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Implementation of factorial design for optimization of forced degradation conditions and development of validated stability indicating RP-HPLC method for Lidocaine hydrochloride

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ABSTRACT

A simple rapid stability-indicating RP-HPLC assay method was developed and validated for quantitative determination of lidocaine hydrochloride in bulk and ampoule dosage form. Lidocaine hydrochloride (drug and drug product) was subjected to acid and alkali hydrolysis, oxidation by hydrogen peroxide and photo-degradation. Experimental factorial design has been used during forced degradation to determine significant factors responsible for degradation and to optimize degradation conditions reaching maximum degradation. 2^3 Full factorial design has also been used to optimize chromatographic conditions. The chromatographic conditions obtained from factorial design involve the use of phosphate buffer (pH 6.0) and acetonitril (55:45, v/v) as mobile phase at a flow rate of 1.7 mL/min. Inertsil ODS-3(250 mm x 4.6 mm, 5µm) column was used as stationary phase and the detection was performed at 220 nm using PDA detector. The method was successfully applied to the determination of Lidocaine hydrochloride in ampoule dosage form, and the percent recovery +/- standard deviation (SD) was 98.95 ± 0.33. The method was validated as per ICH guidelines. The method was found to be simple and rapid with less trial and error experiments by making use of factorial design.

Keywords: Forced degradation, Factorial design, Lidocaine hydrochloride, Stability indicating, Validation

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INTRODUCTION

Forced degradation study is a complementary part of stability testing wherein influence of environmental stress factors like pH, temperature, humidity, oxygen and light are evaluated on drug substances and products. [1] The forced degradation studies are designed to produce the potential degradation products which are essential for the development of the analytical method to be specific for the API without interference from the products resulting from the stress degradation at the different degradation processes.[2] Several approaches are available to conduct forced degradation studies.[3-7] Among those approaches, the approach we followed during this study was that suggested by Singh and Bakshi.[7] The target is to achieve 5-20% degradation of drug. However, degradation conditions may need to be optimized to enrich the degradation products within this range. Generally, trial and error approach is used to select the optimum degradation conditions that maximize drug degradation. Such approach consumes time and money in addition to neglecting inter-dependency of forced degradation parameters. A new systematic approach to optimize forced degradation conditions is applying the concept of Design of Experiment via the use of factorial design. Factorial design is a type of experimental design in which all possible combinations of all levels of factors are investigated.[8] This helps to arrive at a combination of forced degradation conditions that enrich and maximize degradation of drug. Factorial Design was also used for optimization of HPLC chromatographic conditions required for separation of the drug and is degradation products. Literature review reveals the implementation of Design of Experiment and factorial design concepts for optimization of forced degradation conditions.[9-12] In addition; the use of Design of Experiment concept has been recently used for optimization of chromatographic conditions to develop stability indicating methods for different drugs.[13-16] To

ensure that there is no overlap between peak of active pharmaceutical ingredient and peaks of degradation products or excipients, peak purity assessment using Empower 2 ®software was adopted.[17] This includes peak purity plots, 3D chromatograms, purity angle and purity threshold.

The drug under investigation in this study is Lidocaine hydrochloride. It is an amide type local anesthetic. Chemically, it is 2-(Diethylamino)-N-(2,6-dimethylphenyl) acetamide hydrochloride as shown in figure 1. It stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses, thereby effecting local anesthetic action. It is official in BP[18], USP[19] and European Pharmacopeia.[20] Literature review revealed that several methods have been described for the determination of Lidocaine hydrochloride either alone or in a mixture with other drugs. These include UV spectrophotometric [21-23], electrochemical [24-26], AAS[27, 28] , capillary electrophoresis [29], TLC [30], GC [31-33], and HPLC methods.[34-38] Different stability indicating methods were developed for Lidocaine hydrochloride including GC[39], HPLC [40-42] and UPLC [43] methods. However, there is no study on the use of factorial design neither for optimization of forced degradation conditions nor for development of a stability indicating method for this drug.

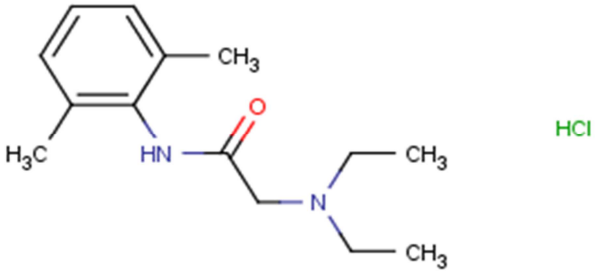

Fig. 1: Chemical structure of Lidocaine hydrochloride

Objectives of the study

The objectives of the current study are: First, optimization of forced degradation conditions for Lidocaine hydrochloride using 2^n full factorial design to enrich degradation products and maximize degradation. Second, Implementation of factorial design for optimization of chromatographic conditions to develop rapid stability indicating method for determination of Lidocaine hydrochloride without interference from degradation products or excipients. Third, ensure that there is no interference from any of degradation product peaks using peak purity assessment.

MATERIALS AND METHODS

Materials and Reagents:

Lidocaine Hydrochloride was kindly supplied by Sigma Tec Pharmaceutical Industries, 6th October, Giza, Egypt, certified to contain 99.9% lidocaine hydrochloride. 2,6-dimethyl aniline was purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade acetonitril was purchased from Fisher Scientific (Pittsburgh, PA, USA). Analytical grade potassium dihydrogen orthophosphate, sodium hydroxide, hydrochloric acid and hydrogen peroxide were used.

Pharmaceutical preparation:

The commercial Lidocaine Hydrochloride –Pharco B® ampoules (Batch No.: 02010613) manufactured by Pharco B International Co. for pharmaceutical industries, Alexandria, Egypt and labeled to contain 1% lidocaine hydrochloride were obtained from local pharmacy.

