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Validation Method of Oxolamine Citrate from Bulk Drug and Pharmaceutical Formulation by High Performance Liquid Chromatography Method

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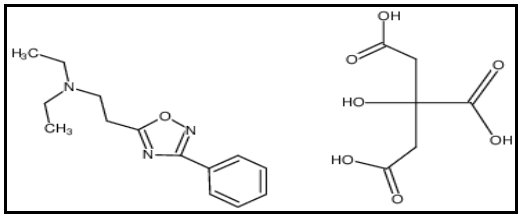
Abstract: A simple, rapid and accurate high performance liquid chromatography method is developed for determination of oxolamine citrates from active pharmaceutical ingredient. The assay of oxalamine citrate was achieved on BDS hypersil C18 (150 x 4.6 mm i.d.) with 5 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of buffer and acetonitrile (72:28 % v/v). The buffer was mixtures of 0.1 % tri-ethyl amine adjusted the pH 3.5 with ortho-phosphoric acid. The mixture of buffer of pH 3.5 and acetonitrile (50:50% v/v) was used as a diluent. The detection was carried out at wavelength 230 nm. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze oxolamine citrate from pharmaceutical formulation.
Keywords : Oxolamine citrate, HPLC, acetonitrile, triethylamine.

Introduction

In this communication the present work proposes high performance liquid chromatographic method 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 official in Chemical Abstracts Service Registry Number[1]. Literature survey reveals liquid chromatography methods[2,3] and spectrophotometric methods[4, 5] for assay of this drug. A rapid, simple and reliable high performance liquid chromatographic method is 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 the proposed work optimization and validation of this method are reported.

Chemical structure of oxolamine citrate



Material and Method

Chemical and reagents:

Reference standard of oxolamine citrate was obtained from reputed firm with certificate analysis. Tri-ethylamine, ortho-phosphoric acid and acetonitrile were used of analytical grade. The HPLC grade water was used of Millipore. Standard and sample solution was prepared in diluent (water: acetonitrile (50:50 % v/v)). Buffer solution was 0.1% tri-ethylamine with pH 3.5.

Instrumentation

The HPLC system Merck-Hitachi equipped with separation module and UV detector (L-7400) was used. The chromatogram was recorded and peaks are quantified by means EZChrom Elite software. A Shimadzu analytical balance with 0.01 mg was used.

Preparation of standard solution:

Standard solution

About 10 mg of standard oxolamine citrate was weighed accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent (water: acetonitrile (50:50 % v/v)) was added and sonicated for 5 minutes. The volume was adjusted to the mark with diluents to give concentration as 1000 µg/ ml. The working standard solution was prepared by diluting 1 ml of 1000 µg/ ml solution to 10 ml with diluent to get concentration 100 µg/ ml.

Sample preparation:

Twenty tablets were weighed accurately and average weight of each tablet was determined. The powder equivalent to 10 mg of oxolamine citrate was weighed accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent (water: acetonitrile (50:50 % v/v)) was added and sonicated for 5 minutes. The volume was adjusted up to mark with diluent to give concentration as 1000 µg /ml. The working sample solution was prepared by diluting 1 ml of 1000 µg/ ml solution to 10 ml with diluent to give 100 µg/ ml.

Chromatographic conditions

Chromatographic separation was performed at room temperature on BDS Hypersil C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. Mobile phase consisted of buffer 0.1% tri-ethylamine where pH is adjusted with ortho-phosphoric acid and acetonitrile (72:28 % v/v). The mobile phase was filtered and degassed. The flow rate of the mobile phase was adjusted to 1.0 ml /min. The detector wavelength was set at 230 nm. The injection volume of the standard and sample solution was 10 µl.

Method Development

Different columns containing octyl and octadecyl silane stationary phase were tried for separation and resolutions. It was found that BDS Hypersil C18 (150 x 4.6 mm i.d.) with 5 µ particle size column offered more advantage over other columns. Drug solution was injected into column. Elution and resolution parameters of drug were recorded at the wavelength range 200 nm to 380 nm and its response optimization was compared. The choice of wavelength 230 nm was considered satisfactory, permitting the detection of the drugs with adequate sensitivity. It produced well shaped peaks for the drug assay. A UV spectrum of the drug is given in fig 1 and typical chromatogram of the drug assayed is depicted in fig. 2.

