

## ESTIMATION OF OXOLAMINE PHOSPHATE BY STABILITY INDICATING HPLC METHOD: METHOD DEVELOPMENT AND VALIDATION

**D. Murali\* and C. Rambabu**

Department of Biochemistry, Acharya Nagarjuna University,  
Guntur, Andhra Pradesh, India-522510.

\*E-mail: murali.dadi@gmail.com

### ABSTRACT

The aim of the present study was to develop a stability-indicating HPLC method with UV detection for the determination of oxolamine phosphate in oral syrup. Oxolamine phosphate was eluted on the Intersil CN (250 x 4.6 mm, i.e., particle size of 5  $\mu$ m) analytical column with a mobile phase consisting of 0.1% formic acid and acetonitrile, 50:50 v/v, pumped at 0.7 mL/min flow rate. The column was maintained at 25 $\pm$ 2 $^{\circ}$ C and 20  $\mu$ l of the solution was injected. UV detection was performed at 292 nm. The procedure eluted the analyte in an overall run time 5 min with oxolamine phosphate eluting at about 2.277 min. The method was validated according to the regulatory guidelines with respect to system suitability, linearity, sensitivity, specificity, precision, accuracy, ruggedness and robustness. Forced degradation studies were performed for oxolamine phosphate oral syrup sample to demonstrate the stability-indicating power of the method. The procedure provided a linear response over the concentration range 50–150  $\mu$ g/mL ( $R^2$ = 0.9998). The applicability of the method was evaluated in the oral syrup form.

**Keywords:** Oxolamine phosphate, cough suppressant, HPLC, stability indicating, syrup

©2016 RASAYAN. All rights reserved

### INTRODUCTION

Oxolamine phosphate is used as cough suppressant<sup>1</sup>. Chemically, it is called as N, N-diethyl-2-(3-phenyl-1, 2, 4-oxadiazol-5-yl) ethanamine. There are two salt forms of oxolamine: oxolamine phosphate and oxolamine citrate. Oxolamine exhibits anti-inflammatory effects on the respiratory tract, specific anti-tussive and bronchospasmodic activity. The anti-inflammatory activity of oxolamine causes reduction in the irritation of the nervous receptors of the respiratory tract. Therefore, as the inflammatory condition subsides, there is a progressive relief of cough<sup>2,3</sup>.

Few analytical techniques are found in the literature for the assay of oxolamine in bulk and pharmaceutical preparations. Four UV spectrophotometric methods using different approaches were developed for the determination of oxolamine citrate in bulk and pharmaceutical formulations<sup>4-6</sup>. First method is based on the measurement of absorbance of oxolamine citrate in methanol at 237 nm<sup>4</sup>. The second method is based on the derivatisation of the zero-order spectrum to get first-order derivative spectra. The amplitudes of the corresponding troughs were measured at 229.2 nm in aqueous media<sup>5</sup>. For the area under curve technique (third method), the area under curve of aqueous solution of oxolamine citrate in the wavelength range 228.6 to 246.4 nm was selected for its determination<sup>5</sup>. The fourth method is based on the determination of aqueous solution of oxolamine citrate at 257.2 nm using second order derivative UV spectra<sup>6</sup>.

A few visible spectrophotometric methods have been reported earlier for the estimation of oxolamine<sup>7,8</sup>. Kumar and Kishore proposed five visible spectrophotometric methods for the quantification of oxolamine in bulk and tablet dosage forms<sup>7</sup>. The reaction schemes involved in the five methods are: oxidation of oxolamine with Fe(III) in acidic medium and the subsequent formation of an colored complex between the liberated Fe(II) and 1,10-phenanthroline (method M1); Ion-pair complex formation of the oxolamine with acidic dyes such as woff fastener blue black (method M2) and alizarin red S (method M3);

diazotization of para nitro aniline with sodium nitrite followed by coupling with oxolamine in alkaline medium (method M4); and formation of coordinate complex between the secondary amine group of oxolamine and cobalt thiocyanate (method M5). Srinivas *et al.*, have reported a method based on charge transfer complex of oxolamine with 2, 3-dichloro-5, 6-dicyanobenzoquinone in acetonitrile medium<sup>8</sup>. Rajan and Sachin developed a non-aqueous potentiometric titration method for quantitative assay of oxolamine citrate from pharmaceutical dosage form. The titration was done using 0.1 N perchloric acid as titrant<sup>9</sup>.

