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STABILITY INDICATING UPLC METHOD OPTIMISATION AND VALIDATION OF PHOLCODINE IN BULK DOSAGE FORM

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ABSTRACT
A selective, precise, accurate and stability indicating UPLC method is validated for estimation of Pholcodine in bulk dosage form. The method employed, with Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column in gradient mode, with mobile phase 1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v. The flow rate was 1ml/min and effluent was monitored at 275nm. Retention time was found to be 2.022±0.10 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 8- 40µg/ml respectively. The LOD and LOQ values for were found to be 0.8379(µg/ml) and 2.53918(µg/ml) respectively. The proposed method was successfully used for estimation of Pholcodine in bulk dosage form.

KEYWORDS: Pholcodine, UPLC, Validation, stability indicating method.

1. INTRODUCTION
Pholcodine, (4R,4aR,7S,7aR,12bS)-3-methyl-9-(2-morpholin-4-ylethoxy)-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7-ol (Fig. 1)), is an opioid cough suppressant also expressed as an antitussive agent.[1-3] It helps suppress unproductive coughs and also has a mild sedative effect, but has little or no analgesic effects. It is also known as morpholinylethylmorphine and homocodeine.

The stability of Pholcodine is a matter of great concern as it affects the safety and efficacy of the finished bulk product.[4-6] Forced degradation studies provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the Pholcodine molecule and validation of stability indicating analytical procedures.[7-9]

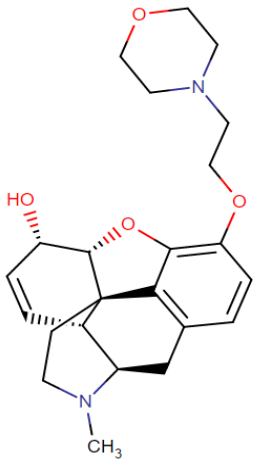


Fig.1: Molecular Structure of Pholcodine, (4R,4aR,7S,7aR,12bS)-3-methyl-9-(2-morpholin-4-ylethoxy)-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7-ol

Regulatory agencies recommend the use of stability indicating methods (SIMs) for the analysis of stability samples. [10-11] This requires stress studies in order to generate the potential related impurities under stressed conditions, method development and validation. With the evident of the International Conference on Harmonization (ICH) guidelines, requirements for the establishment of SIMs have become more clearly

mandated. [12] Environmental conditions including light, heat and the susceptibility of the drug product towards hydrolysis or oxidation can play an important role in the production of potential impurities. Stress testing can help identifying degradation products and provide important information about intrinsic stability of the drug product. Therefore, herein we report the results of stability study of Pholcodine with the aim of determining the extent of the influence of different stress conditions on the stability of the bulk product.

