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Analytical Method Development for Assay of Diacerein, Validation and Forced Degradation Studies

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ABSTRACT

A stability-indicating HPLC method was developed and validated for the quantitative determination of diacerein in capsule dosage form. An isocratic separation was achieved using a C18, 250×4.6 mm, 5 µm particle size column with a flow rate of 1 ml/min and using a UV detector to monitor the eluent at 258 nm. The mobile phase consisted of Water: acetonitrile (70:30v/v). The drug was subjected to oxidation, hydrolysis, neutral, thermal and photolytic degradation. Diacerein was found to degrade under acidic, basic, oxidative, neutral and also dry heat condition. Complete separation of degraded products was achieved from the parent compound. All degradation products were eluted in an overall analytical run time of approximately 10 min with the parent compound diacerein eluting at approximately 6.44 min. The method was linear over the concentration range of 5-15 µg/ml (R^2 = 0.999) with a limit of detection and quantitation of 0.0093 and 0.028 µg/ml respectively. The method has the requisite accuracy, selectivity, sensitivity, precision and can be used to assay diacerein in tablet. Degradation products resulting from the stress studies did not interfere with the detection of diacerein and the assay is thus stability-indicating.

Keywords: Diacerein, Degradation, HPLC-UV.

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INTRODUCTION

Diacerein 1, 8-diacetoxy-3-carboxy anthraquinone is a novel osteoarthritis drug which selectively inhibits the IL-1. It is semi-synthetic anthraquinone derivative extracted from certain plants[1]. It directly inhibits IL-1 synthesis and release which plays a fundamental role in osteoarthritis pathophysilogy and cartilage destruction. After thorough literature survey only one HPLC method was found to be reported[8]. But the reported method was found to be more time consuming and more solvent consuming as it shows long retention time for pure drug. The present work, aim for development and validation of more economical, precise, accurate and specific HPLC method for the quantitative determination of diacerein in pharmaceutical dosage form[3].

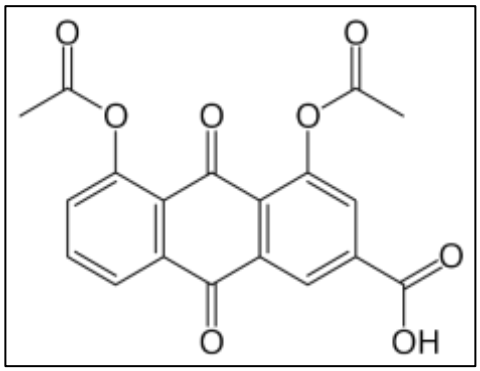

Figure 1: Structural formula of diacerein

MATERIALS AND METHOD

Diacerein, pure compound was kindly supplied by Meyer Pharmaceuticals, Thane, India and was used without further purification. Diacerein Tablet containing 50 mg diacerein as per label claim was purchased from local pharmacy. All the chemicals used were of analytical grade. Purified HPLC grade water was obtained by double distillation and filtered through filter (Millipore) and was used to prepare all the solutions.

HPLC instrumentation and conditions:

The HPLC system consisting of a pump with injecting facility programmed at 20 µl capacity per injection was used. The detector consists of a UV (Shimadzu) operated at a wavelength of 258 nm. The chromatographic separation was performed using a HiQsil, C18 5µm, 250×4.6 mm column. Separation was achieved using a mobile phase consisting of water: acetonitrile (70:30 v/v) solution at a flow rate of 1 ml/min. The eluent was monitored using UV at a wavelength of 258 nm. The column was maintained at ambient temperature and injection volume of 20 µl was used. The mobile phase was filtered through 0.45 µm membrane filter prior to use.

HPLC METHOD DEVELOPMENT

Selection of detection wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected [4, 5]. In the present study drug solution of 10µg/ml was, therefore scanned in the UV region of 200-400 nm using UV visible spectrophotometer. (Shimadzu 1800) and λ max was determined.

