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STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF OXOLAMINE CITRATE IN A PHARMACEUTICAL FORMULATION

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ABSTRACT

A stability indicating HPLC method for the estimation of oxolamine citrate in tablets was developed and validated. Oxolamine citrate is a cough suppressant. The HPLC method was performed with a reversed phase Zodiac C18 column (250 mm X 4.6 mm id, 5mm particle size), detection at 245 nm and a mixture of methanol, water and Acetonitrile as mobile phase. Typical retention time for oxolamine citrate was 7.25 min. Forced degradation studies were carried out. The drug was found to be stable to the dry heat, photo-degradation, oxidation, basic, and acidic condition attempted which indicate drug is highly stable. Quantification was achieved with ultraviolet detection at 245 nm over the concentration range 15 –90µg/ml with range of recovery 98.9 – 100.3 % for oxolamine citrate by the RP-HPLC method. The method was statistically validated for linearity, accuracy, precision and selectivity following ICH recommendations. Due to its simplicity and accuracy, the method can be used for routine quality control analysis.

Key Words: Validation; RP-HPLC; Oxolamine citrate.

INTRODUCTION

Oxolamine citrate is one of the synthetic derivatives of 3, 5-disubstituted 1,2,4-oxodiazole. It is used against the common cold, particularly for its antitussive activity [1-4]. It is a highly potent and effective drug used to treat all types of cough of various etiologies [5-8]. It is available in single or in combination form. The usual dose of the drug is 200 mg given four times a day.

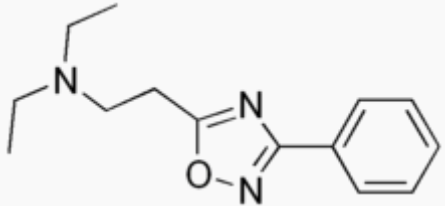

Fig. 1 Structure of Oxolamine citrate

Its use was limited by side-effects of nausea and vomiting. In order to prevent the disadvantages caused by taking the drug four times daily, and to reduce the side-effects, a sustained-release dosage form of oxolamine citrate was prepared by the microencapsulation technique and microcapsules thus formed were pressed into tablets. Very few studies have been reported on Oxolamine citrate [9-15]. Only one HPLC method in the literature report the simultaneous quantification of acetaminophen, chlorpheniramine and phenylephrine in combined pharmaceutical dosage forms [16].

EXPERIMENTAL

APPARATUS

A Series HPLC system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Zodiac C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Materials

Working standard of Oxolamine citrate was obtained from well reputed research laboratories. HPLC grade Acetonitrile, water, Methanol was purchased from E. Merck (Mumbai, India).

Chromatographic Conditions

The Zodiac C18 column was used at ambient temperature. The mobile phase consisted of methanol and Acetonitrile and the flow rate was maintained at 0.9ml/min. The mobile phase was passed through nylon 0.45µm– 47mm membrane filter and degassed before use. The elution was monitored with UV detector at 245nm, and the injection volume was 20µL.HPLC method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

Preparation of Standard Stock Solutions (100µg/ml)

Accurately weighed 25mg of Oxolamine citrate transferred to a 25ml volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard solution of1000µg/ml. This solution (1ml) was further diluted to10 ml with mobile phase to obtain a working standard stock solution of 100µg/ml for the RP- HPLC method. Required concentrations Oxolamine citrate was prepared from working standard.

Preparation of Sample Solutions

Twenty tablets were weighed and finely powdered. A mass equivalent to 10mg of Oxolamine citrate was weighed and transferred in a 100ml volumetric flask, mixed with mobile phase (60ml), and sonicated for 20min. The solution was filtered through Millipore filter paper. An aliquot of this solution was further diluted to with mobile phase to obtain a solution containing 45µg/ml of Oxolamine citrate and subjected to RP-HPLC analysis.

Method Validation
Linearity and range

Calibration curves were constructed by plotting peak areas versus concentrations of Oxolamine citrate, and the regression equations were calculated. The calibration curves were plotted over the concentration range 15–90µg/ml. Accurately measured standard working solutions of Oxolamine citrate 15, 30, 45, 60, 75, 90µg/ml were prepared with mobile phase. Aliquots (20µl) of each solution were injected under the operating chromatographic conditions described above.