Apparatus and software:

Waters HPLC system was used, (Waters Corporation, Milford, MA, USA), equipped with a model 600 pump, 2707 auto-sampler injector and 2996 PDA detector. The data acquisition and peak purity analysis were performed using Empower 2® software. Thermostatic controlled water bath (Memmert, Germany) was used for forced degradation studies. pH measurements were made with HANNA pH 211 Microprocessor pH Meter with double junction glass electrode. Photo stability studies were carried out using SUNSET CPS+ Photo cabinet equipped with 1500 W air cooled xenon lamp (ATLAS Material Testing Technology, Illinois, USA). Statistical analysis of data including factorial design was made using Minitab 16® software.

Chromatographic conditions:
Separation and quantitation were carried out on Inertsil ODS-3(250 mm x 4.6 mm, 5µm) column using phosphate buffer (pH 6.0) and acetonitril (55:45, v/v) as mobile phase at a flow rate of 1.7 mL/min. The detection was performed at 220 nm using PDA detector.

Preparation of stock standard solution
Stock standard solution was prepared by dissolving accurately weighed 50 mg of lidocaine hydrochloride powder with distilled water into 50 mL volumetric flask to obtain a solution of 1 mg/mL.

Construction of calibration curve
Different aliquots of 1 mg/mL lidocaine standard solution were quantitatively transferred into a series of 10 mL volumetric flasks, volumes were adjusted to 10mL with mobile phase so as to contain the drug within the concentration range of 5-150 µg/mL. 20.0 µL portions of each solution were injected in replicates into the chromatographic system.

Forced Degradation Studies
The drug concentration for all stress studies was taken 1 mg/mL as per standard literature. A minimum of two samples were prepared for every stress condition. For each stress condition, a blank (control) experiment subjected to stress in the same manner as the drug was chromatographed using the proposed HPLC method.

Acid degradation studies:
Acidic Degradation Solutions were prepared by dissolving 25 mg of Lidocaine Hydrochloride in 5.0 N HCl in 25-mL volumetric flask, then transferred to round bottom flask-condenser assembly, the solution was heated in a boiling water bath for 24 hours. Aliquots of these solutions (1 mL each) were transferred to 10 mL volumetric flasks, cooled and neutralized with 5.0 N NaOH then complete to volume with mobile phase.

Alkaline degradation studies:
Alkaline Degradation Solutions were prepared by dissolving 25 mg of Lidocaine Hydrochloride in 5.0 N NaOH in 25-mL volumetric flask, then transferred to round bottom flask-condenser assembly, the solution was heated in a boiling water bath for 24 hours. Aliquots of these solutions (1 mL each) were transferred to 10 mL volumetric flasks, cooled and neutralized with 5.0 N HCl then complete to volume with mobile phase.

Oxidative degradation studies:
Oxidative Degradation Solutions were prepared by dissolving 25 mg of Lidocaine Hydrochloride in Hydrogen peroxide solution (3% and 10%) in 25-mL volumetric flask and heated in water bath at different temperatures for different periods of time. Aliquots of these solutions (1 mL each) were transferred to 10 mL volumetric flasks, cooled and complete to volume with mobile phase.

Thermal Degradation studies:
Thermal degradation studies were performed by exposing solid drug to dry heat of 50°C in a conventional oven for 7 days.25mg sample was taken every day and transferred to 25-mL volumetric flask and complete to volume with distilled water. 1mL of this solution is transferred to 10-mL volumetric flask and complete to volume with mobile phase.

Photolytic degradation studies:
Photolytic studies were carried out by exposing the drug in solid state to 1.2×10^6 lx hour and 6.0×10^6 lx hour in photostability chamber as per ICH guidelines[44]. Then accurately weighed 25 mg of this powder is transferred to 25-mL volumetric flask and complete to volume with distilled water. 1mL of this solution is transferred to 10-mL volumetric flask and complete to volume with mobile phase.

RESULTS AND DISCUSSION

Forced degradation Studies:
Acid degradation: Lidocaine hydrochloride was found to undergo 22.9% degradation when refluxed in 5.0 N HCl at 100˚C for 24 hours. The high stability of lidocaine is due to the steric hindrance towards attack on the amide group exhibited by the two ortho methyl groups. [45] The degradation product formed (2,6-dimethyl aniline) was confirmed by comparing chromatogram and UV spectrum of acid degraded sample and laboratory prepared mixture of lidocaine and 2,6 DMA. Chromatogram of acid stressed sample is shown in figure 2