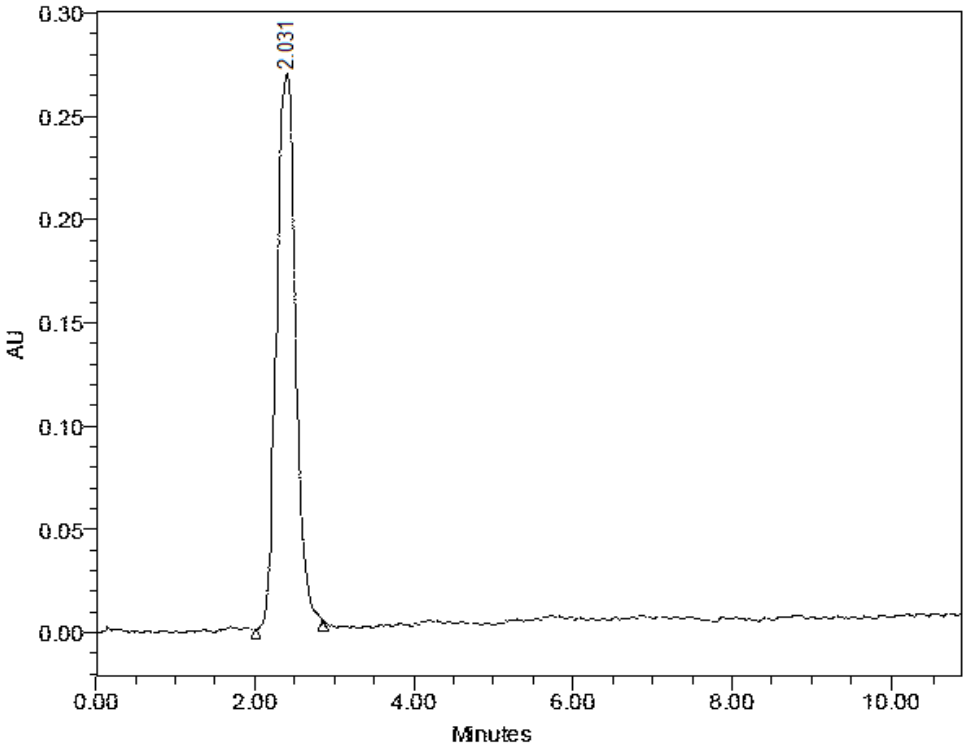


Fig. 2: Standard Chromatogram of Pholcodine, using mobile phase of 1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v

2. EXPERIMENTAL

Materials

Pholcodine (98.50 % purity) used as analytical standard was procured from Active Pharma Labs (Hyderabad).

HPLC grade methanol, Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were analytical grade. All chemicals used were of pharmaceutical or special analytical grade.

Instrumentation

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis.

The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerex sonicator was used for enhancing dissolution of the compounds. A Labindia pH System 362 was used for pH adjustment.

Chromatographic Conditions

Table 1: Chromatographic Conditions of the validating method

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm)
Mobile Phase	1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v
Flow rate	1 mL/min
Run time	10 Min.
Column Temperature	Maintained at ambient temperature
Injection volume	20 µL
Detection wavelength	275nm
Diluent	Mobile Phase

Preparation of Standard Stock Solution
Preparation of Diluent
In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.0 mL min-1 shows good resolution. The PDA detector response of Pholcodine was studied and the best wavelength was found to be 275 nm showing highest sensitivity.

The mixture of two solutions 1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v. Finally, the pH was adjusted to 7.65 by sodium hydroxide. with gradient programming was used as mobile phase at 1.0mL/min was found to be an appropriate mobile phase for separation of Pholcodine. The column was maintained at ambient temperature.

Preparation of internal standard solution
Weighed accurately about 10 mg of ephedrine hydrochloride working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Pholcodine standard solution
Weighed accurately about 10 mg of Pholcodine and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. Linearity was determined in the range of 8- 40 µg mL-1.

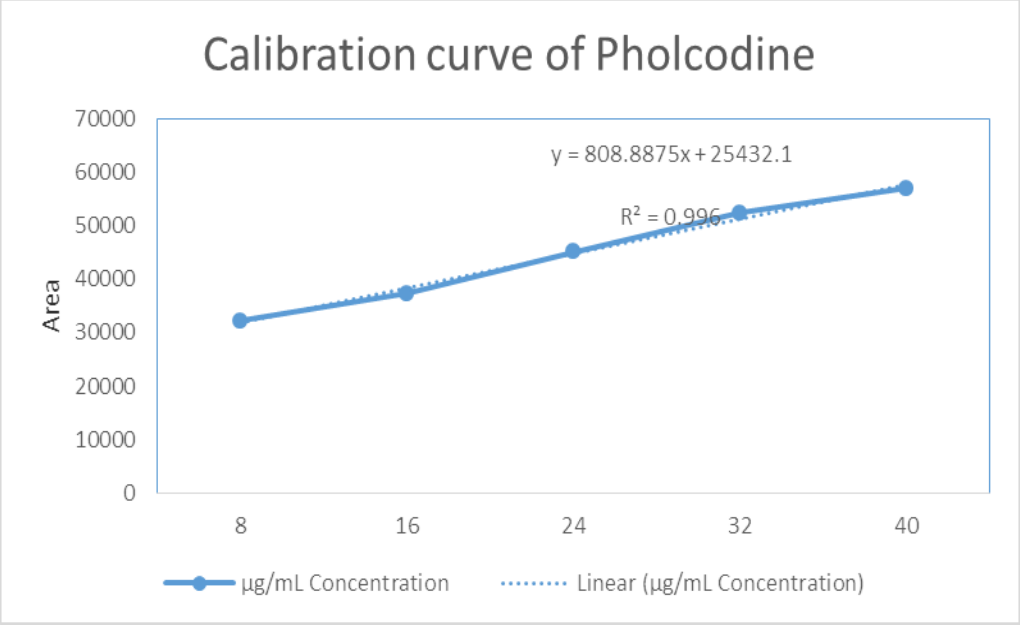
Stability Indicating Studies
Stability Indicating studies like acid hydrolysis, basic hydrolysis, dry heat degradation, wet heat degradation and oxidative degradation were carried out.