Accuracy (recovery)

The accuracy of the method was determined by calculating recoveries of Oxolamine citrate by the standard addition method. Known amounts of standard solutions of Oxolamine citrate (50, 100, and 150%) were added to pre quantified sample solutions of tablets. The amounts of Oxolamine citrate were determined and given in table 3.

Method precision (repeatability)

The precision of the instruments was checked by repeatedly injecting (n = 6) solutions of Oxolamine citrate (45μg/ml) for the RP-HPLC method. Intermediate precision was evaluated in terms of intraday and interday precision. The intraday precision was investigated using different concentrations of standard solutions and sample solutions. The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses three times on the same day and on three different days over a period of 1 week for different concentrations of Oxolamine citrate standard and sample, respectively. The results were reported in terms of % RSD.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the effect on resolution was recorded. There was no detrimental effect on the method performance as shown. Low value of relative standard deviation was indicating that the method was robust.

LOD and LOQ

The LOD was determined by the analysis of samples with known concentrations of analyte and by establishing through visual evaluation the minimum level at which the analyte could be reliably detected. The LOQ was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be quantified with acceptable accuracy and precision.

System suitability

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of Oxolamine citrate to be performed. The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values with the acceptance criteria. A system suitability test of the Chromatography system was performed before each validation run. Six replicate injections of a system suitability/calibration standard and one injection of a check standard were made. Area, retention time (RT), tailing factor, asymmetry factor, and theoretical plates for the six suitability injections were determined.

Stability of standard and sample solutions

Stability of standard and sample solution of Oxolamine citrate was evaluated at room temperature for 48 hr. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were stable up to 48 hr at room temperature.

Forced degradation study

All solutions prepared for use in forced degradation studies were prepared to yield a starting Oxolamine citrate concentration of 45µg/ml.
(a) Oxidation: Solutions of Oxolamine citrate (45µg/ml) for oxidation studies were prepared using 3% H2O2 and the resultant solutions of was stand for 24 hr to facilitate oxidation of the Oxolamine citrate.

(b) Acid degradation: Solutions of DONE (45µg/ml)for acid degradation studies were prepared using0.1N HCl, in methanol and the resultant solution was stand for 24 hr.

(**c**) Alkali degradation: Solutions of Oxolamine citrate (45µg/ml)for alkali degradation studies were prepared using 0.1N in methanol and the resultant solution was stand for 24 hr.

(d) Dry heat: Solutions for dry heat studies were prepared by exposing powder to dry heat (80oC) in an oven for 2 days. The powder was removed from the oven, and Oxolamine citrate was accurately weighed and transferred to a volumetric flask to give a final Oxolamine citrate concentration of 45µg/ml.

(**e**) Sunlight radiation.: Solutions of Oxolamine citrate (45µg/ml) in methanol for photolytic study were prepared by exposing powder to sunlight (35oC) to determine the effects of light irradiation on the stability of Oxolamine citrate. Samples were placed in direct sunlight for 24 hr. The sample was removed from the sunlight, and solutions were prepared for analysis as previously described.

Determination of Oxolamine citrate in Tablets
Tablets of Oxolamine citrate were purchased from local market .The responses of tablet solutions measured with the UV detector showed a wavelength maximum at 245nm for the RP-HPLC method. The amounts of Oxolamine citrate present in sample solution were determined by fitting the responses into the regression equation for Oxolamine citrate.