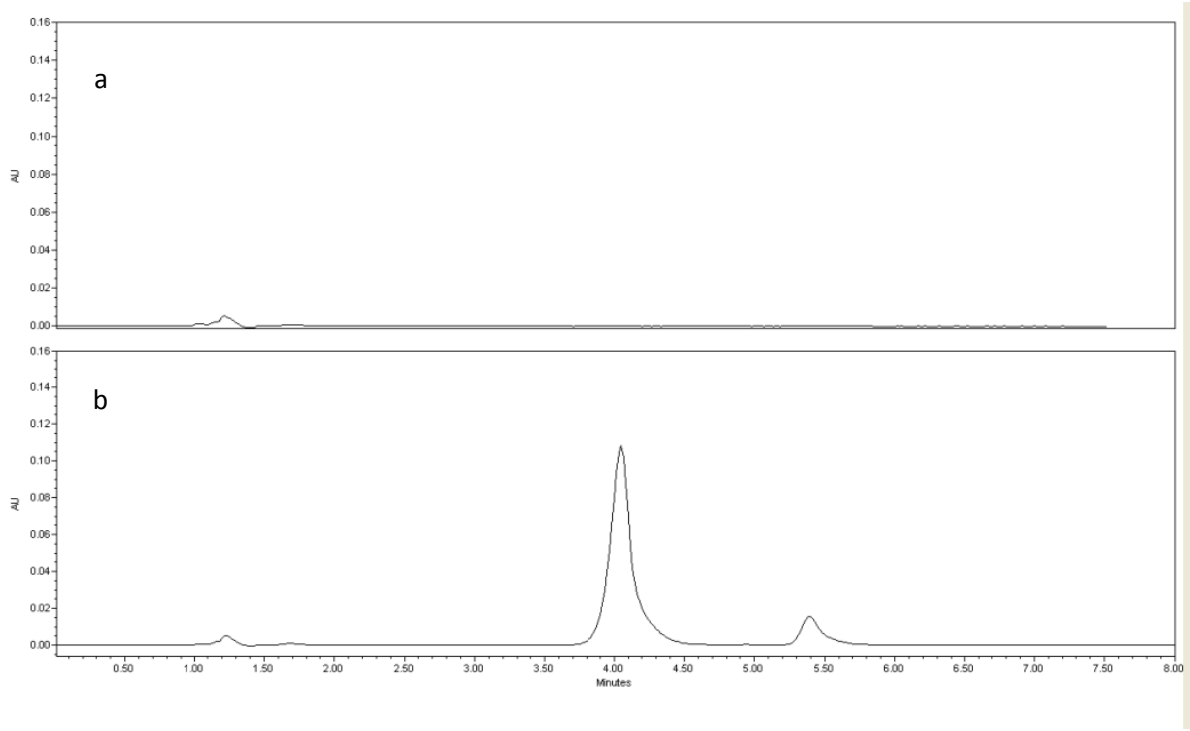

Fig. 2: Chromatogram of (a) blank and (b) Lidocaine alcid stressed sample using the proposed HPLC method

Alkaline degradation: Lidocaine hydrochloride was found to be practically stable in alkaline medium. This is due to the inhibited mesomerism - due to loss of planarity between the benzene nucleus and the amide group - which gives rise to higher electron densities at the amide nitrogen and acyl carbon atoms, thus inhibiting nucleophilic attack.[45] Chromatogram of alkaline stressed sample is shown in figure 3

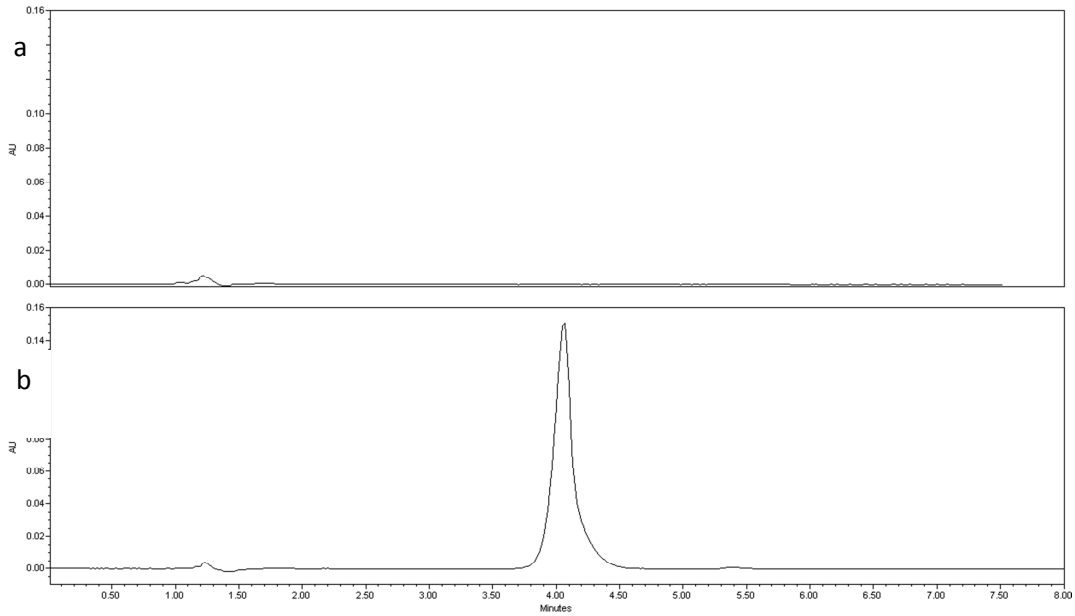

Fig. 3: Chromatogram of (a) blank and (b) Lidocaine alkaline stressed sample using the proposed HPLC method

Thermal Degradation: Lidocaine hydrochloride was found to be thermally stable as it does not undergo any significant degradation after seven days at 50ºC.

Photolytic degradation: Lidocaine hydrochloride does not undergo any significant degradation when exposed to photo-stress conditions in photostability chamber.

Oxidative degradation: For oxidative degradation, Flow chart was followed, and lidocaine hydrochloride was degraded by 7.25% using 3% H_2O_2 for 24 hours at room Temperature.

Optimization of forced oxidative degradation conditions by factorial design:
To maximize oxidative degradation, Factors affecting lidocaine hydrochloride oxidation were studied and optimized by factorial design. Three factors were found to affect Lidocaine hydrochloride oxidation. These include hydrogen peroxide concentration, Temperature and time. Two levels of each of those factors were selected and 2^3 full factorial design was performed. A set of eight experiments were carried out and % degradation of drug was obtained as shown in table 1. Using Minitab 16® Software, pareto chart and contour plot were obtained as shown in figures 4 and 5. Optimum degradation conditions were 10% hydrogen peroxide for 24 hours at 40ºC as shown by response optimizer figure 6. Chromatogram of oxidative stressed sample is shown in figure 7.

Table .1: Factorial Design experiments for optimization of Lidocaine hydrochloride % Oxidative degradation

Experiment no.	H_2O_2 conc. (%)	Time (hour)	Temp. (°C)	% Degradation
1	3	24	40	12.34
2	10	24	40	18.99
3	10	6	25	10.28
4	10	6	40	14.56
5	3	24	25	7.52
6	3	6	40	7.77
7	10	24	25	15.39
8	3	6	25	3.08