3. RESULTS AND DISCUSSIONS

Validation
The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

Linearity and Range
The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample.

The calibration curve showed good linearity in the range of 8 – 40 µg/ml, for Pholcodine (API) with correlation coefficient (r2) of 0.9963. A typical calibration curve has the regression equation of y = 808.8875x +25432.1 for Pholcodine. Results are given in Table 2.



Limit of Detection (LOD) and Limit of Quantitation (LOQ):
The LOD and LOQ of Pholcodine were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of Pholcodine was found to be 0.8379(µg/ml) and the LOQ of Pholcodine was found to be 2.53918(µg/ml). Results are given in Table 2.

Table 2: Summary of validation parameters for the proposed method

PARAMETER	Pholcodine
Linearity	8 – 40 µg/ml
Intercept (c)	25432.1
Slope (m)	808.8875
Correlation coefficient	0.9963
LOD	0.8379(µg/ml)
LOQ	2.53918(µg/ml)

Precision

The Precision of the method was studied in terms of intraday and interday precision of sample injections (16 µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.30%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.31%. Low % RSD values indicate that the method is precise. The results are given in table 3.

Accuracy

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favour of (% RSD < 2%) the developed UPLC method for the analysis of Pholcodine. The results are given in table 5.

Table: 3, Results of Precision Studies

Pholcodine			
Precision Studies			
Parameter	**Peak Area**	**% RSD**	**%LC**
Intraday precision	37312	0.30%	99.28%
	37458		99.67%
	37536		99.88%
Inter day precision	37304	0.31%	99.26%
	37420		99.57%
	37533		99.87%
Instrument:1 Acquity UPLC Waters,2695H	37558	0.19%	99.94%
	37423		99.58%
	37459		99.68%
Instrument:2 Agilent Technologies,1290	37564	0.19%	99.98%
	37428		99.59%
	37462		99.68%
Average			**99.66**
Std.Dev			**0.233**
%RSD			**0.23%**

Table: 4, Results of accuracy study

Pholcodine						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	08.16	08.09	99.14	99.13	0.625	0.63%
100	16.32	16.28	99.75			
150	24.48	24.36	98.50			

Table: 5, Results of Robustness Studies

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	37312	0.15%
	Actual	37365	
	Plus	37421	
Temperature	**Low**	37406	0.16%
	Actual	37476	
	Plus	37529	
Wavelength	**Low**	37312	0.17%
	Actual	37367	
	Plus	37438	

Results of Stability Indicating Studies

According to Singh and Bakshi, the stress testing suggests a target degradation of 20-80 % for establishing stability indicating nature of the method. UPLC study of samples obtained on stress testing of Pholcodine under different conditions using mixture 1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v as a mobile solvent system suggested the following degradation behaviour.

a. Acid hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of 1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 3.

