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VALIDATED GRADIENT STABILITY-INDICATING UPLC METHOD FOR THE DETERMINATION OF LIDOCAINE AND ITS DEGRADATION IMPURITIES IN PHARMACEUTICAL DOSAGE FORM

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Abstract

Objective: Aim of the present work is to develop a stability indicating ultra performance liquid chromatography (UPLC) method to determine Lidocaine and its degradation impurities in pharmaceutical dosage forms.

Method: Chromatographic separation was achieved by gradient elution on Agilent eclipse plus C18 (100x4.6) mm, and 1.8µm column with potassium dihydrogen phosphate buffer (pH 4.50) and acetonitrile within a short runtime of 14.0 min. The eluted compounds were monitored at wavelength of 230 nm using photodiode array (PDA) detector, the flow rate was 1.0 mL/min, and the column oven temperature was maintained at 40 ◦C.

Result: The resolution of Lidocaine and six (potential, bi-products and degradation) impurities was greater than 2.0 for all pairs of components. The repeatability and intermediate precision, expressed by the RSD, were less than 1.0%. The accuracy and validity of the method were further ascertained by performing recovery studies. The specificity of the method was investigated under different stress conditions including hydrolytic, oxidative, photolytic and thermal as recommended by ICH guidelines. Relevant degradation was found to take place under oxidative condition.

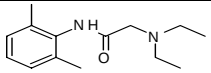
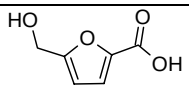
Conclusion: Method was Robustness against small modification in pH, column oven temperature, flow rate and percentage of the mobile phase composition was ascertained. All these results provide that the method has stability indicating properties being fit for its intended purpose; it may find application for the routine analysis of the related substances of Lidocaine formulations.

***Keywords*:** Ultra Performance Liquid chromatography (UPLC); Lidocaine; Validation; Stress Degradation products

1. Introduction

Lidocaine Hydrochloride Injection,[1] USP is chemically designated 2-(diethylamino)-N-(2,6-dimethylphenyl)-acetamide mono hydrochloride monohydrate, commonly used as local anesthetic and anti-arrhythmic drug. Lidocaine stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses, thereby effecting local anesthetic action. It is a white colored powder which is freely soluble in water and diethyl ether and insoluble in cold & hot water. Lidocaine is having a chemical formula of $C_{14}H_{22}N_2O$ with molecular weight of 288.82. [Fig-1]. UPLC[2] is a new separation technique takes advantage of technological strides made in particle chemistry performance, system optimization, detector design, and data processing and control. Using sub-2mm particles and mobile phases at high linear velocities, and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity, and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance. UPLC presents the ability to extend and expand the utility of separation science at a time when many scientists have reached separation barriers, pushing the limits of conventional HPLC. New chemistry and instrumentation technology can provide more information per unit of work as UPLC begins to fulfill the promise of increased speed, resolution, and sensitivity predicted for liquid chromatography

Figure-1 Structures of Lidocaine & its related impurities:

S.No	Name	IUPAC Name	RRT	Mol. Weight	Structure
1.	Lidocaine ($C_{14}H_{22}N_2O$)	2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide	-	234.34	
2.	5-Hydroxy Methyl Furfural ($C_6H_6O_3$)	5-(hydroxymethyl)-2-furaldehyde	0.46	126.11	

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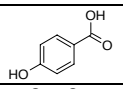
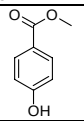
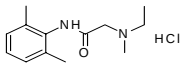
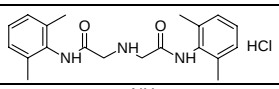
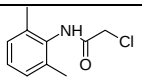
3.	4-Hydroxy Benzoic Acid ($C_7H_6O_3$)	4-Hydroxybenzoic acid	0.56	138.12	
4.	Methylparaben ($C_8H_8O_3$)	Methyl 4-hydroxybenzoate	1.34	152.15	
5.	Lidocaine Impurity K ($C_{13}H_{20}N_2O$. HCl)	N-(2,6-Dimethylphenyl)-2-(ethylmethylamino)acetamide Hydrochloride	1.64	220.32	
6.	Lidocaine Impurity E ($C_{20}H_{25}N_3O_2$. HCl)	2,2'-(Azanediyl)bis[N-(2,6-dimethylphenyl)-acetamide] Hydrochloride	1.79	339.44	
7.	2,6-Dimethylaniline ($C_8H_{11}N$)	2,6-dimethylbenzene-1-amine	2.01	121.18	
8.	Lidocaine Impurity H ($C_{10}H_{12}ClNO$)	N-(2,6-Dimethylphenyl) chloro acetamide	2.28	197.66	