To the best of our knowledge, only two reports are found in the literature regarding the assay of oxolamine using HPLC<sup>10,11</sup>. Rajan reported an HPLC-UV detection method for the assay of oxolamine citrate in pharmaceutical formulation<sup>10</sup>. The chromatographic separation of oxolamine was achieved on BDS hypersil C18 (150 x 4.6 mm, 5 µm particle size) with a mobile phase of 0.1 % triethyl amine buffer, adjusted to pH 3.5 with dilute orthophosphoric acid and acetonitrile (72:28 v/v). The flow rate and UV detector was set 1 mL/min and 230 nm, respectively. Sekhar Reddy and Bhaskar Rao reported a stability indicating HPLC method for the quantification of oxolamine citrate in pharmaceutical formulations<sup>11</sup>. In this method, quantitative determination of oxolamine citrate in the presence of stress degradation products was carried out on a Zodiac C18 column (250 mm x 4.6 mm, 5 µm particle size), using a methanol, water and acetonitrile as mobile phase in a isocratic elution mode with a flow rate of 1.0 mL/min and UV detection at 245 nm. Earlier reported HPLC methods for measuring the oxolamine citrate were found to have drawbacks such as lack of sensitivity, precision and accuracy. The retention time of oxolamine citrate is more (>3 min) in methods proposed by Rajan<sup>10</sup> and Sekhar Reddy and Bhaskar Rao<sup>11</sup>. Due to the lengthy retention time, the time taken for the analysis of a single sample increases. The use of triple solvent system as mobile phase in turn increases the cost of the analysis. In the Rajan method forced degradation studies were not reported<sup>10</sup>.

The present study describes, for the first time, the development of a rapid, sensitive, precise and accurate stability indicating HPLC method with UV detection for the determination of oxolamine phosphate in the presence of its stress degradation products. The developed method was validated as per the guidelines of ICH<sup>12</sup> and successfully applied to the determination of oxolamine phosphate in commercially available oral syrup samples.

## EXPERIMENTAL

### Apparatus

Chromatographic analysis was performed on Shimadzu HPLC class LC series consisted of two LC-10 AT, VP pumps, Rheodyne manual injection valve with variable loop volume of 20 µl and variable wavelength programmable UV detector. The system was controlled and data was acquisitioned by LC solution software.

### Materials

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 Labororios was used in the present study. Milli-Q-water was used all the way through the process. The HPLC grade acetonitrile and analytical reagent grade formic acid were procured from Merck chemicals Pvt Ltd, (Mumbai, India). All other materials were of analytical grade. Hydrochloric acid, sodium hydroxide and hydrogen peroxide were purchased from Sdfine-Chem limited (Mumbai, India).

### Chromatographic conditions

The chromatographic separation of oxolamine phosphate and its forced degradation products were performed on Intersil CN column (250 x 4.6 mm, i.d., particle size of 5 µm). The column temperature was kept at 25 ± 2°C. Separations were performed in the isocratic mode with a mobile phase consisting of 0.1% formic acid and acetonitrile (50:50 v/v) at a flow rate of 0.7 mL/min. The mobile phase was filtered

by a millipore membrane filter paper and degassed by ultrasonic bath before use. The sample injection volume was 20  $\mu$ L. The UV detector was set at 292 nm. The peak area of oxolamine phosphate was used for its quantitation.

#### Preparation of standard solutions

An accurately weighed amount (100 mg) of oxolamine phosphate was quantitatively transferred into a 100 mL calibrated flask, dissolved in 20 mL of mobile phase, completed to volume with the same solvent to obtain a stock solution of 1 mg/mL. The stock solution was further diluted with mobile phase to obtain working solutions in the range of 50-150  $\mu$ g/mL oxolamine phosphate.

#### Preparation of sample solution of syrup

The viscous oxolamine phosphate syrup was shaken thoroughly to make homogenous mixture. A volume of the syrup equivalent to 100 mg of oxolamine phosphate was transferred accurately into a 100 mL calibrated volumetric flask containing 20 mL of mobile phase. The content of the flask was shaken for about 10 min and diluted to volume with the same solvent. The solution was then filtered through a millipore membrane filter. Desired concentration (100  $\mu$ g/mL) of the drug was obtained by exact dilution with the mobile phase.

#### General analytical procedure

Working standard solutions equivalent to 50, 60, 80, 100, 130 and 150  $\mu$ g/mL oxolamine phosphate were prepared by appropriate dilution of the stock standard solution (1 mg/mL) with the mobile phase. Twenty  $\mu$ L of each solution was injected automatically onto the column in triplicate and the peaks were determined at 292 nm. The peak areas of oxolamine were plotted vs corresponding concentrations to obtain calibration curve. The concentration of the oxolamine phosphate was calculated either from the calibration curve or from the regression equation derived from linearity data.