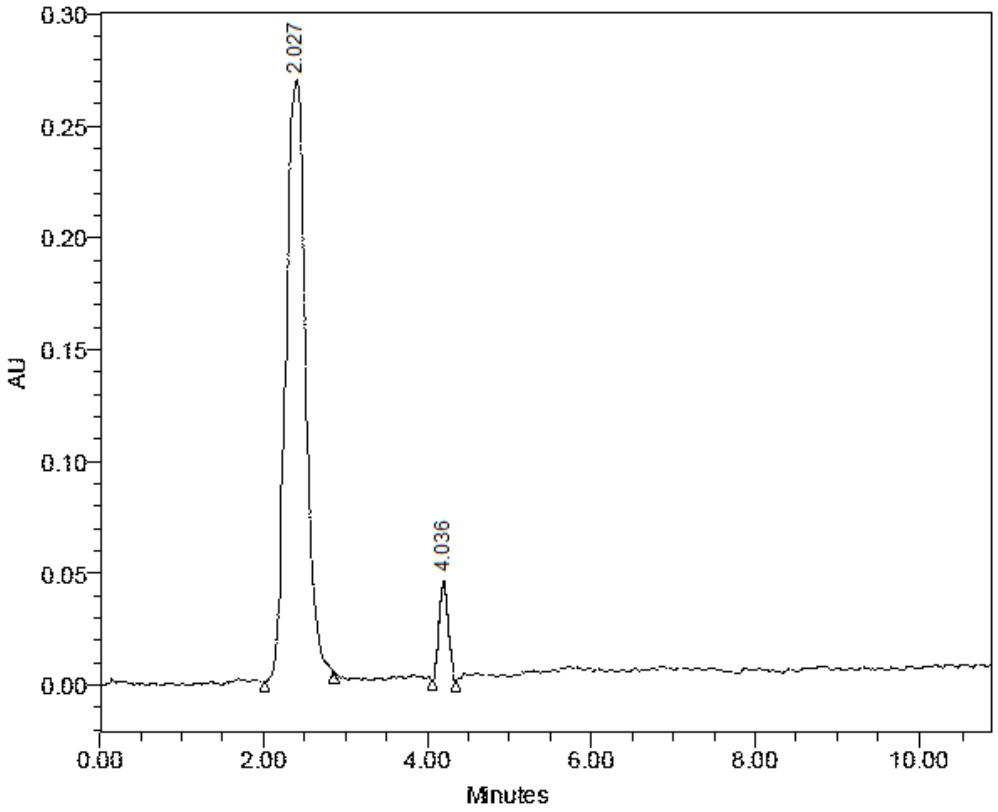


Fig. 3: Chromatogram showing the degraded products in Acidic degradation

b. Basic hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of 1

M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 4.

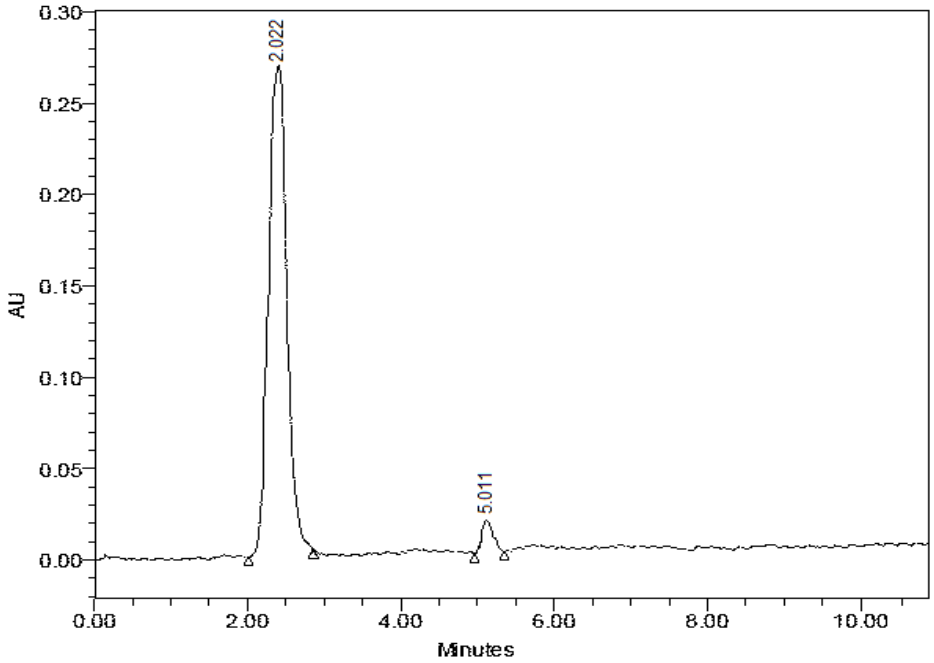


Fig. 4: Chromatogram showing the degraded products in Basic degradation

c. Wet heat degradation

Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of 1 M KH_2PO_4-acetonitrile in the ratio of 80:20%v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Fig. 5.