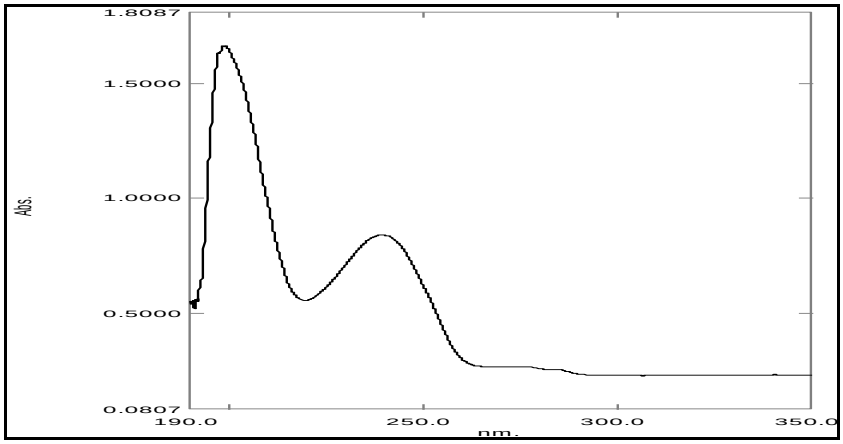


Fig. 1 UV spectrum of oxolamine citrate

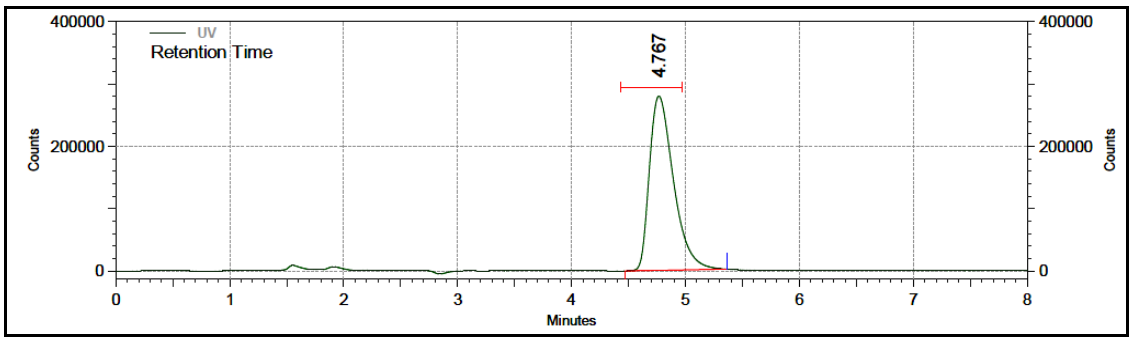


Fig 2.: Chromatogram of oxolamine citrate (standard)

Method validation

System suitability

System performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N) and symmetry factor were shown in Table-1.It indicated good performance of the system.

Table – 1: System performance parameters for oxolamine citrate. (n = 6).

Drug	Retention time	symmetry factor	No. of plates
Oxolamine citrate	4.767	1.54	2461

***Calculated at 5% peak height , $^{+}$Calculated as N = $16(t_R/W)^2$**

Linearity

The linearity of the method was determined for oxolamine citrate at six concentrations level ranging from 50 to 150µg/ml. The calibration curve was constructed by plotting response factor against concentration of the drugs. The regression equation was given as y = 39149 x + 99738. The correlation coefficient (r^2) was 0.9993 and concentration range indicated above. The results of the same are tabulated in the table 2.

Table 2 : Linearity – regression analysis data

Parameters	Values
Correlation Coefficient (r)	0.9993
Intercept (y)	99738
Slope (m)	39149

Y =mx + c

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and percentage recovery was calculated and presented in Table 3.