#### Assay of oxolamine phosphate in perebron syrup

Twenty  $\mu$ L of the syrup sample solution prepared in the section, Preparation of sample solution of syrup, was injected into the HPLC system thrice. The analysis was followed up as in the general analytical procedure. The nominal content of oxolamine phosphate in perebron syrup was determined by using the calibration curve or regression equation.

## RESULTS AND DISCUSSION

#### Optimization of chromatographic conditions

Attempts were made by using two different stationary phases like hypersil ODS C18 Column (250 x 4.6 mm, 5  $\mu$ m particle size) and Intersil CN column (250 x 4.6 mm, 5  $\mu$ m particle size) and using a combination of buffers and organic modifiers like acetonitrile and methanol in the mobile phase. Selection of a suitable HPLC column was of the major concern. The chromatographic separation was achieved using a mobile phase containing a mixture of 0.1% formic acid and acetonitrile in the ratio of 50:50 (v/v) using the Intersil CN, 250 x 4.6 mm, 5 $\mu$ m column. A sharp peak with a good symmetry factor of oxolamine phosphate was observed when the Intersil CN column was employed.

In the optimized conditions, oxolamine phosphate and its possible stress degradants were well separated. This shows that the stress degradation products did not have any effect on the elution of oxolamine phosphate. The retention time of oxolamine phosphate was about 2.277 min (Fig.-1).

#### Method validation

The developed method was validated as per ICH guidelines<sup>12</sup>.

**System suitability**

The system suitability was studied by performing the experiments and looking for changes in retention time, peak area, USP tailing factor and USP plate count. Six injections of the standard solution of the drug (100 µg/mL) were injected for this purpose. The results are presented in Table-1. The values obtained demonstrated the suitability of the system for the analysis of the oxolamine phosphate.

**Linearity and sensitivity**

Under the above stated optimum chromatographic conditions, linear relationship with good correlation coefficient ( $R^2 = 0.9998$ ) was found between the peak area of oxolamine phosphate vs oxolamine phosphate concentration in the range of 50–150 µg/mL. The regression equation of the calibration curve obtained from six points was:  $y = 47.75x + 42$  (where  $y$  = peak area and  $x$  = concentration of oxolamine phosphate in µg/mL)

The sensitivity parameters, limit of detection (LOD) and the limit of quantitation (LOQ), were calculated according to the ICH guidelines for validation of analytical procedure based on the standard deviation of the response and the slope of the calibration curve. The LOD and LOQ were found to be 2.10 and 6.35 µg/mL, respectively.

**Precision**

The precision was evaluated in terms of system precision and method precision. The system and method precision was established by analyzing the standard and syrup sample solution, respectively in six replicates at a concentration 80 µg/mL of oxolamine phosphate. The relative standard deviation (RSD) values were used as a measure for the precision. The method gave satisfactory precise results as the RSD values did not exceed 1% (Table-2).

**Accuracy**

Accuracy was determined by analyzing a sample of known concentration and comparing the measured value with the true value. The accuracy was evaluated by using the method of standard addition technique. The accuracy results are expressed as percent recovery and are summarized in Table-3. The proposed method gave satisfactory results as the recovery values were in the range 100.20 – 102.66%.

**Robustness**

Robustness was examined by evaluating the influence of small and deliberate variation in the method variables (mobile phase composition, flow rate of mobile phase, column temperature and analytical wavelength) on the analytical performance of the method. During these experiments, one parameter was changed whereas the others were kept unchanged. The peak area was determined each time. The method robustness is evaluated at a concentration level of 80 µg/mL of oxolamine phosphate. It was found that small variation in the method variables did not significantly affect the method; %RSD values were less than 1% (Table-4). This indicated the reliability of the proposed method during its routine application for the quantification of oxolamine phosphate.

**Ruggedness**

Ruggedness was tested by applying the proposed method to the assay of oxolamine phosphate using the same operational conditions but using two different analysts, two different columns and two different systems. Results obtained were reproducible as the RSD did not exceed 1% (Table-5).

**Specificity (Forced Degradation Studies)**

Specificity is the ability of the method to estimate the drug in the presence of its excipients and degradants. The specificity of the proposed method was carried out in the presence of its degradants. Stress studies were performed for oxolamine phosphate syrup to provide an indication of the stability-

indicating property and specificity of the method as per the guidelines of ICH<sup>13</sup>. The degradation studies were done on syrup sample solution with concentration 100 µg/mL of oxolamine phosphate. Deliberate degradation was tried with the stress conditions of acid (0.5 N HCl at 80 °C reflux condition for 2 hrs), alkali (0.5 N NaOH at 80 °C reflux condition for 2 hrs), oxidative (20% H<sub>2</sub>O<sub>2</sub> at 80 °C reflux condition for 2 hrs), thermal (105 °C in hot air oven for 2 hrs) and photolytic (exposed to sun light for 24 hrs) to evaluate the ability of the proposed method to separate oxolamine phosphate from its degradation products.