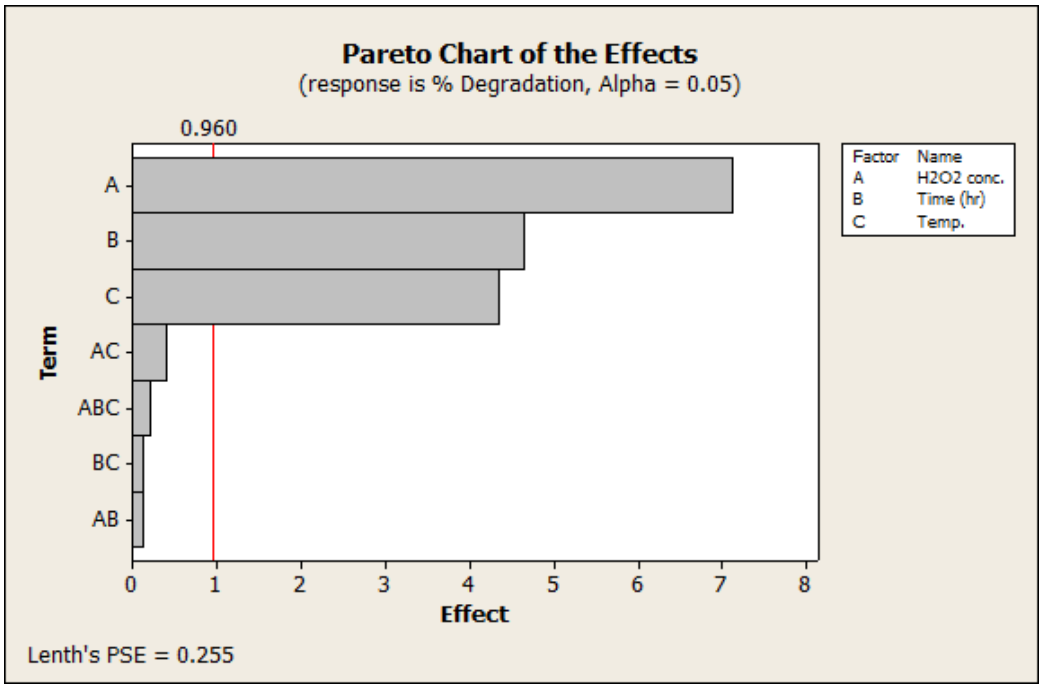

Fig. 4: Pareto chart of the effect of different factors on lidocaine hydrochloride % oxidative degradation

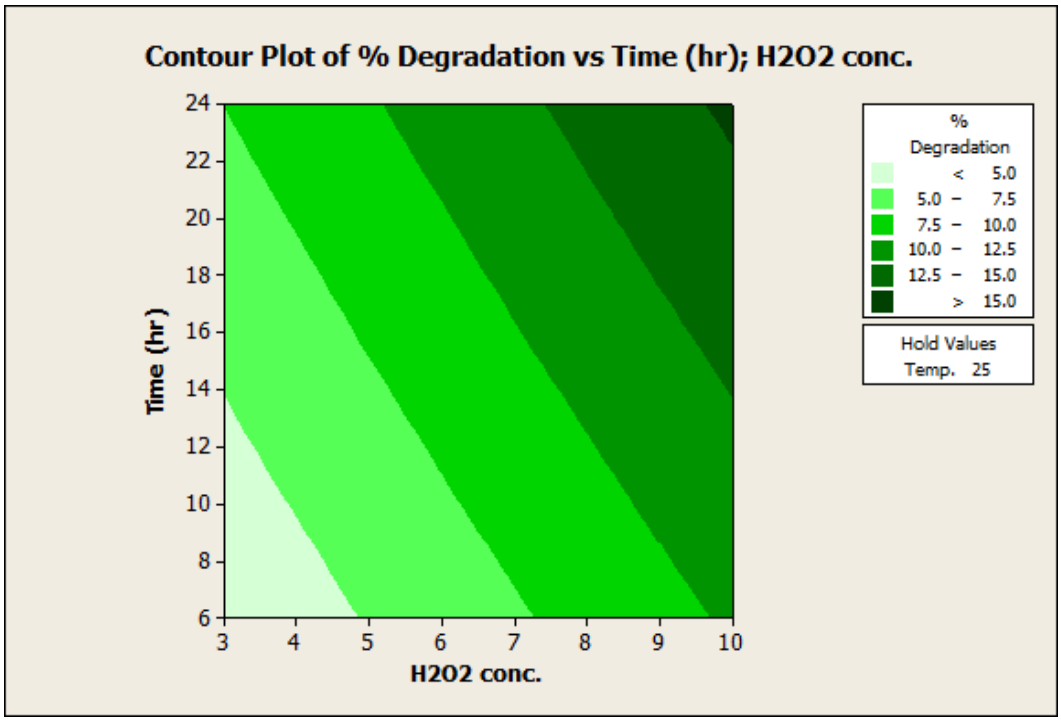

Fig. 5: Contour plot of lidocaine hydrochloride % oxidative degradation versus time and hydrogen peroxide concentration