Parameter	**Oxolamine citrate**
Retention times (RT)	7.25 Min
HPLC Plate Count	12339
Tailing factor	1.78
Area	287611

Table1.System suitability test parameters for Oxolamine citratae

Parameter	**Oxolamine citrate**
Linearity (µg/ml)	15 – 90
Correlation co –efficient (r)	0.999
Slope of Regression(S)	6579.9
Intercept of Regression	-839.714

TABLE 2: Regression analysis of calibration graphs for Oxolamine citrate by proposed HPLC method

% Recovery	Oxolamine citrate				
	Target Conc., (µg/ml)	**Spiked conc, (µg/ml)**	**Final Conc, (µg/ml)**	**Conc., Obtained**	**% of Recovery**
50%	30	15	45	44.8	99.7
	30	15	45	45.1	100.2
	30	15	45	44.8	99.5
100%	30	30	60	59.3	98.9
	30	30	60	60.07	100.1
	30	30	60	59.8	99.6
150%	30	45	75	75.2	100.3
	30	45	75	74.4	99.2
	30	45	75	74.7	99.7

TABLE 3 : Data derived from accuracy of Oxolamine citrate the proposed HPLC method

Parameters		**Oxolamine citrate**
LOD		0.25µg/ml
LOQ		0.8µg/ml
Accuracy%		98.9 – 100.3 %
Ruggedness, (% RSD, n = 6)		0.81
Precision (% RSD)	Interday (n = 6)	0.79
	Intraday(n=6)	1.16

TABLE 4: Summary of validation parameters for Oxolamine citrate the proposed HPLC method

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	287611	……
2	MP	Acetonitrile : Water Mp-1 82:17 Mp-2 87:13	 287659 283231	 0.01 1.52
3	FL change	0.8 1.0	283093 283133	1.57 1.55
4	WL	242nm 248nm	284380 283133	1.12 0.7

TABLE 5: Data derived from robustness of Oxolamine citrate the proposed HPLC method

Condition after 48 hours	Observation
Standard	No degradation
3% Peroxide	Oxalamine Citrate degraded in to three compounds.
0.1 N Basic	Oxalamine Citrate degraded in to two compounds
0.1 N Acidic	Oxalamine Citrate degraded in to three compounds
Sun light	Oxalamine Citrate degraded in to one compounds
UV light	Oxalamine Citrate degraded in to two compounds
Aqueous (HPLC)	Oxalamine Citrate degraded in to two compound
Thermal (thermal)	Standard peak was spited into two peaks

TABLE 6: Data derived from deagradation of Oxolamine citrate the proposed HPLC method

Time (in Hours)	**Area found**	**% Assay**
0	287611	100
2	292303	101.6314
4	289428	100.6318
6	287990	100.1318
12	283970	98.73405
18	283799	98.6746
24	291184	101.2423
36	282823	98.33525
48	284415	98.88878

TABLE 7: Data derived from stability of Oxolamine citrate the proposed HPLC method

Formulation	**Brand name**	**Sample conc**	**Area**	**Amount found**	**% Assay**
Tablet	Oledro Tablet (100mg) combination drug	45µg/ml	283434	44.35	98.55

