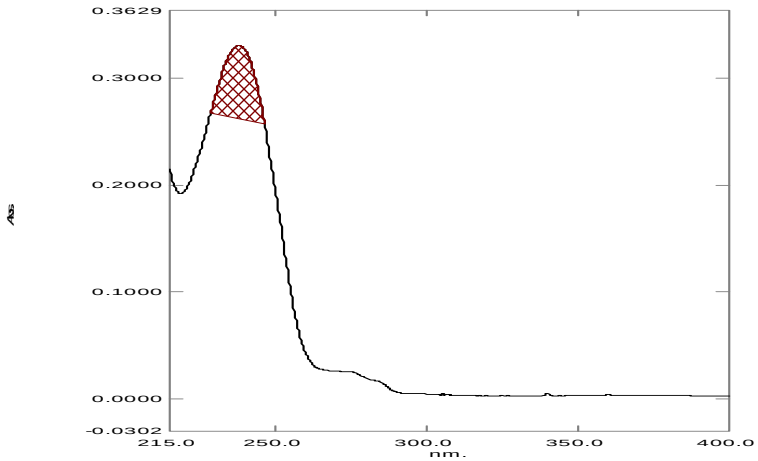
Fig. 3. Calibration curve for oxolamine citrate at 229.2 nm by first order derivative spectroscopy



Method B: Area under curve (AUC) method

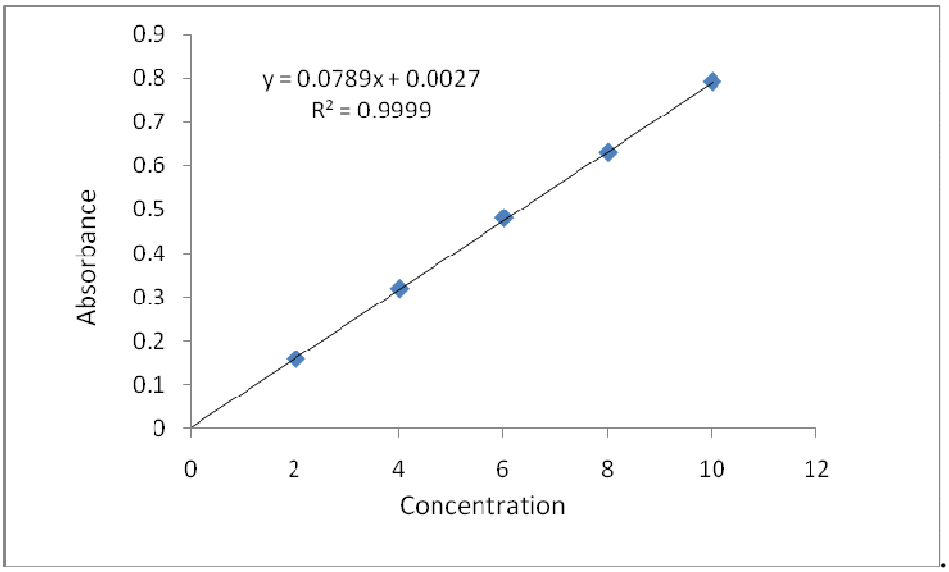
Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 was calculated by UV probe 2.42 software. In this method, 10 µg/ml solution of oxolamine citrate was scanned in the spectrum mode from 400 nm to 190 nm. From zero order spectrum the AUC calculation was done. The AUC spectrum was measured between 228.6 nm to 246.4 nm (Fig. 4).

Fig. 4. Area under curve spectrum of oxolamine citrate (10 µg/ml) showing area from 228.6 nm to 246.4 nm



Into series of 10 ml graduated flask, varying amount of sample solutions of oxolamine citrate were pipetted out and volume was adjusted with distilled water. Solutions were scanned between 400 nm to 190 nm in spectrum mode. The AUC calculations were done and the calibration curve for oxolamine citrate was plotted in the concentration range of 1 to 14 µg/ml (Fig. 5).