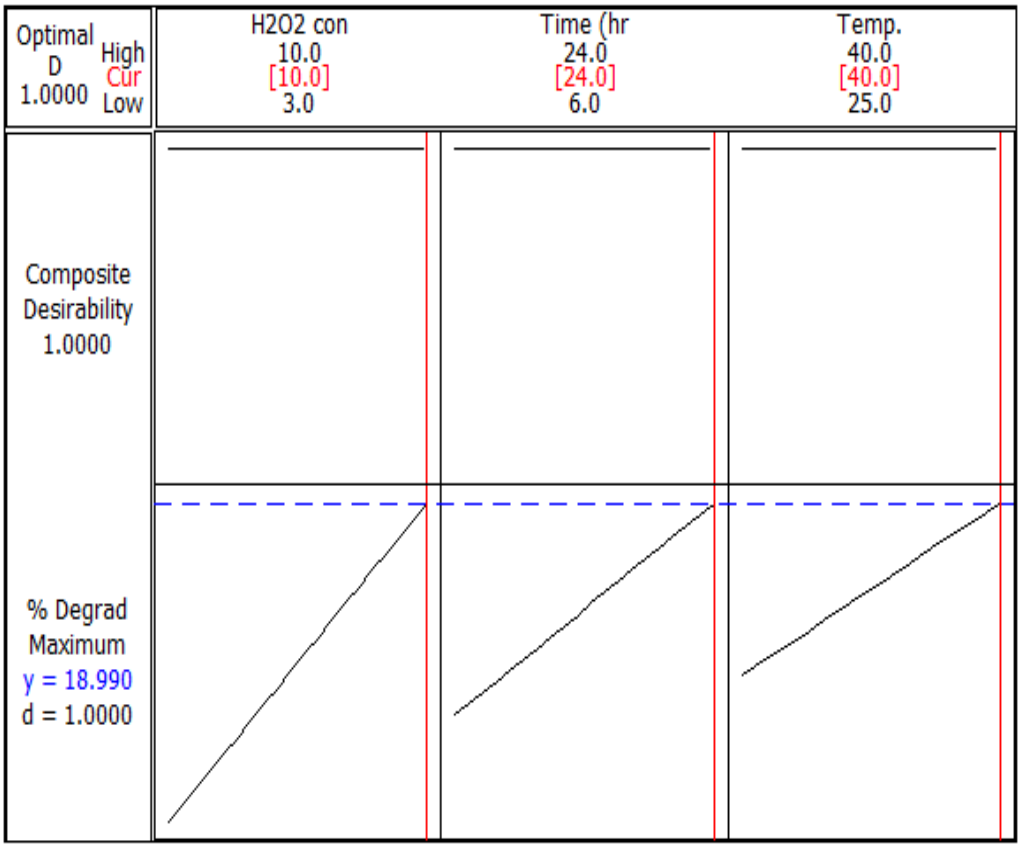

Fig. 6: Response optimizer for lidocaine hydrochloride % oxidative degradation showing optimum degradation conditions

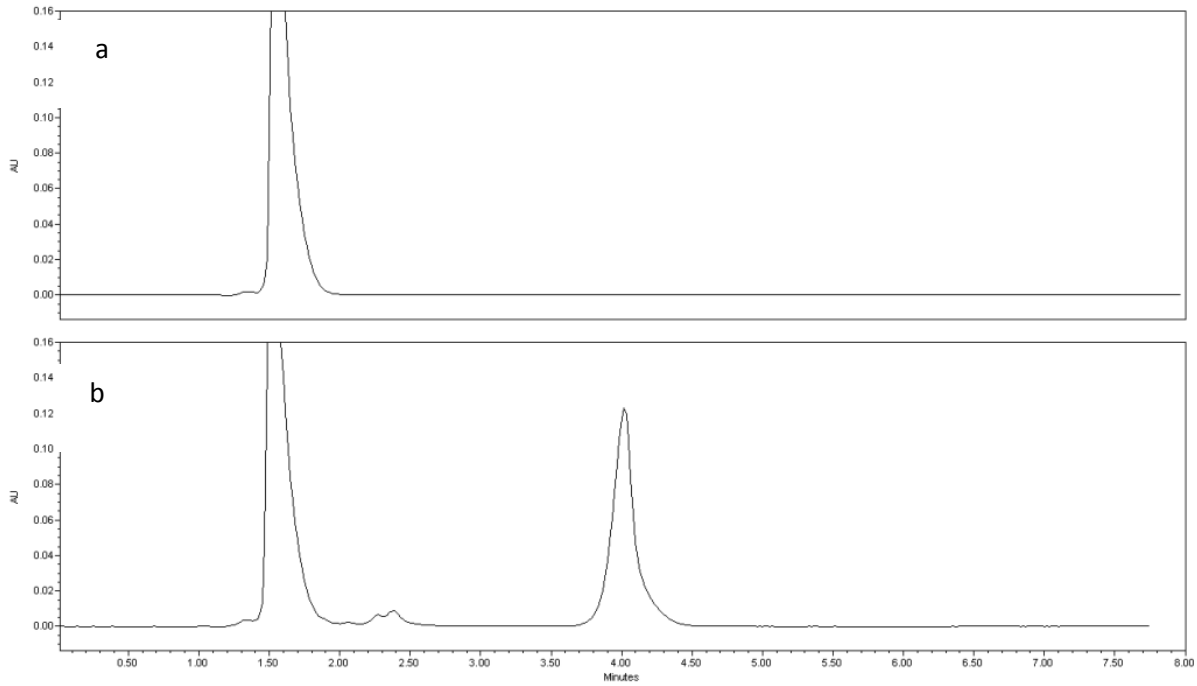

Fig. 7: Chromatogram of (a) blank and (b) Lidocaine oxidative stressed sample using the proposed HPLC method

Optimization of HPLC chromatographic conditions using factorial design:

From preliminary experiments three factors were found to greatly affect HPLC method performance which are pH of phosphate buffer, ratio of acetonitril in the mobile phase and flow rate. A set of preliminary studies was performed to establish the low and high levels of each factor. 2^3 full factorial design was carried out for optimization of chromatographic conditions. Four responses were selected, run time, tailing of Lidocaine peak, resolution between Lidocaine and 2,6- DMA and resolution between Lidocaine and its oxidative degradation product as shown in table 2. Some of pareto charts, contour plots and interaction plot results are shown in figures 8-10. Optimum chromatographic conditions obtained from factorial design are shown in figure 11. System suitability criteria of the proposed HPLC method are listed in table 3.

Table .2: Factorial Design experiments for optimization of HPLC conditions

Exp. no.	pH	% ACN	Flow rate (mL/min)	Rs LH & DMA	Rs LH & Ox degradation	Tailing	Run time (minutes)
1	5.8	40	1.7	13.76	4.55	1.01	11.0
2	5.8	40	1.3	13.24	4.88	1.02	14.5
3	7.0	60	1.3	3.95	7.89	1.93	8.0
4	7.0	60	1.7	4.58	8.42	1.88	6.5
5	5.8	60	1.7	3.59	3.65	1.12	5.0
6	7.0	40	1.3	10.23	15.26	1.69	24.0
7	5.8	60	1.3	3.58	2.41	1.18	6.0
8	7.0	40	1.7	10.53	15.04	1.65	18.0