Table-1: System suitability of the method

Parameters	Value	Recommended limits
Retention time	2.283 (%RSD – 0.056)	RSD ≤2
Peak area	681.467 (%RSD – 0.042)	RSD ≤2
USP plate count	28882.5	> 2000
USP tailing factor	1.1	≤ 2

Table-2: System and method precision

System precision		Method precision	
Concentration of drug (µg/mL)	Peak area	Concentration of drug (µg/mL)	% Recovery
70	571.957	70	571.959
70	582.747	70	575.957
70	582.747	70	577.955
70	581.786	70	571.956
70	571.957	70	571.951
70	571.957	70	571.957
Mean peak area – 577.191		Mean recovery - 575.29	
SD – 5.745		SD – 3.050	
% RSD – 0.995		%RSD – 0.533	

Table-3: Accuracy of the method

Spiked level (%)	Concentration of drug (µg/mL)				Mean recovery	% RSD
	Taken	Spiked	Recovered	% Recovery		
50	100	50.10	50.00	100.20	100.60	1.354
50		50.30	49.10	102.44		
50		50.20	48.90	102.66		
100		100.11	99.20	100.92	98.50	0.109
100		100.20	99.50	100.70		
100		100.40	99.60	100.80		
150		150.20	148.30	101.28	98.30	0.120
150		150.10	148.20	101.28		
150		150.10	148.50	101.08		

Table-4: Robustness of the method

Parameter	Investigated range	Peak area	%RSD
Mobile phase ratio (v/v)	48:52	582.262	0.181
	50:50	582.746	

	52:48	582.254	
Column temperature (°C)	24	582.428	0.090
	25	581.783	
	26	582.435	
Wavelength (nm)	290	582.924	0.354
	292	581.763	
	294	582.599	
Flow rate (mL/min)	0.6	583.252	0.562
	0.7	582.615	
	0.8	581.920	

Table-5: Ruggedness of the method

Parameter	Concentration of drug (µg/mL)		% Recovery	% RSD
	Taken	Found (n=6)		
Analyst I	70	69.895	99.85	0.567
Analyst II	70	70.012	100.02	0.562
Column I	70	69.950	99.93	0.245
Column II	70	69.978	99.97	0.362
System I	70	69.986	99.98	0.486
System II	70	70.054	100.08	0.157

Oxolamine phosphate was found to degrade significantly in alkali hydrolysis and less degradation was observed in all the remaining other stress conditions. Figures 2-6 shows the representative chromatogram of degradation studies. Assay studies were carried out for the stress samples against oxolamine phosphate working standard. The results are presented in Table-6. The assay of oxolamine phosphate was unaffected by the presence of its degradation products and thus confirms the specificity and stability-indicating power of the developed method.