Fig. 5. Calibration curve for oxolamine citrate by area under curve spectroscopy



Results of the analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	First order derivative method	Area under curve method (AUC)
Detection wavelength in nm	229.2	228.6-246.4
Beer's law limits (µg/ ml)	1-14	1-14
Correleation cofficient (r^2)	0.9991	0.9990
Regression equation (y = mx + c)		
Slope (a)	0.0010	0.0789
Intercept (b)	0.0001	0.0027

VALIDATION

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table (2, 3).

Table 2: Results of recovery of oxolamine citrate for first order derivative method

Amount of sample added in (µg/ ml)	Amount of standard added in(µg/ ml)	Total amount recovered in µg/ ml	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (%) C.O.V.)
2	0	2.000	100.000	0.0777	3.885
2	2	3.9456	98.640	0.0749	1.8983
2	4	5.9723	98.54	0.0921	1.5417
2	6	7.9183	98.98	0.1394	1.7606

Table 3: Results of recovery of oxolamine citrate for area under curve (AUC) method

Amount of sample added in (µg/ ml)	Amount of standard added in(µg/ ml)	Total amount recovered in µg/ ml	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (%) C.O.V.)
2	0	2.0061	100.305	0.0477	2.3769
2	2	4.0025	100.125	0.2323	0.5566
2	4	5.9723	100.278	0.0416	0.6911
2	6	7.9183	100.344	0.0523	0.6520

Precision

The methods precision were established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical methods in seven replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the methods. The results obtained are tabulated in table 4.

Table 4: Precision- method precision

Experiment no.	Weight of oxolamine citrate taken in mg	contents of oxolamine citrate in mg.	Contents of oxolamine citrate in mg
		First order derivative method	Area under curve method
1	10	10.010	10.007
2	10	9.529	10.125
3	10	10.476	9.9765
4	10	10.008	10.009
5	10	9.529	10.009
6	10	10.476	10.100
7	10	10.010	9.765
	Standard deviation	0.3866	0.2381
	%R.S.D.	3.8641	2.3732

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of oxolamine citrate was transferred to 100 ml of volumetric flask. A 30 ml of distilled water was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with distilled water to give concentration as 100 µg /ml. Such solution was used for analysis.

For first order derivative method

Solution was scanned between 400 nm to 190 nm in spectrum mode. The first order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at between 230 nm to 228 nm by using distilled water as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 229.2 nm for first order derivative (method A). Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of oxolamine citrate was estimated by comparison with standard at 229.2 nm for first order derivative, table 5.

For area under curve method

Solution was scanned between 400 nm to 190 nm in spectrum mode. The area under curve of resulting solutions was measured at between 246.4 nm to 228.6 nm by using distilled water as blank. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 246.4 nm to 228.6 nm (method B). Similarly area under curve of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of oxolamine citrate was estimated by comparison with standard at 246.4 nm to 228.6 nm, table 5.

Table 5: Summary of validation parameter for intra-day and inter-day

Sr. No.	Parameters	First order derivative method	Area under curve method
1	Intra day precision (N=3) amount found ± % R.S.D.	99.60% 0.24847	99.45% 0.03446
2	Intra day precision (N=3) amount found ± % R.S.D.	98.484 0.13607	98.762% 0.00768
3	Rugadness analyst to analyst % R.S.D.	100.12% 0.6786	99.87% 0.00812

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$$LOD = 3.3\ \sigma/S \quad \text{and} \quad LOQ = 10\ \sigma/S$$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 6.

Table 6: Values of results of optical and regression of drug

Parameter	First order derivative method	Area under curve method
Limit of Detection (µg/ml)	0.26944	0.09929
Limit of Quantification (µg/ml)	0.81649	0.300090

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of oxolamine citrate sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of oxolamine citrate was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The first order derivative and area under curve UV-spectroscopic methods are useful for routine analysis of oxolamine citrate in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedure [4]. Oxolamine citrate has the absorbance maxima at 229.2 nm (method A) and in the AUC spectrum method areas were measured between 228.6 nm to 246.4 nm (method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 14 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2, 3. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the methods were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of oxolamine citrate in pharmaceutical formulation.

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