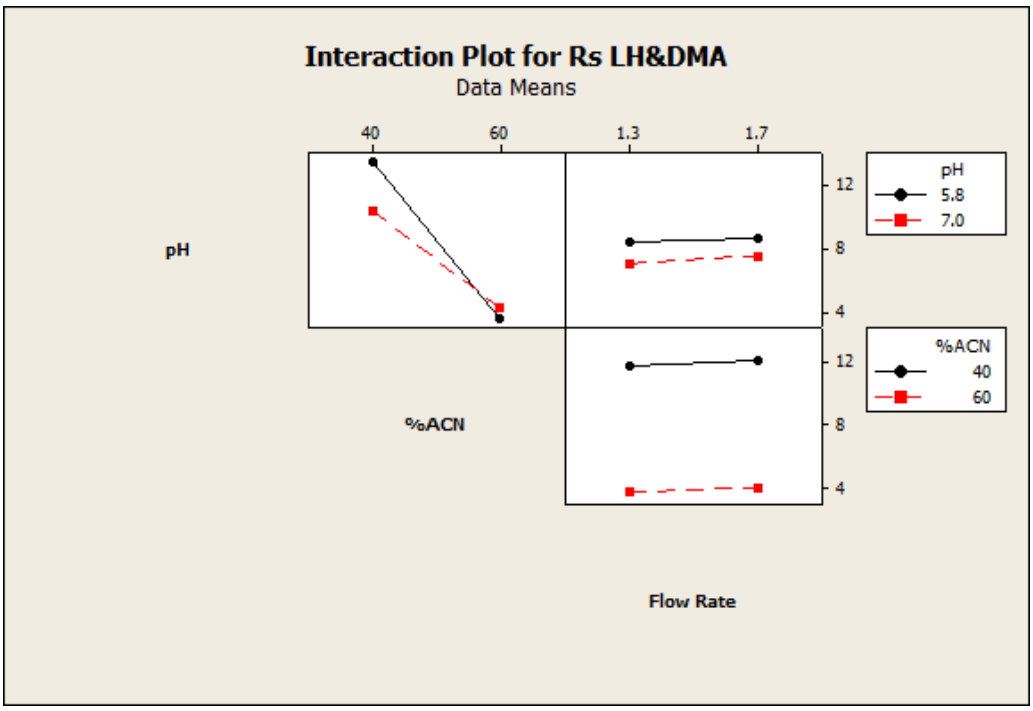

Fig. 8: Interaction plot of the effect of factors' interaction on resolution between peaks of lidocaine hydrochloride and 2,6- dimethyl aniline

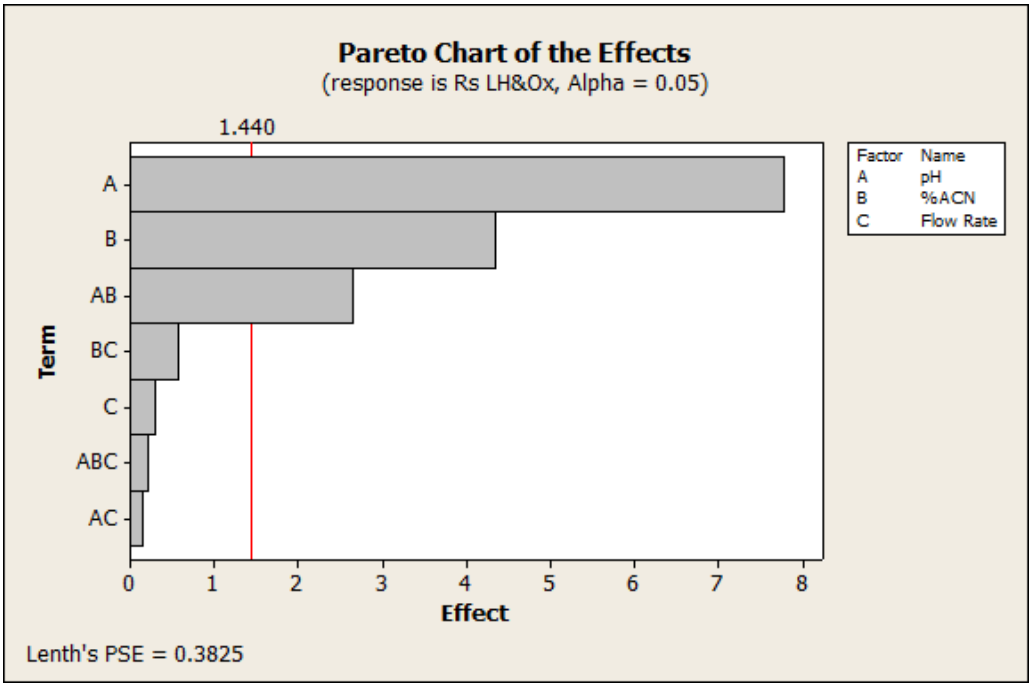

Fig. 9: Pareto chart of the effect of different factors on resolution between peaks of lidocaine hydrochloride and its oxidative degradation product

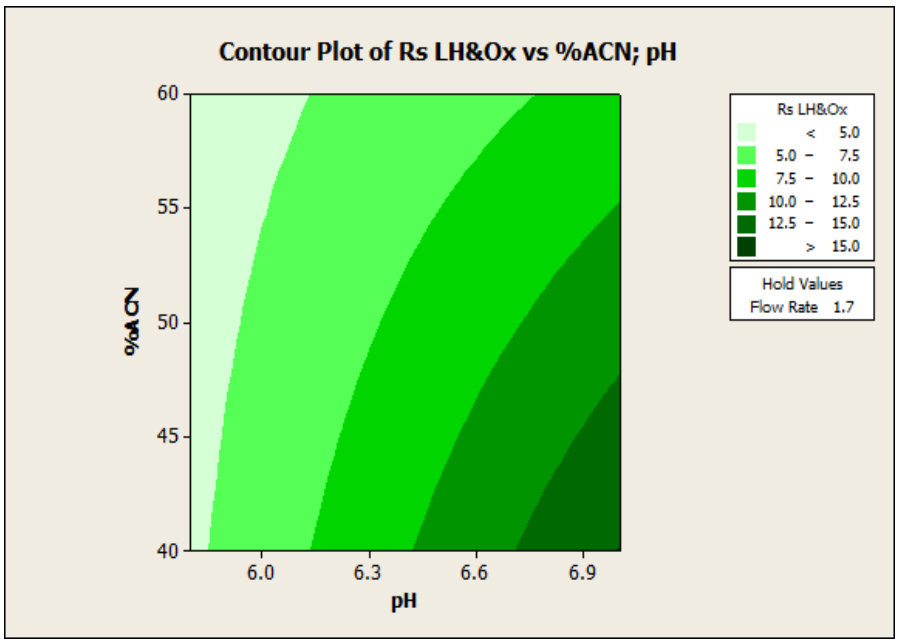

Fig. 10: Contour plot of Resolution between peaks of lidocaine hydrochloride and its oxidative degradation product versus % ACN and pH