Optimization of chromatographic condition

Proper selection of the HPLC method depends upon the nature of the sample (ionic or ionisable or neutral molecule), its molecular weight and solubility. The drug selected for the present study is non-polar in nature and hence either reversed phase or ion-pair or non-aqueous chromatography can be used. Reversed phase HPLC was selected for the initial separations because of its simplicity and suitability. The standard solution of Diacerein was prepared and run through the system and different combinations of mobile phase were tried for isocratic mode to get well resolved, symmetric peaks. The resulting chromatograms were recorded.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines[5, 6, 7].

Preparation of stock and standard solutions:

Standard stock solution

Accurately weighed 25mg of Diacerein was transferred to 25ml of volumetric flask to which 2 ml of DMSO was added and sonicated to dissolve completely. The volume was made up to the mark with ACN, to obtain a solution of 1000µg/ml; resultant solution was sonicated for 5 min and filtered through 0.45µ membrane filter.

Preparation of diluent

Mobile phase [Water and acetonitrile (70:30% v/v)] was used as a diluent.

Preparation of working standard solution

1 ml of stock solution was pipetted out and transferred to a 10 ml volumetric flask and volume was made up to mark with diluent to get final concentration of 100µg/ml.

Validation of the proposed method

The developed method was validated for its system suitability, linearity, accuracy, precision and sensitivity. System suitability tests were carried out on freshly prepared solution of drug from working standard solution and the parameters like theoretical plates, tailing factor, resolution and area of the peaks were calculated (Table 1). Calibration curve was plotted over a concentration

range of 5-15µg/ml by plotting peak area v/s concentration (Figure 2). The accuracy of the method was determined by performing recovery studies on prepared solutions containing known amount of drug by standard addition method at three different levels (Table 2). Also, the experiment was repeated three times in a day to determine intra-day precision and on two different days to determine inter-day precision. The percent relative standard deviation (% RSD) was calculated at each concentration level (Table 3 and Table 4).Limit of detection (LOD) and quantification (LOQ) were estimated from the Standard Deviation of the Response and the Slope. The LOD and LOQ were calculated by the use of the equations:

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

ASSAY

Sample Preparation:

Marketed product of Diacerein was weighed and finely powdered. A quantity equivalent to 25mg was transferred into 25 ml volumetric flask to which 2 ml of DMSO was added. The contents were sonicated to dissolve completely and the volume was made up to the mark with ACN and filtered through 0.45µm membrane filter to get concentration of 1000 µg/ml. 1 ml of this solution was pipetted out and transferred to a 10 ml volumetric flask and volume was made up to the mark with diluent to get concentration of 100µg/ml. 1 ml from 100 µg/ml solution was pipetted out and transferred to a 10 ml volumetric flask and volume was made up to the mark with diluent to get final concentration of 10µg/ml. Twenty micro litres of standard solutions was injected into HPLC and chromatogram was recorded.

Standard Preparation:

25mg of Diacerein was weighed and was transferred into 25 ml volumetric flask to which 2 ml of DMSO was added. The contents were sonicated to dissolve completely and the volume was made up to the mark with ACN and filtered through 0.45µm membrane filter to get concentration of 1000 µg/ml. 1 ml of this solution was pipetted out and transferred to a 10 ml volumetric flask and volume was made up to the mark with diluent to get concentration of 100µg/ml. 1 ml from 100µg/ml solution was pipetted out and transferred to a 10 ml volumetric flask and volume was made up to the mark with diluent to get final concentration of 10µg/ml. Twenty micro litres of standard solutions was injected in HPLC and chromatogram was recorded.

FORCED DEGRADATION STUDIES[8-12]

Preparation of stock solution

Accurately weighed 25mg of Diacerein was transferred to 25ml volumetric flask to which 2 ml of

DMSO was added and sonicated to dissolve it completely and the volume was made up to the mark with ACN, to obtain a solution of 1000µg/ml, resultant solution was sonicated for 5min and filtered through 0.45µm membrane filter.1 ml of this solution was pipetted out and transferred to a 10 ml volumetric flask and Volume was made up to the mark with diluent to get final concentration of 100µg/ml.

Neutral degradation studies

The drug was refluxed in water for 3 hours at a temperature of 70ºC. The resultant solution was diluted to obtain 20µg/ml.