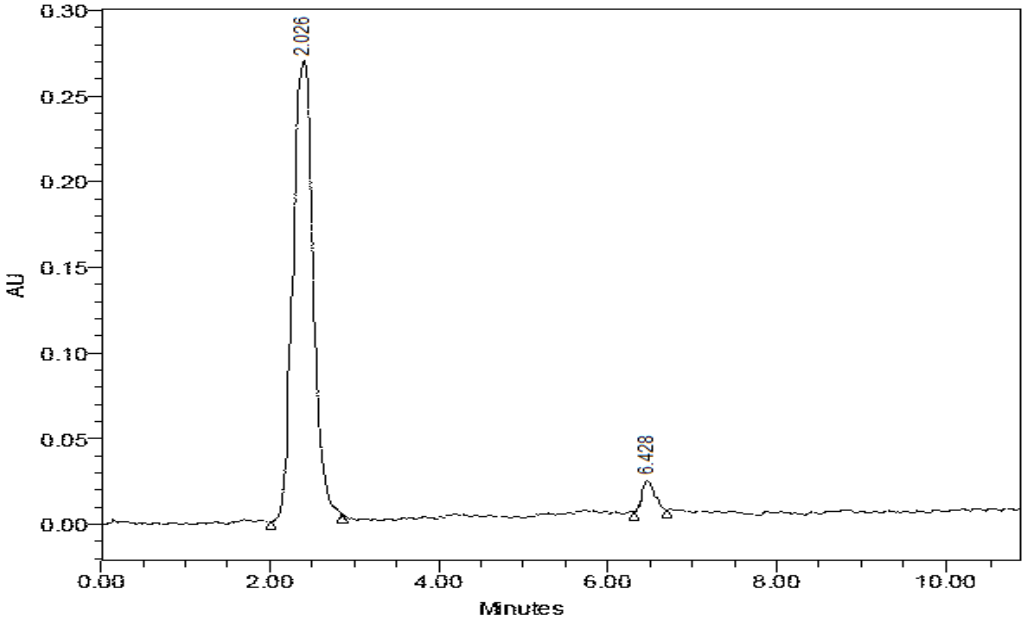


Fig. 5: Chromatogram showing the degraded products in Wet heat degradation

d. Oxidation with (3%) H_2O_2

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Fig. 6.

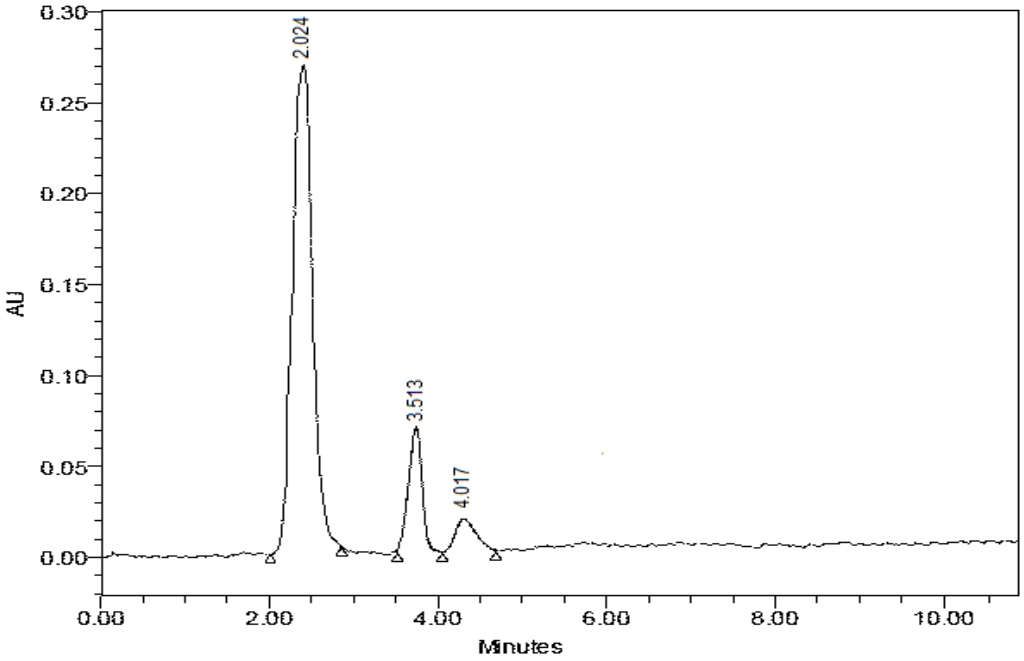


Fig. 6: Chromatogram showing the degraded products in H_2O_2

In all degradation studies, there was a significant formation of degradation products when compared to that of a standard. This indicates that, the drug may be degraded to low molecular weight non-chromophoric compounds.

Table:6, Stability Indicating study for the developed method

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)
Acidic	60°C	3	1 (4.036)
Basic	60°C	9	1 (5.011)
Oxidative	RT	48	2 (3.513, 4.017)
Wet Heat	105°C	24	1 (6.428)

4. CONCLUSION

A selective and sensitive stability indicating UPLC method has been validated for the analysis of Pholcodine in bulk drug form. Based on peak purity results, obtained from the analysis of stability indicating studying samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Pholcodine indicated that the developed method is specific for the estimation of Pholcodine in presence of degradation products. Further the proposed UPLC method has excellent precision, sensitivity and reproducibility. Even though no attempt has been made to identify the degraded products, proposed method can be used as stability indicating method for assay of Pholcodine in commercial formulations.

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