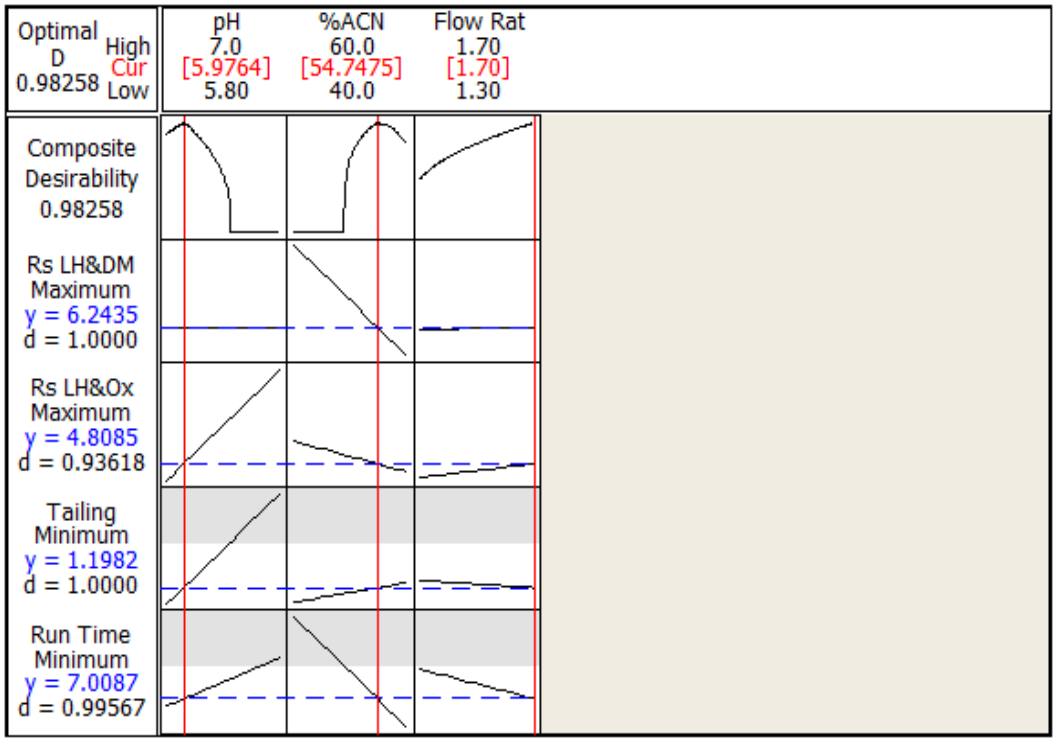

Fig. 11: Response optimizer showing optimum chromatographic conditions

Table .3: Results of system suitability tests

parameter	Value
Retention time (R_T) (min)	4.03
Theoretical plates (N)	3922
HETP (cm)	0.0064
Tailing factor	1.19
K'	1.69
R_s between LH and 2,6-DMA	5.13
R_s between LH and oxidative degradation product	6.21

Peak purity assessment

Peak purity analysis was carried out using Empower 2® software to make sure there is no overlap from any of degradation products peaks with Lidocaine peak. Table 4 demonstrates purity angle and purity threshold for each stressed sample. Peak purity plot of Lidocaine are shown in figure 12. Three dimensional plots of HPLC chromatogram of stressed samples are shown in figure13.

Table .4: Degradation results, purity angle and purity threshold of Lidocaine hydrochloride

Stress condition	Purity angle	Purity threshold	% Degradation
Acid degradation	0.222	0.354	22.9%
Alkali degradation	0.101	0.325	2.1%
Oxidative degradation	0.183	0.328	18.9%
Thermal degradation	0.111	0.321	No degradation
Photolytic degradation	0.123	0.343	No degradation