Acid degradation studies

2 ml of 0.01 N HCL was added to 2 ml of stock solution and kept for 15 min at RT. The resultant solution was diluted to obtain 20µg/ml.

Base degradation studies

2 ml of 0.01 N NaOH was added to 2 ml of stock solution and kept for 15 min at RT. The resultant solution was diluted to obtain 20µg/ml.

Oxidation degradation studies

2 ml of 1% $H_2O_2$was added to 2 ml of stock solution and kept for 30 min at RT. The resultant solution was diluted to obtain 20µg/ml.

Dry heat degradation studies

Standard drug was placed in oven at 80ºC for 6 hrs. It was diluted to get concentration of 20µg/ml.

Light degradation studies

Solid drug powder was exposed for 36 hours to daylight.

% degradation = Area of Standard Peak – Area of Sample Peak/ Area of standard peak x 100

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Initially acetonitrile and water in different ratios were tried. It was found that acetonitrile and water system (70:30) gives good result, Acetonitrile: water in the ratio of 50:50 was not able to give good peak symmetry with acceptable retention time. An attempt to improve peak symmetry was made by adding acetonitrile to the mobile phase. The presence of more acetonitrile in mobile phase resulted in an ideal chromatograph with appropriate peak symmetry and complete base line resolution. Finally the mobile phase consisting of Water and acetonitrile (70:30 v/v) was selected for validation purpose and stability studies. The method was validated with respect to parameters

including system suitability, linearity, accuracy, precision, and sensitivity [limit of detection (LOD) & limit of quantitation (LOQ)].

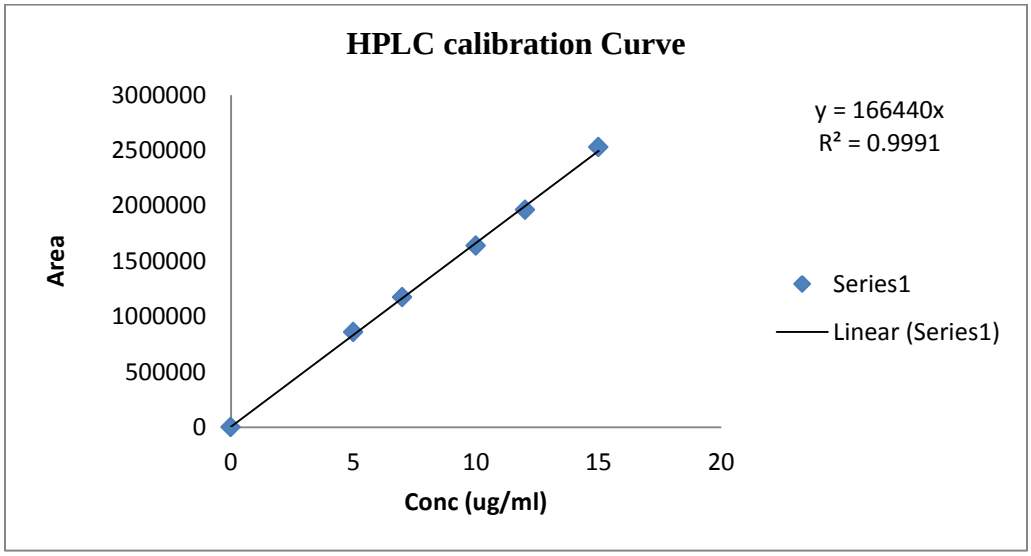

Figure 2: Linearity of Diacerein

The system suitability was done at the 100% level of analyte. The data derived from system suitability test is shown in Table 1.

Table 1: System suitability test parameters for Diacerein

Sr. No.	RT (min)	Theoretical plates	Tailing factor
1	6.89	2319	1.21
2	6.86	2387	1.23
3	6.89	2290	1.21
4	6.89	2302	1.2
5	6.89	2365	1.2
Avg	6.88	2332.6	1.21
SD	0.013	41.66	0.012
% RSD	0.19	1.78	1.01

Diacerein showed linearity in the concentration range of 5-15 µg/ml (R^2 = 0.9991) for HPLC. Linearity was evaluated by ten standard working solutions containing 5-15 µg/ml in triplicate. Peak areas of diacerein were plotted versus Diacerein concentration and linear regression analysis is performed on the resultant curve. For HPLC method the linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient and the standard deviation for intercept value less than 2%.