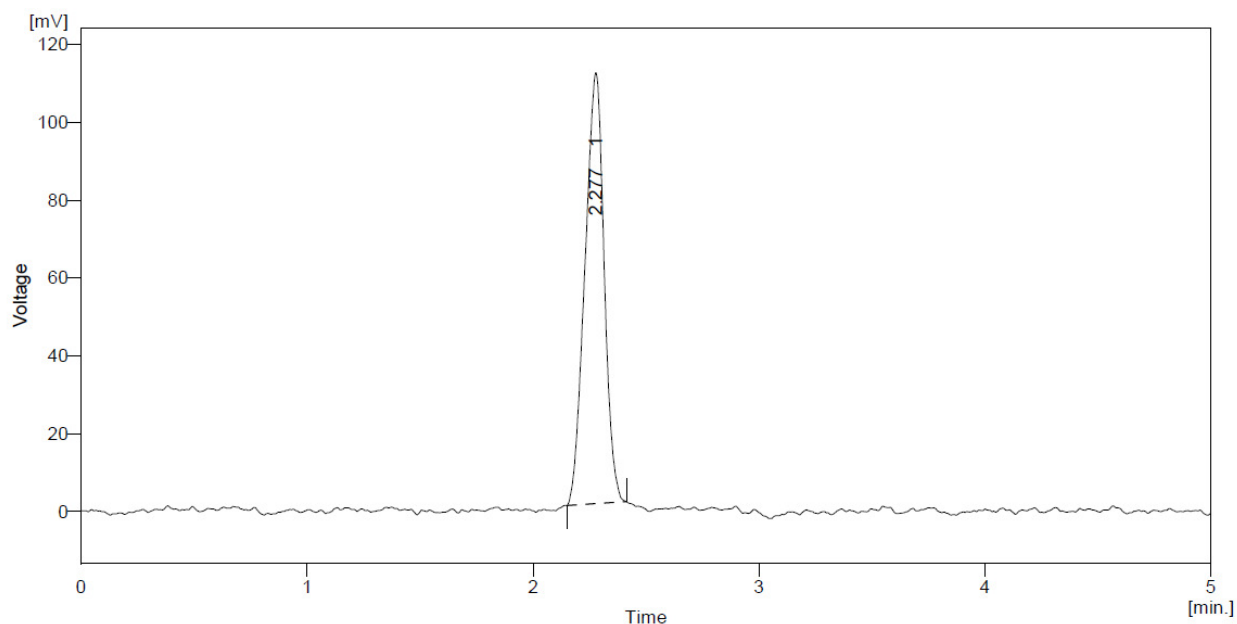


Fig.-1: Chromatogram of oxolamine phosphate under optimized conditions

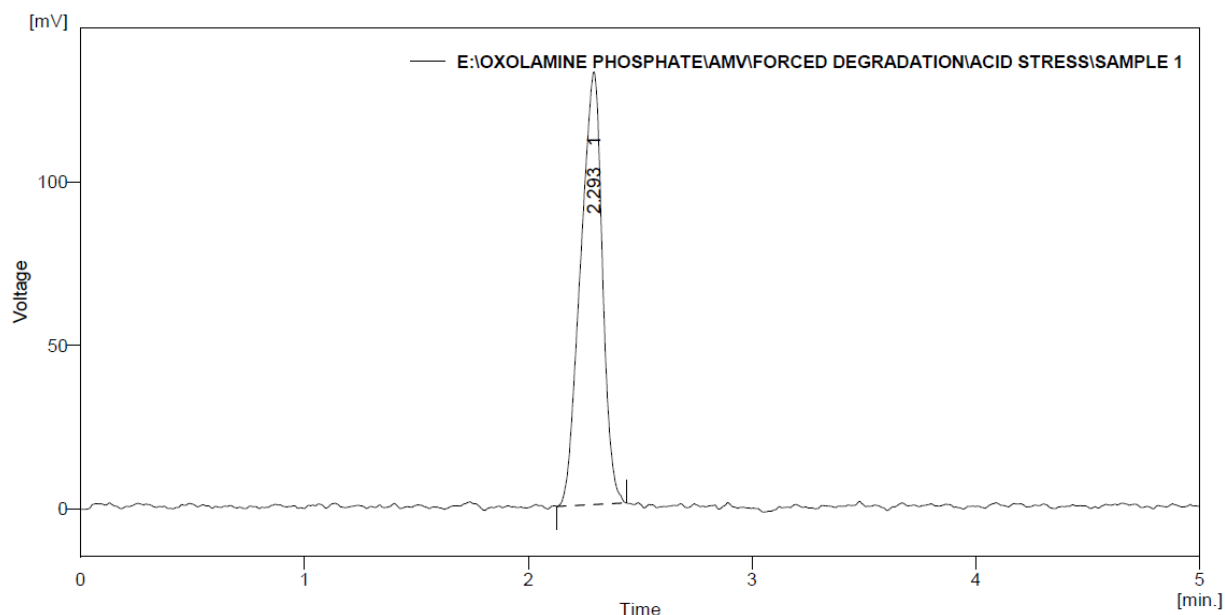


Fig.-2: Chromatogram of perebron syrup sample after acid hydrolysis

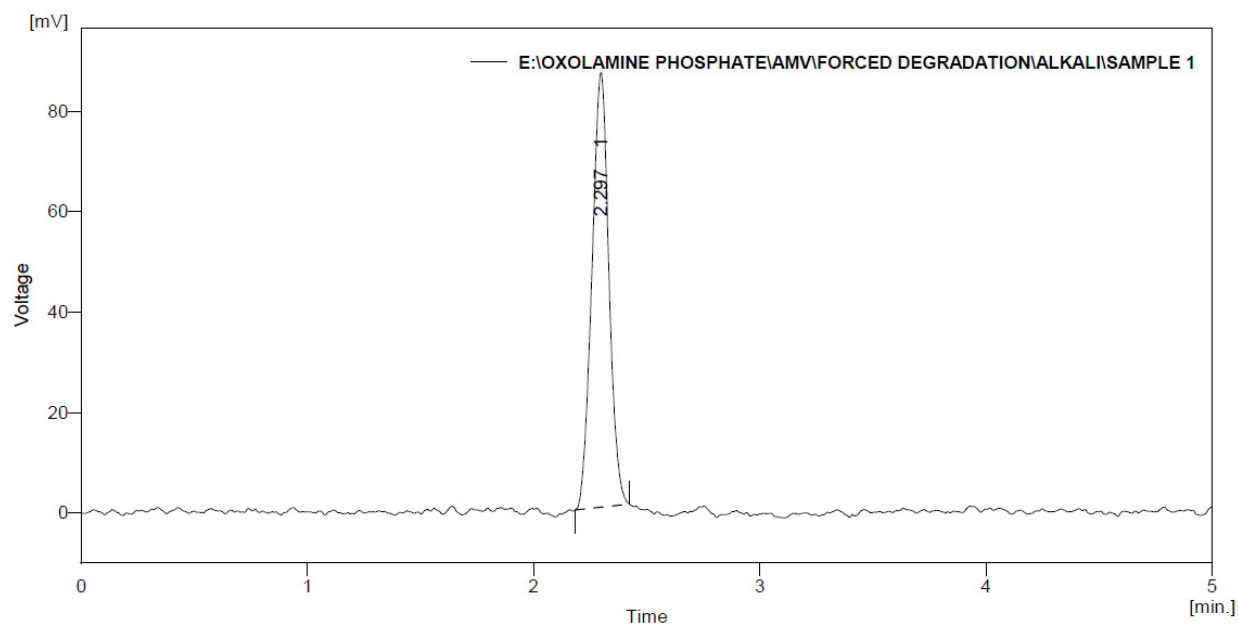


Fig.-3: Chromatogram of perebron syrup sample after alkali hydrolysis

### Stability of solutions

The oxolamine phosphate standard and syrup sample solutions stability was determined by storing them at room temperature for a varied period of time. The peak areas of the solutions were checked at 6, 12, 20, 26, 30 and 36 hours of storage. The peak area of the stored solution was compared with freshly prepared sample. The results are shown in Table-7. The results indicated that the standard and syrup sample solutions were stable for at least 36 hours at room temperature.

### Application of the method

The applicability of the method to the estimation of oxolamine phosphate in perebron syrup (labeled to contain 50 mg/5 mL) was investigated. The results were satisfactorily in terms of the accuracy and precision as the recovery value were ~100 % with low RSD <1% (Table-8). These data indicated the applicability of the proposed HPLC method for the accurate and precise determination of oxolamine phosphate in perebron syrup.