TABLE 8: Formulation analysis of Oxolamine citrate

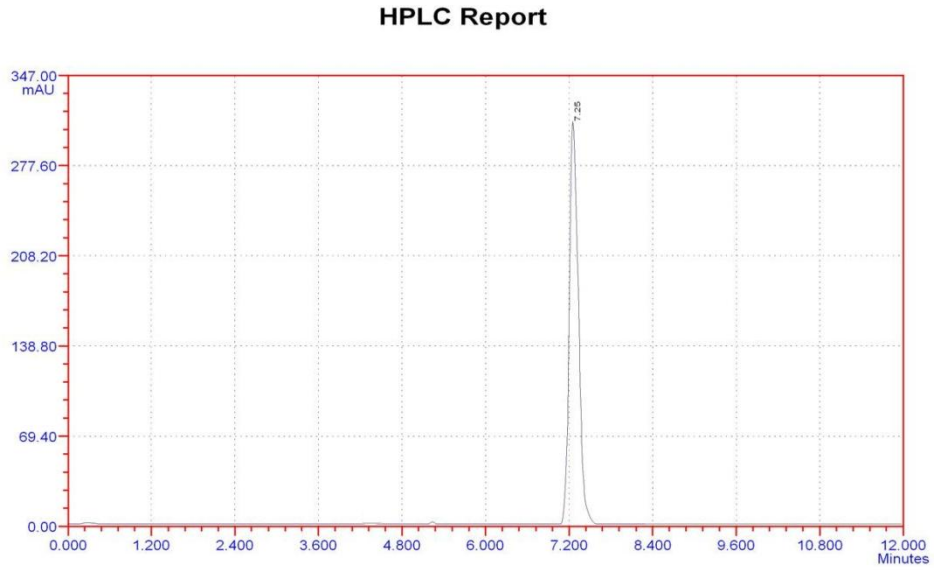

Figure 2: standard chromatogram of Oxolamine citrate

RESULT AND DISCUSSION

Optimization of the chromatographic condition

Several mobile phases were tried to resolve Oxolamine citrate but the resolution was not satisfactory. So modification was made in the above mobile phase. Finally the system containing methanol: Acetonitrile as the mobile phase at a flow rate of 0.9ml/min was found to be satisfactory and gave well resolved peak for Oxolamine citrate. The retention time for Acetonitril was 7.25 min. For the selection of detection wavelength, the spectrum of 10µg/ml Acetonitril revealed that, at 245 nm the drug possesses significant absorbance. So considering above fact, 245 nm was selected as a detection wavelength for estimation of Oxolamine citrate using HPLC. Complete resolution of the peaks with clear base line separation was obtained (Figure 2). The system suitability test parameters are shown in table 1.

Validation of the Proposed Method

The developed method was validated, as described below, for various parameters like linearity and range, accuracy, precision, ruggedness, system suitability, specificity, LOQ and LOD. Linearity of the method was evaluated at six concentration levels by diluting the standard stock solution to give solutions in the range of 15-90µg/ml. The calibration curve for Oxolamine citrate was prepared by plotting area v/s concentration. Calibration data for Oxolamine citrate was shown in Table 2. The linearity plot of Oxolamine citrate was found to be linear with the linear equationy = 6579.848- -839.714

and correlation coefficient 0.999662for Oxolamine citrate. Linearity was observed in the expected concentration range, demonstrating suitability of the method for analysis. This indicates that the method is linear in the specified range for the analysis of Oxolamine citrate in dosage form. The recovery experiments were carried out by the standard addition method. The method was found to be accurate with % recovery 98.9% – 100.3%.There recoveries obtained by the RP-HPLC method for Oxolamine citrate are shown in Table 3. Precision was calculated as repeatability and intraday and interday variation for Oxolamine citrate. The method was found to be precise with RSD 1.16 for intraday and 0.79 for interday for Oxolamine citrate. The low value of RSD (i.e. NMT 2%) has observed for the three results shown in Table 4 hence it concluded that the method is precise for the analysis of Oxolamine citrate in their dosage form. There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of Oxolamine citrate in their dosage form. The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in Table 5. The low value of relative standard deviation was indicating that the method was robust. Standard and sample solution stability was evaluated at room temperature for 78 hr. The relative standard deviation was found below 2.0%.It showed that both standard and sample solution were stable up to 48 hrs at room temperature. These data shows, the method was found to be sensitive for the determination of Oxolamine citrate. The LOD and LOQ were measured by a visual method and were found to be 0.25nd 0.8µg/ml, respectively.

Degradation Behavior

Forced degradation study was carried out by subjecting the drug to acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photolytic (sunlight) conditions. The Oxolamine citrate was found to be stable to sunlight degradation, dry heat degradation, UV light. The study indicated that Oxolamine citrate was highly stable to chemical oxidation study, dry heat, photolytic condition, acid, and alkali hydrolysis.

Analysis of marketed formulation

The proposed method was applied for the determination of Oxolamine citrate in tablets of Oxolamine citrate. The results of these assays were 98.55% of the label claim for the formulation. The results of the assay indicated that the method is selective for the assay of Oxolamine citrate without interference from excipients used in the tablets.

CONCLUSIONS

A validated stability-indicating HPLC analytical method has been developed for the determination of Oxolamine citrate in bulk and in tablet dosage form. The results of stress testing undertaken according to the ICH guidelines revealed that the method is selective and stability-indicating. The proposed method is simple, accurate, precise, and specific, and it has the ability to separate the drug from degradation products and excipients found in the dosage form but from all stability conditions the Oxolamine citrate was found to be highly stable molecule. The method is suitable for the routine analysis of Oxolamine citrate in tablets. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

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