The accuracy of assay was determined by interpolation of replicate (n=3) peak areas of three accuracy standards (3, 7.5, 13.5 µg/ml) from a calibration curve prepared as previously described. In each case the recovery was calculated. Data derived from accuracy experiment was given in

Table 2.

Table 2: Recovery of diacerein

Accuracy level	Amt. added in	Control Samples	Amt. recovered in µg (Avg)	% Recovery
20	6	3		
20	6	3	5.88	98.03
20	6	3		
50	10.5	7.5		
50	10.5	7.5	10.32	98.29
50	10.5	7.5		
90	16.5	13.5		
90	16.5	13.5	16.50	100.05
90	16.5	13.5		

The precision was determined with respect to both repeatability and reproducibility. An amount of the product powder equivalent to 100% of the label claim (50mg) of diacerein was accurately weighed and assayed. Method repeatability was obtained from RSD value by repeating the assay three times in same day for intra-day precision. Inter-day precision was assessed by the assay of three sample sets on different days inter-day precision. The intra-day and inter-day variation for determination of diacerein was carried out at three different concentration levels 3, 7.5, 13.5 µg/ml shown in Table 3 and Table 4 respectively.

Table 3: Determination of Intra-day Precision

Conc.	3 ppm		7.5 ppm		13.5 ppm	
	Area	RT	Area	RT	Area	RT
SD	841.21	0.0435	3564.0990	0.025	4696.839	0.01
% RSD	0.17	0.62	0.30	0.36	0.22	0.14

Table 4: Determination of Interday Precision

Conc.	3 ppm		7.5 ppm		13.5 ppm	
	Area	RT	Area	RT	Area	RT
SD	469.27	0.015	4317.27	0.011	12122.47	0.005
% RSD	0.091	0.21	0.36	0.16	0.57	0.08

The LOD and LOQ were determined based on a signal-to-noise ratios and were based on analytical responses of 3 and 10 times the background noise, respectively. The LOD was found to be 0.0093µg/ml. The LOQ was found to be 0.0093µg/ml. Summary of validation parameters were presented in Table 5.The proposed method was applied to the determination of diacerein in Marketed Tablet. The result of these assay yielded 99.87% of label claim of the tablet. The results

of the assay indicate that the method is selective for the assay of diacerein without interference from the excipients used in these tablets. Results are shown in Table 6.

Table 5: Summary of validation parameters

Parameters	HPLC
Linearity range	5-15
Correlation coefficient	0.9991
% Recovery	98-102%
% RSD	< 2%
LOD	0.0093µg/ml
LOQ	0.028µg/ml

Table 6: Determination of diacerein in tablet dosage form

Sr. No	Area	% Assay
1 (Sample)	1632709	
2 (Standard)	1630689	99.87

The results of stress testing studies indicated a high degree of selectivity of this method for diacerein. Typical chromatographs obtained from the assay of pure sample and stressed samples are shown in Figures (4, 5, 6, 7, 8) respectively. The average retention time for diacerein was found to be 6.50 min. The peak obtained was sharp and had clear baseline separation.

Diacerein is characterized by an anthraquinone moiety with a side chain of acetoxy group and carboxylic acid group (Figure 1), which is prone to hydrolysis. In neutral conditions when the drug was refluxed with water for 3 h, around 27% degradation was shown (Figure 4). Also it was unstable in acidic conditions (Figure 5) when kept for 15 min at room temperature. The drug was degraded approximately to 11.53%.The drug was unstable under basic stress conditions (Figure 6) when kept for 15 min at room temperature. The drug was degraded approximately to 17.61%. When kept under oxidative stress conditions with 1% H_2O_2 for 30 min at room temperature, the drug was degraded to around 27.86% (Figure 7). Diacerein was found to be stable under dry heat conditions (Figure 8) and also no decomposition was seen on exposure of solid drug powder for 36 hours to day light. The stability of stock solution was determined by quantitation of diacerein and comparison to freshly prepared standard. No significant change was observed in the stock solution response, relative to freshly prepared standard.