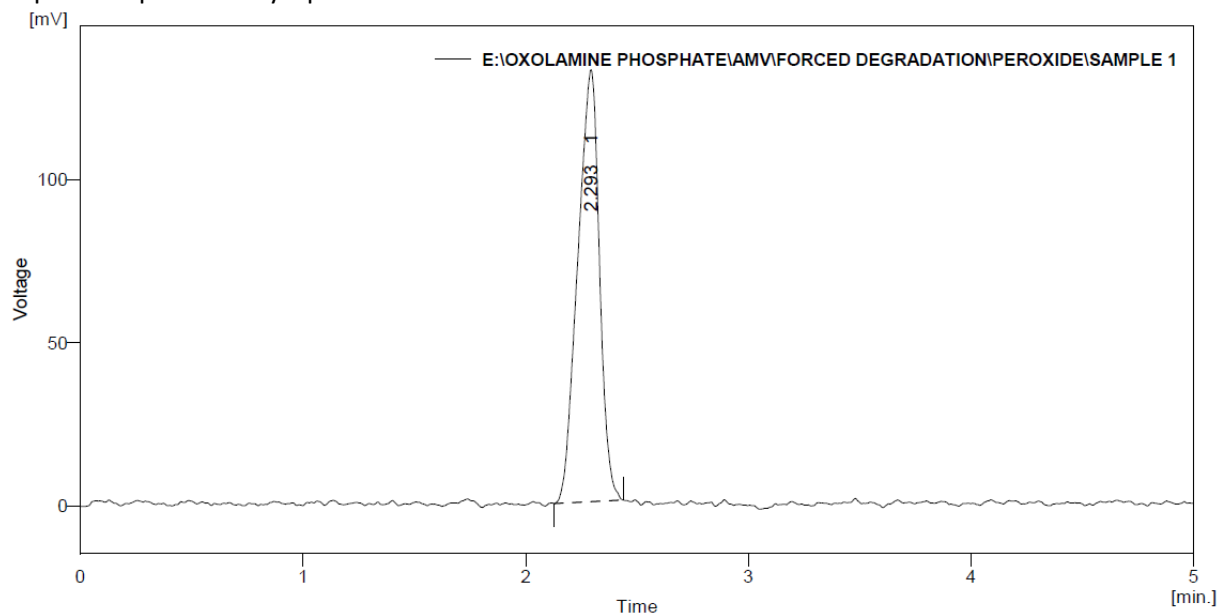


Fig.-4: Chromatogram of perebron syrup sample after hydrogen peroxide hydrolysis

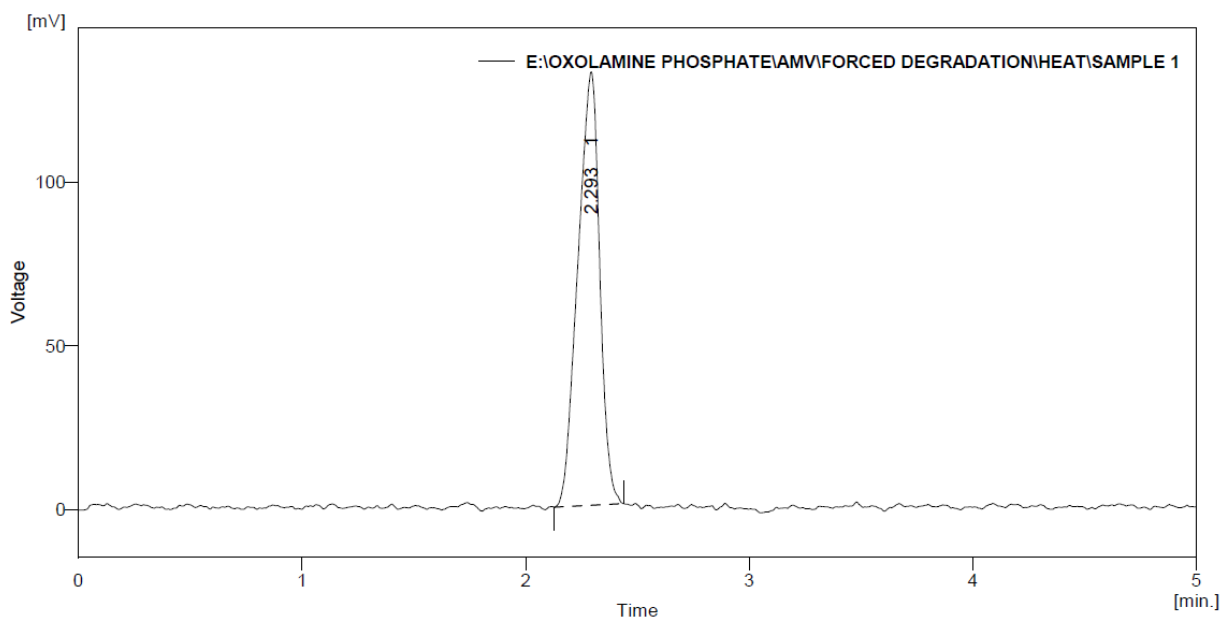


Fig.-5: Chromatogram of perebron syrup sample after thermal hydrolysis

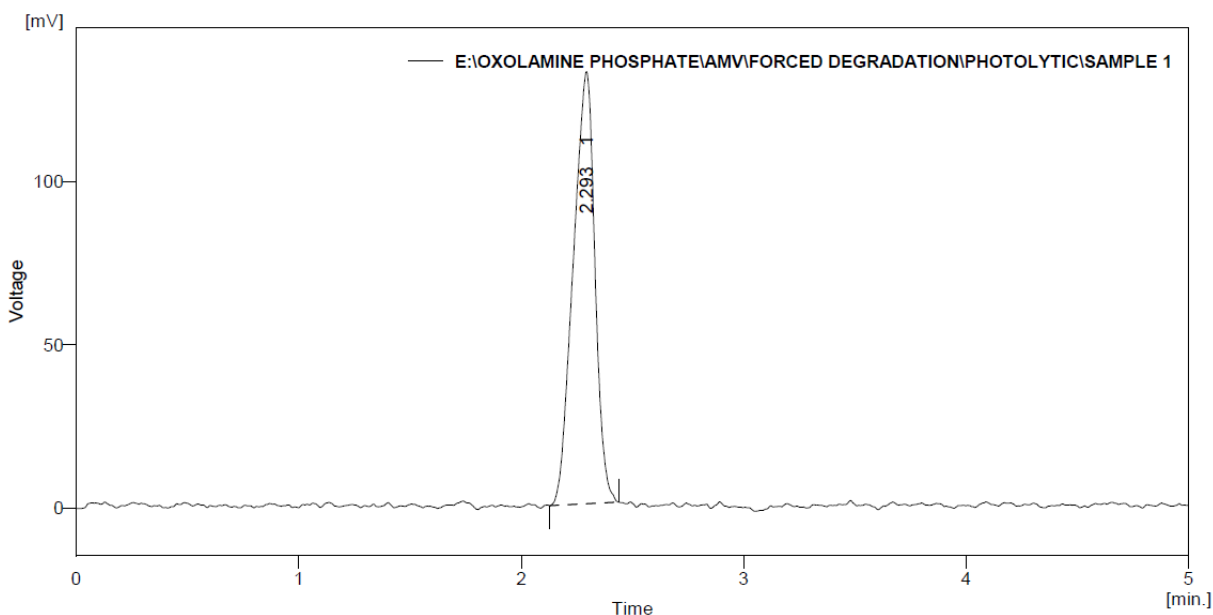


Fig.-6: Chromatogram of perebron syrup sample after photolysis

Table-6: Results of stress degradation studies

Stress condition	Condition applied	Peak area	% Recovered	% Degraded
Undegraded	-----	893.484	100.20	0.00
Acid	0.5 N HCl at 80 °C reflux condition for 2 hrs	891.980	99.83	0.17
Alkali	5 N NaOH at 80 °C reflux condition for 2 hrs	451.650	50.55	49.45
Oxidative	20% H <sub>2</sub> O <sub>2</sub> at 80 °C reflux condition for 2 hrs	887.984	99.38	0.62
Thermal	105 °C in hot air oven for 2 hrs	891.984	99.83	0.17
Photolytic	exposed to sun light for 24 hrs	889.484	99.55	0.45

Table-7: Stability of solutions

Oxolamine phosphate standard solution				
Time (hr)	Peak area			% Difference
0	582.747	581.747	582.247	NA
6	582.747	582.747	581.247	0.172
12	582.747	585.747	584.247	-0.516
20	582.747	580.747	581.747	0.428
26	582.747	582.747	582.747	-0.172
30	582.747	582.747	582.747	0.000
36	582.747	582.747	582.747	0.000
Perebron syrup sample solution				
0	581.747	582.747	582.747	NA

6	580.747	581.747	581.747	0.1716
12	582.747	580.747	580.747	0.17
20	584.747	581.747	583.247	-0.4305
26	582.747	581.747	582.247	0.17
30	581.747	582.747	582.247	0.00
36	582.747	582.747	582.747	-0.09

Table-8: Analysis of oxolamine phosphate perebron syrup

Concentration of drug taken (µg/mL)	Peak area	Mean peak area	% Recovery	% RSD
100	896.23	893.90	99.78	0.421
100	893.23			
100	892.23			