To date, all analytical methods described in literature [Table-1] include determination of Lidocaine in combination of different molecules in biological fluids involve liquid chromatography[3-4], determination of Lidocaine in combination of different molecules involve liquid chromatography[5-9], determination of Lidocaine in combination of different molecules involve gas chromatography[10] and assay methods for determination Lidocaine involves UV and liquid chromatography[11-12]. Literature survey shows that no stability indicating method has been reported for the determination of Lidocaine and its related substances. Hence an attempt was made to develop a single method for all paraben, dextrose and epinephrine combination formulations which is a stability indicating, reliable, accurate and sensitive method for the determination of Lidocaine and its Degradation Impurities in Pharmaceutical Dosage Form.

2. Experimental

2.1. Chemicals and Reagents

Lidocaine (purity 99.0%) and standard materials of degradation products were obtained from Hospira Health Care India Pvt Ltd, Chennai, India. Monobasic potassium phosphate, Orthophosphoric acid and acetonitrile were purchased from Ranbaxy Chemicals, New Delhi, India. All chemicals were of HPLC grade and used as received. Water was purified by a milli-Q-water purification system (Millipore, Bedford, MA, USA) and used for preparation of all the solutions.

2.2 UPLC Instrumentation and Condition

The analysis was performed using Waters Acquity UPLC system (H-Class) equipped with a quaternary solvent delivery pump, an auto sampler, and a PDA /UV detector. Data acquisition and processing was done by using Empower2 software version FR5 (Waters Corporation, USA). The chromatographic separation was performed using Agilent eclipse plus C18 column (100 x 4.6 mm), 1.8 µ particle column. The mobile phase was a mixture of mobile phase A and mobile phase B. Mobile phase A was 20mM of mono potassium phosphate adjusted to pH 4.5 with orthophosphoric acid and acetonitrile was used as mobile phase B. The mobile phase A & B were pumped through the column at a flow rate of 1.0mlmin−1 with a gradient elution (Table 2). The optimum wavelength selected was 230nm which represents the wavelength of maximum response for all impurities in order to permit simultaneous determination of related impurities of Lidocaine. The stressed samples were analyzed using a PDA detector covering the range of 200–400 nm.

Table 1.Comparison of the performance characteristics of the present method with the published methods

S. No	Method	λ (nm)	Remarks	Ref
1.	LC-MS/Lidocaine & MEGX	-	Human Plasma	[3]
2.	LC-MS/Lidocaine	-	Human Plasma	[4]
3.	HPLC/ Lidocaine & Nystatin	230	Assay/combinational drugs	[5]
4.	HPLC/ Lidocaine & Tolperisone	210	Assay/combinational drugs	[6]
5.	HPLC/ Lidocaine & Tribenoside	254	Assay/combinational drugs	[7]
6.	HPLC/ Lidocaine & Prilocaine	225	Assay	[8]
7.	HPLC/ Lidocaine & Cetrimonium	208	Assay	[9]
8.	GC/Lidocaine & Bupivacaine	-	Human Plasma	[10]
9.	UV/Lidocaine	263	Derivatization method	[11]
10	HPLC/Lidocaine	210	Narrow linearity	[12]
11.	UPLC/ Lidocaine	230	Related substances Stability Indicating	Present work

2.3 Preparation of buffer, diluent, standard and sample solution

2.3.1. Buffer: About 2.72 g of monobasic phosphate phosphate dissolved in 1000 mL of water, adjusted to pH 4.5 ± 0.05 with dilute ortho phosphoric acid solution was used as buffer(mobile phase A).

2.3.2. Diluent: The diluent used for the standard and sample preparation was a mixture of acetonitrile and water in the ratio of 25:75 (v/v).

2.3.3. Standard: A stock solution of Lidocaine Hydrochloride (500 µg/ mL) was prepared by dissolving an appropriate amount in the diluent. Standard solution containing 2 µg/ mL was prepared from this stock solution.