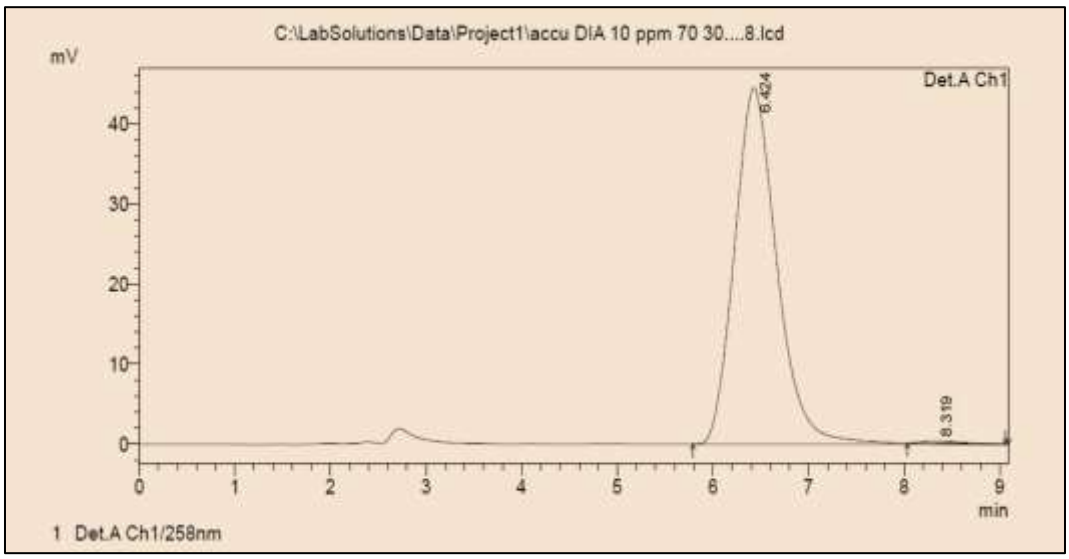

Figure 3: Chromatograph of Standard solution

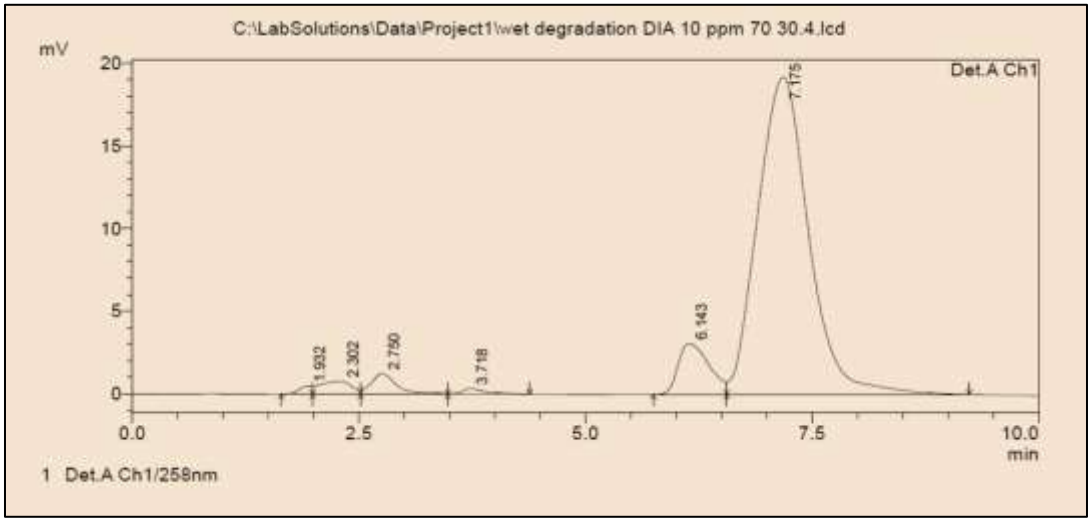

Figure 4: Chromatograph of Aqueous degradation

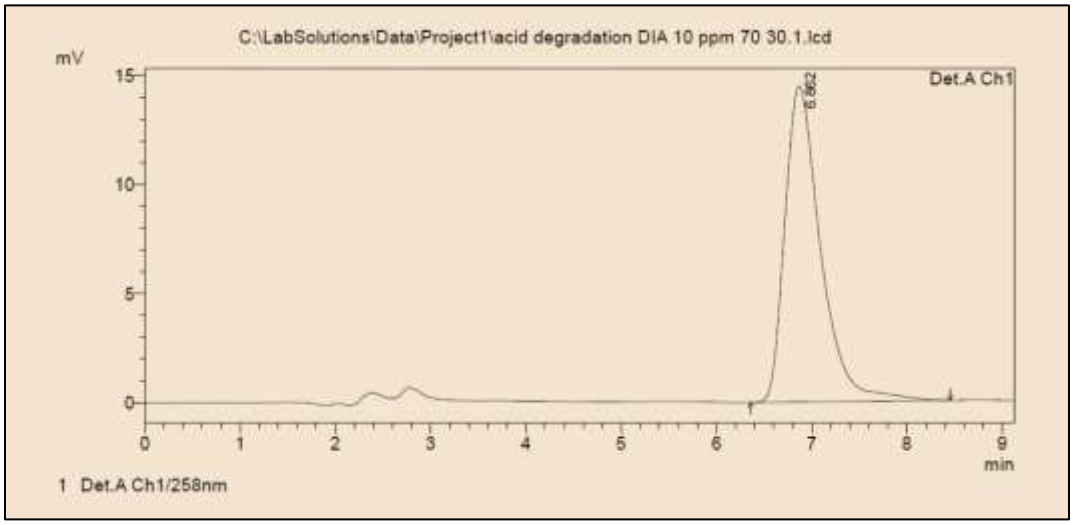

Figure 5: Chromatograph of acid degradation

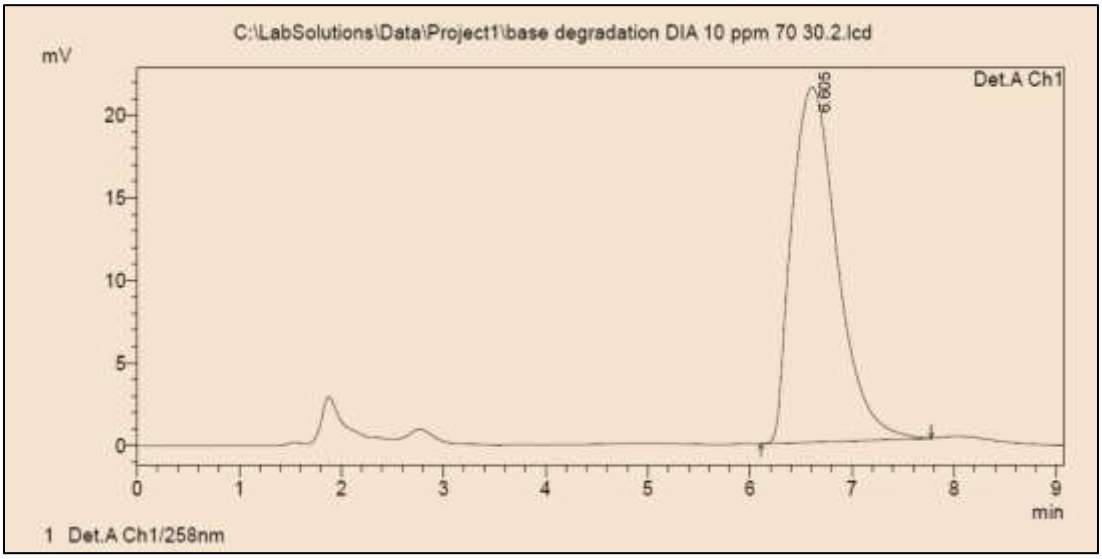