### CONCLUSION

A stability-indicating HPLC assay method with UV detection was developed for the quantitation of oxolamine phosphate in bulk and syrup. The developed method is simple, sensitive, specific, accurate, precise, rugged and robust. The procedure permitted the quantitative determination of oxolamine phosphate in the presence of its stress degradation products. The degradation products did not interfere with the oxolamine phosphate peak and demonstrated that the developed method was specific and stability-indicating. This method can be used to perform the analysis of oxolamine phosphate in routine quality control and stability samples.

### ACKNOWLEDGEMENTS

The authors are thankful to authorities of Acharya Nagarjuna University, Guntur and Chalapathi College of Pharmacy, Guntur for providing facilities to carry out the present work.

### REFERENCES

1. B.B. Ceyhan and S. Karakurt, *Res. Med.*, **96**, 61 (2002).
2. M. De Gregorio, *Panminerva Medica.*, **4**, 90 (1962).
3. M. Repaci, *Minerva Medica.*, **51**, 4072 (1960).
4. R.V. Rajan and S.A. Sawant, *Der Pharma Chemica.* **4**, 2389 (2012).
5. R.V. Rajan, S.A. Sawant, S.S. Patil and P.J. Gurav, *J. Chem. Pharma. Res.*, **5**, 12 (2013).
6. R.V. Rajan, *Res. J. Pharma. Tech.*, **7**, 1150 (2014).
7. V.P. Kumar and C.H.V. Kishore, *Int. J. Sci. Inv. Today*, **2**, 163 (2013).
8. B. Srinivas, P. Yadagiriswamy and G. Venkateshwalru, *Int. J. Anal. Bioanal. Chem.*, **5**, 88 (2015).
9. V.R. Rajan and S.P. Sachin, *Amer. J. Pharmatech.*, **3**, 445 (2013).
10. V.R. Rajan, *Int. J. PharmTech. Res.*, **7**, 549 (2014).
11. B.R.C. Sekhar Reddy and N.V. Bhaskar Rao, *Int. J. Res. Rev. Pharm. Appl. Sci.*, **3**, 578 (2013).
12. ICH Validation of analytical procedures; Text and methodology; Q2 (R1), International Conference on Harmonization, 2005.
13. ICH Stability Testing of New Drug Substances and Products, Q1A (R2), International Conference on Harmonization, 2003.

[RJC-1375/2016]