2.3.4: Sample: 2 mL of Lidocaine injection USP solution containing 50000 µg/ mL was dissolved in 50 mL of diluent to give a solution containing 2000 µg/ mL was used as sample solution.

2.4 Forced degradation sample solution for specificity study

The study was intended to ensure the separation of Lidocaine and its degradation impurities. Forced degradation study was performed to evaluate the stability indicating properties and specificity of the method[13]. As per the ICH guide line Q2(R1)[14] provides the stress conditions to be performed for degradation impurities which states that the stress samples should be stored under relevant stress conditions such as light, heat, humidity, acid/base hydrolysis and oxidation. As per the guide line multiple stress studies were performed as indicated below and they were chromatographed along with an un-stressed sample.

2.4.1.Hydrolytic conditions: acid, base, water induced degradation: Solution containing 2mg/mL of Lidocaine was treated with 1N HCl, 1N NaOH and water respectively. These samples were refluxed at 80°C for 5 hrs. After cooling the solutions were neutralized and diluted with diluent.

2.4.2. Oxidative condition: Hydrogen peroxide-induced degradation. Solution containing 2mg/mL of Lidocaine was treated with 6% w/v H_2O_2 at 40°C for 6 hrs was cooled and diluted with diluent.

2.4.3. Thermal degradation study: The drug solution (5mg/mL) was subjected to heat at 105°C for 24 hrs. After cooling 2 ml of the above solution was transferred in 50 ml volumetric flask, diluted to the volume with diluent.

2.4.4. Photolytic degradation study: The drug solution (5mg/mL) was exposed to the UV light in the photolytic chamber for 184 hrs. 2 ml of the above solution was transferred in 50 ml volumetric flask, diluted to the volume with diluent.

2.4.5. Humidity degradation study: Metoclopramide injection USP (5mg/mL) was subjected to25°C/90%RH for 7 days. 2 ml of the above solution was transferred in 50 ml volumetric flask, diluted to the volume with diluent.

3. Results and discussion

3.1. Optimization of chromatographic conditions

3.1.1. Selection of mobile phase:

Considering that Lidocaine and its related compounds acidic buffer in combination of organic modifiers was selected, following mobile phases with gradient elution were tested,

1. $KH_2PO_4 \cdot H_2O$ (50mM) of pH 4, 4.5, 5, 6 as a buffer in combination with acetonitrile.
2. $(NH_4)H_2PO_4$ (50mM) of pH 4, 4.5, 5, 6 as a buffer combination with acetonitrile.
3. $KH_2PO_4 \cdot H_2O$ (20mM) of pH 4, 4.5, 5, 6 as a buffer combination with acetonitrile.
4. $KH_2PO_4 \cdot H_2O$ (20mM) of pH 4, 4.5, 5 as a buffer combination with methanol.

Trails with different combination of buffer and organic modifier were performed, Potassium phosphate buffer of 50mM strength have given broad peak shape with more tailing for Lidocaine and Impurity H. To have better tailing trails were performed with Ammonium phosphate buffer of 50mM strength, with Ammonium Phosphate buffer 5-HMF & 4-HBA were completely merging with each other and poor resolution was observed between Lidocaine impurity-k & impurity- E. Hence $KH_2PO_4 \cdot H_2O$ (20mM) as a buffer (pH 4.5) in combination with acetonitrile was chosen to improve resolution among the impurities and peak shape of Lidocaine.

3.1.2. Selection of stationary phase

The possible impurities of Lidocaine are very similar to Lidocaine. To obtain a good resolution among the impurities and main drug substances different stationary phases were tested considering;

a. The feature of stationary phase (C8 and C18).

b. The particle size of the column (1.7µm and 3µm).