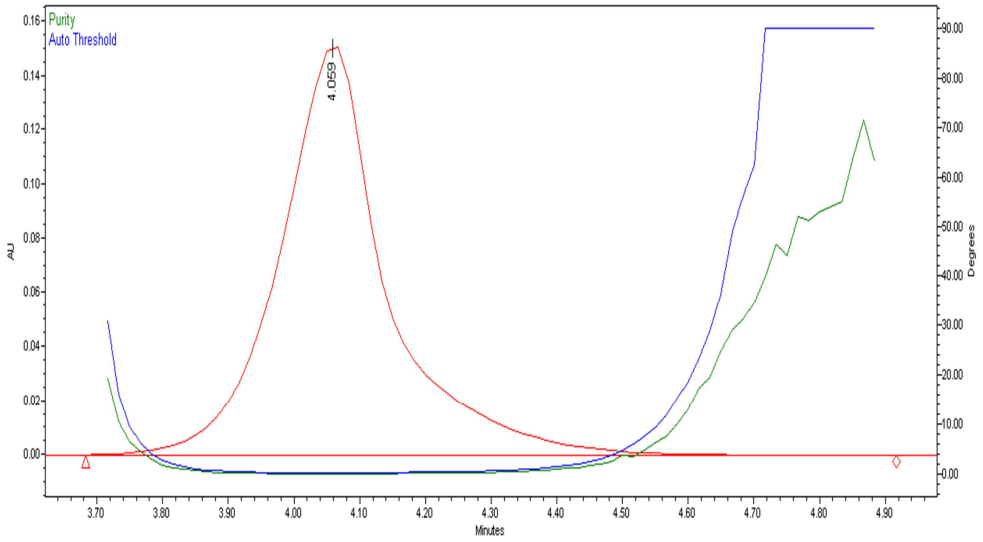

Fig.12: Peak purity plot of Lidocaine hydrochloride in acid stressed sample

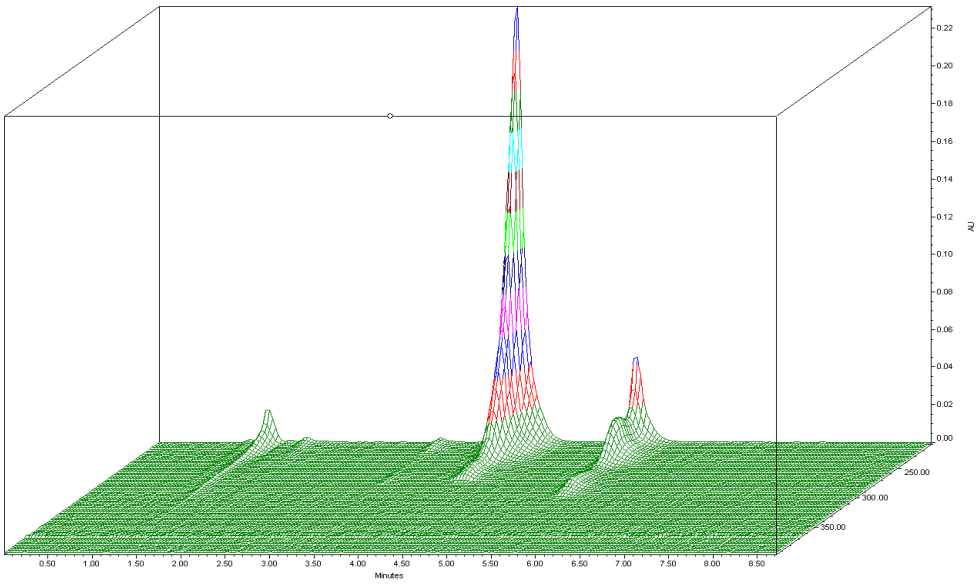

Fig.13: Three dimensional plot chromatogram of Lidocaine acid stressed sample

Method validation

The proposed method was validated regarding linearity, accuracy, repeatability and intermediate precision according to ICH Q2 (R1) recommendations. [46]

Linearity and range

The linearity of the method was established by preparing a calibration curve in the range 5-150 µg/mL. Triplicates of each of the solution were injected and chromatograms recorded. The mean area under peak (mAU*min) was plotted against concentration (µg/mL) to construct the calibration curve. The correlation coefficient and regression equation were determined. The results are shown in table 5.

Accuracy

The accuracy of the method was evaluated by analyzing freshly prepared solutions of the drug in triplicate at concentrations of 50, 75 and 100µg/mL. The percentage recoveries, the mean recovery and the standard deviation were then calculated. The results are shown in table 6.

Precision

The Interday and intraday precision were determined by calculation of the % RSD values on injection of triplicates of each concentration on the same day (intraday) precision and on three consecutive days (interday). The mean (n=3) area under peak (mAU*min) of each concentration was compared with that of second run on the same day (intraday) and with that on the next day (interday) and the percent relative standard deviation was calculated. The results are shown in tables 7 and 8.

Robustness

Deliberate changes in the mobile phase flow rate (+/- 0.1 units), mobile phase composition (+/- 2%) and pH of aqueous (+/- 0.1) were made. The %RSD between the area under peak (mAU*min) and that obtained under optimized chromatographic conditions were determined. The results are shown in table 9.

Limit of detection and limit of Quantitation

The limit of detection and limit of Quantitation were calculated based on standard deviation (σ) of responses for triplicate blank injections and the slope (S) of the calibration plot, using the formulae LOD= 3.3σ/s and LOQ=10σ/S as defined by ICH. The LOD and LOQ values were found to be 0.080and 0.244 µg /mL respectively.

Table .5: Linearity regression data for the calibration plot of Lidocaine hydrochloride

Linearity range	1 – 150 µg/mL
slope	17099.52
SE of slope	72.25
Intercept	- 12845.43
SE of Intercept	4875.42
Correlation coefficient (r)	0.9999
SE of estimation	10452.24

Table .6: Recovery data of Lidocaine hydrochloride using the proposed HPLC method

conc. taken (µg/ml)	conc. found (µg/ml)	* % Recovery
50	49.80	99.61
75	75.08	100.10
100	99.88	99.88
Mean % recovery ± SD	99.86 ± 0.25	

**mean of three determinations.*

Table .7: Intraday precision experiments for determination of Lidocaine hydrochloride using the proposed HPLC method

conc. taken (µg/ml)	conc. found (µg/ml)	%RSD
50	49.90	0.38
	49.58	
	49.92	
75	75.38	0.36
	74.98	
	74.87	
100	99.60	0.25
	99.95	
	100.09	

Table .8: Intermediate (inter day) precision for determination of Lidocaine hydrochloride using the proposed HPLC method

conc. taken (µg/ml)	conc. Found (µg/ml)	%RSD
50	49.80	0.82
	49.52	
	50.33	
75	75.08	0.73
	74.25	
	75.30	
100	99.88	0.54
	99.05	
	100.07	

Table .9: Robustness results for the HPLC method

Parameters		Recovery %	Mean recovery %	S.D	R.S.D
pH of aqueous component of mobile phase	5.9	98.87	99.54	0.64	0.64
	6.0	99.60			
	6.1	100.14			
% ACN	53	100.43	99.65	0.76	0.76
	55	99.60			
	57	98.91			
Flow rate (mL/min)	1.6	100.24	99.48	0.82	0.83
	1.7	99.60			
	1.8	98.61			

CONCLUSION

Forced degradation behavior of Lidocaine hydrochloride was studied. Using 2^n full factorial design, the oxidative degradation conditions were optimized to obtain the targeted level of degradation product. Factorial design was also implemented for development of simple, sensitive and rapid stability indicating HPLC method for the determination of lidocaine hydrochloride. The developed method determines it in the presence of its potential degradation products as well as in ampoule dosage form within six minutes. This study highlights significant utility of factorial design in optimization of forced degradation conditions as well as the development of Stability Indicating Methods.

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