Figure 6: Chromatograph of Base degradation

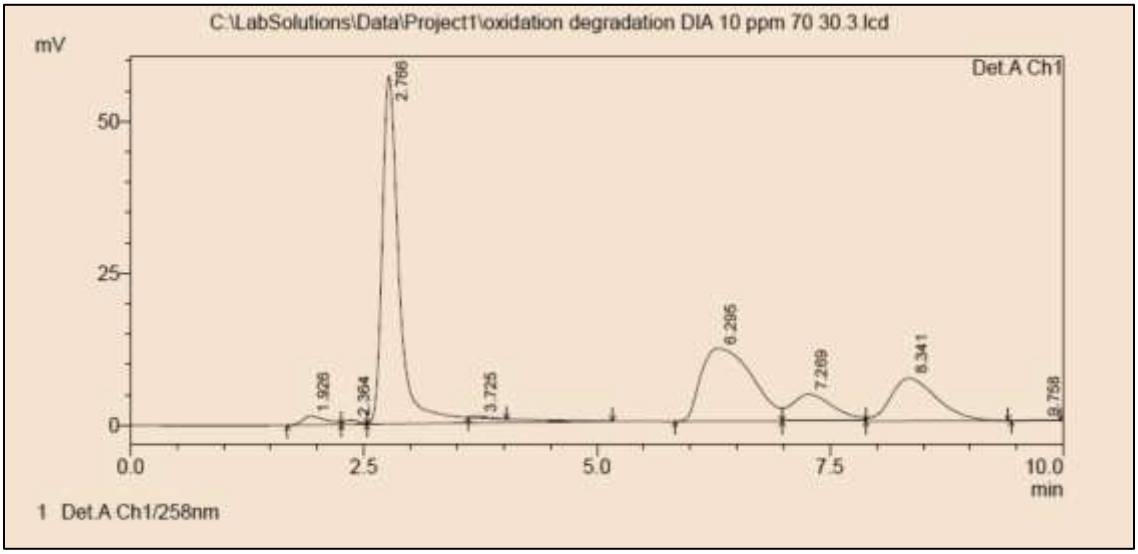

Figure 7: Chromatograph of Oxidation degradation

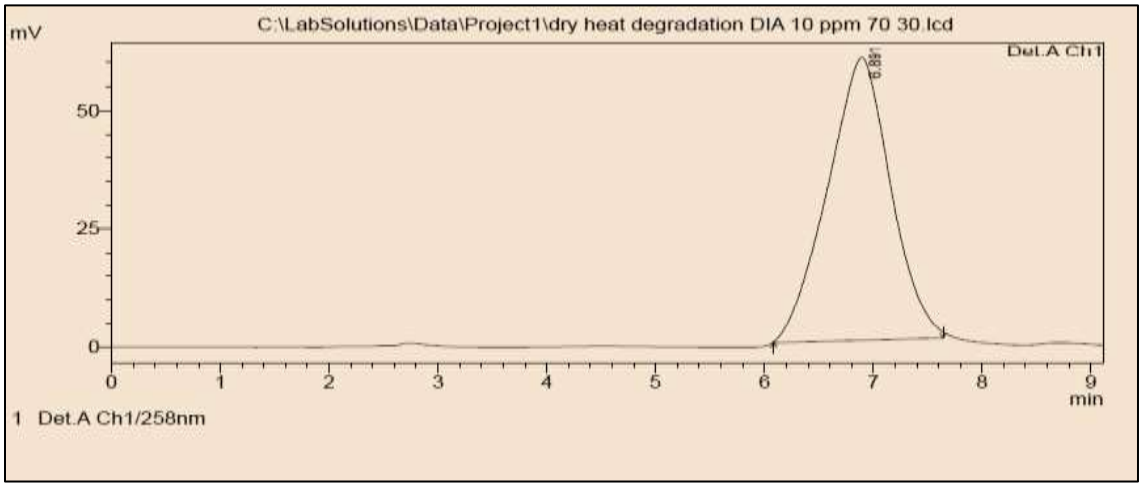

Figure 8: Chromatograph of Dry heat degradation

The chromatograms of wet acidic and basic stress conditions show that peaks of main degradation products were well resolved from that of parent compound.

CONCLUSION

From the present study it can be concluded that the optimized and validated method is simple, sensitive, accurate, precise, and reproducible. It can be utilized for analysis of drug from marketed product.

ACKNOWLEDGMENTS

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