It is clear from the molecular structure (Fig. 1) that all compounds do not possess a functional group which can readily ionize indicating non-polar in nature. Hence we started the development activity with C8 stationary phase of various manufacturers using different mobile phases. The poor resolution between 5-HMF and 4-HBA, Lidocaine Impurity-K&E and broad peak shape for Lidocaine implies that C8 stationary phase is not suitable for this application. Hence C18 stationary phase was chosen to improve resolution among the peaks and peak shape for Lidocaine. The development activity was further carried out with Waters acquity C18 (100x 2.1) mm 1.7µ column, the 5-HMF and 4-HBA were completely merged with each other; very poor resolution was observed between Lidocaine Impurity K& E implies that 1.7µm column is not suitable for this application. Hence 1.8µm column was used to improve resolution among all components, with Agilent eclipse plus C18, (100×4.6) mm, 1.8µm column the peak shapes and resolution among all components was found to be satisfactory. The stationary phase is not only the parameter which can give better separation among all impurities. Mobile phase, pH and organic modifies also plays very important role which leads to the best separation.

Hence in order to get the best separation among the impurities and main peaks the further trails were

proceeded with Agilent eclipse plus C18, 100mm×4.6mm, 1.8µm column as stationary phase.

3.1.3. Influence of organic modifier

Initially the methanol was used as an organic modifier which gives the poor baseline with baseline drift. Hence the response for the related compounds was reduced. The retention for all impurities was increased leading to poor resolution among the peaks. To improve the resolution among the peaks and response, acetonitrile was tried as an organic modifier. The base line was found good and response for all components was improved. The peak shape for all components was also improved and hence acetonitrile was selected as organic modifier. The composition of the acetonitrile was altered [Table 2] accordingly depending on the molecule and impurity to obtain the best separations among the impurities and main peaks.

Table 2: Mobile phase program for gradient elution:

Time (min)	Flow (mlmin−1)	Solvent A (%)	Solvent B (%)
0	1.0	90	10
4	1.0	70	30
5	1.0	68	32
11	1.0	65	35
12	1.0	90	10
14	1.0	90	10

Solvent A: potassium dihydrogen phosphate buffer (pH 4.5); solvent B: Acetonitrile

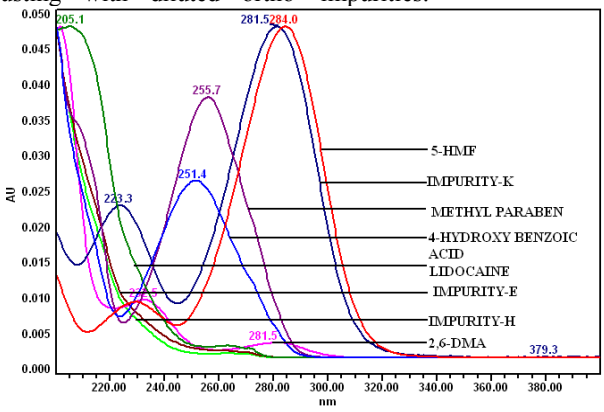
3.1.4. Influence of pH of mobile phase buffer

Different pH values of the mobile phase were checked to establish the optimum separation and the highest analytical sensitivity for Lidocaine and its degradation products. The mobile phase was buffered because of the existence of ionizable groups in the chemical structure of the drug, which could ionize at different pH values. The pH values tested were 4.0, 4.5 and 5.0. Finally, the best results were obtained at pH 4.5 ±0.1 by adjusting with diluted ortho phosphoric solution. The choice of this mobile phase is justified by the excellent symmetry of the peaks and adequate retention times of Lidocaine and its degradants.

3.1.5. Selection of wavelength

Based on the spectra [Fig. 2] of Lidocaine and its related substances 230 nm was selected as detection wavelength for the method.

Figure- 2 Spectra of Lidocaine and its related impurities:



3.1.6. Flow rate optimization

Different mobile phase flow rates (0.8, 1.0and 1.2 mL min-1) were investigated. The optimum flow rate for which the column plate number was maximum, with the best resolution between all compounds and with a shortest runtime of 14 min observed was 1.0 mL min-1.

3.1.7. Column temperature optimization

Column thermostat temperatures were used at 35ºC, 40ºC and 45ºC for better peak shapes, baseline and resolution. At the column oven temperature of 40ºC the finest baseline resolution was observed between all the components

After an extensive study, the method has been finalized on Agilent eclipse plus C18, 100mm×4.6mm, 1.8µm columns using variable composition of solvent A: $KH_2PO4.H_2O$ (20mM), pH 4.5 with diluted ortho phosphoric acid and solvent B: acetonitrile as mobile phase. The mobile phase pumped through the column with gradient elution [Table 2] at a flow rate of 1.0 ml min−1 and column compartment temperature kept at 40 ◦C. Depending on the λ max [Fig. 2] of the Lidocaine and its related impurities the detector response for all the components found maximum at 230 nm, hence the typical chromatogram was recorded at this wavelength. The typical UPLC chromatograms (Fig. 3) represent the satisfactory separation of all components among each other.