Table 3 : Accuracy - %Recovery

level	test	Weight in mg	area	Quantity added in µg /ml	Quantity recovered in µg /ml	% recovery	mean recovery
80%	1	10.33	3254164	40.36	39.94	98.97	**98.97**
	2	10.45	3258212	40.36	39.99	99.09	
	3	10.3	3250080	40.36	39.89	98.84	
100%	1	10.11	4052668	50.45	49.74	98.60	**98.68**
	2	10.21	4054979	50.45	49.77	98.66	

	3	10.13	4060044	50.45	49.83	98.78	
150%	1	10.26	4847096	60.54	59.50	98.27	**98.28**
	2	10.26	4850050	60.54	59.53	98.33	
	3	10.28	4845672	60.54	59.48	98.25	
					Mean recovery of all level		**98.64**

** Average of triplicate analysis.*

Precession

The method precision was established by carrying out the analysis of oxolamine citrate. The assay was carried out of the drug using analytical method in six replicates. The value of relative standard deviation lies well with the limits (0.10 %). The results of the same are tabulated in the table 4.

Table 4 : Precision – method precision.

Test	Weight of standard	Area	% assay
Test solution -1	10.31	4102018	99.13
Test solution -2	10.41	4099102	100.02
Test solution -3	10.45	4089068	100.16
Test solution -4	10.39	4092060	99.66
Test solution -5	10.36	4100105	99.56
Test solution -6	10.4	4102615	100.01
	Mean Assay		**99.76**
	SD		**0.383**
	RSD		**0.384**

Stability of solution

The stability studies of the solutions under study were established by keeping the solutions at room temperature for 24 hours. The results indicated no significant change in the assay results of the same solutions. It confirmed the stability of the drug in the solvents used for the analysis.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions.
Variation in flow rate ± 0.2˚ C.
Variation in mobile phase ± 0.2 units
Variation in wavelength ± 0.5 units
No marked changes in the chromatograms demonstrated that HPLC method developed was robust.

Method Application

The validated high performance liquid chromatographic method was applied for determination of oxolamine citrate its formulation. Twenty tablets of oxolamine citrate were used. A portion equivalent to 10 mg of oxolamine citrate was weighed accurately. It was dissolved in 10 ml of diluent to obtain final concentration 1000 µg/ml. The working sample solution was prepared by diluting 1 ml of 1000 µg/ ml solution to 10 ml with diluent to give 100 µg/ ml. 10 µl of this solution was injected under specified conditions. The analyte peaks were identified by comparison with respective standard and chromatogram was recorded. (Fig. no.3)

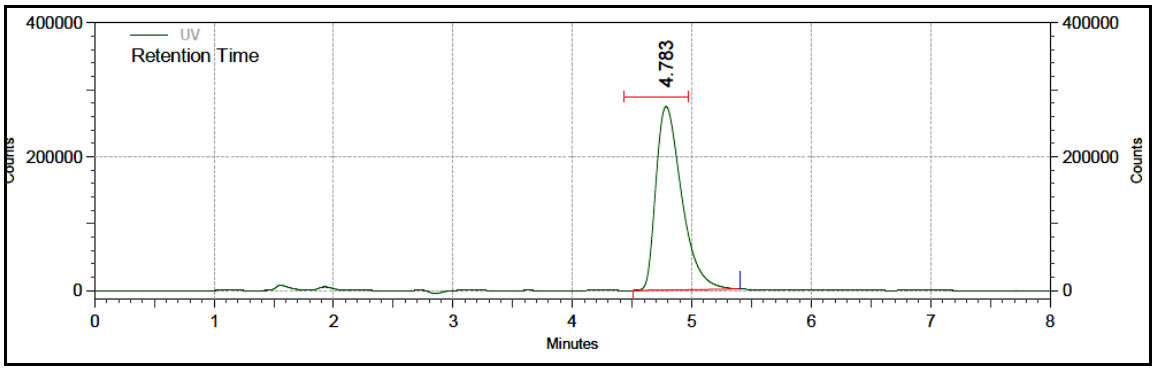


Fig 3: Chromatogram of oxolamine citrate (sample)

The assay results expressed as mg / tablets are shown in Table-3. It indicated the amount of each drug in the product meet the requirement.

Conclusion

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation in comparison to previous methods. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drugs to the pre analyzed formulation and reanalyzing the mixture by proposed method. The percent recovery obtained indicates non- interference from the excipients used in the formulations.

Thus the proposed RP-HPLC method for the estimation of oxolamine citrate in dosage forms is precise, accurate, linear, robust, simple and rapid. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, formulations and dissolution studies.

Acknowledgement

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