Figure -3 Impurity spiked chromatogram Lidocaine:

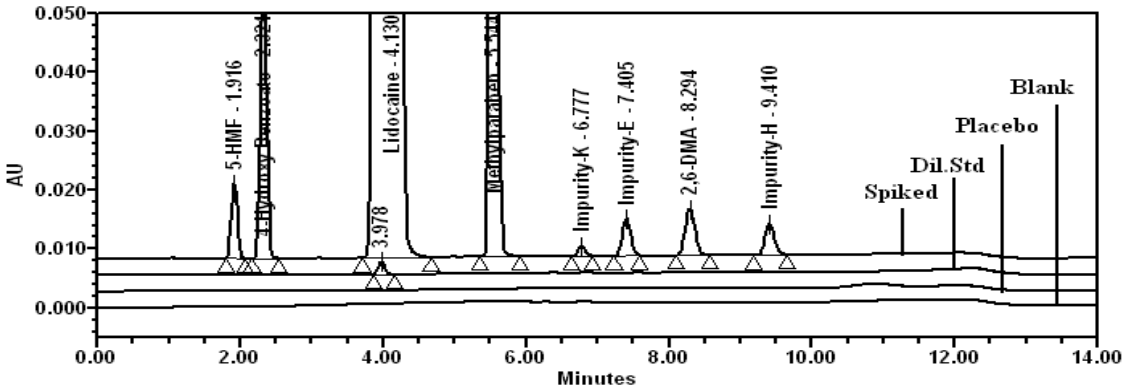


Fig-3: Overlay of Blank, Placebo, Diluted standard and Impurity Spiked chromatogram

3.2. Results of specificity/ Force degradation study:

The peak purity indices for the analytes in stressed solutions were determined with PDA detector under optimized chromatographic conditions found to be better (purity angle < purity threshold) indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference. Baseline resolution was achieved for all investigated compounds. The FDA guidelines indicated that well separated peaks, with resolution, Rs > 2 between the peak of interest and the closest eluting peak, are reliable for the quantification[15]. Degradation was not observed in a Lidocaine sample during photolytic, hydrolytic, thermal and humidity stress. About 9.25% of degradation was observed in oxidative stress. The method is linear in the tested range. Peaks meet this specification, visibly confirmed in Fig 4.

Figure 4 Specificity / Force Degradation Chromatograms:

Figure 4.1: Acid Stress:

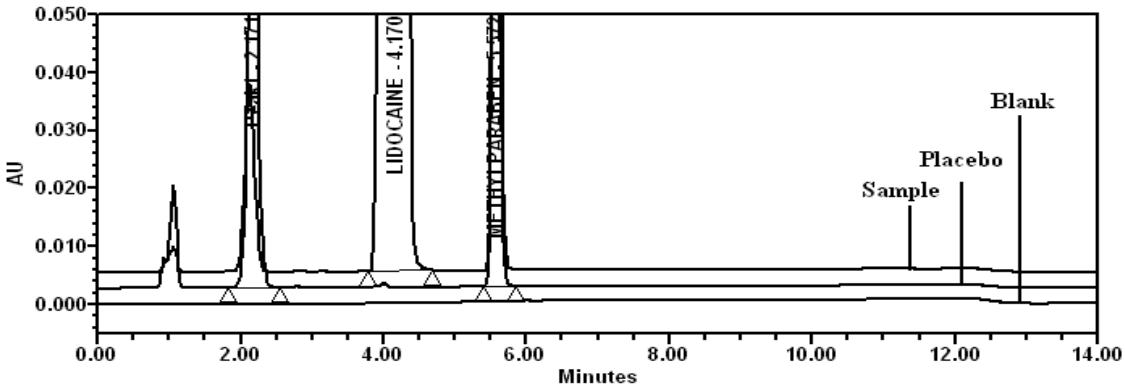


Figure 4.2: Base Stress:

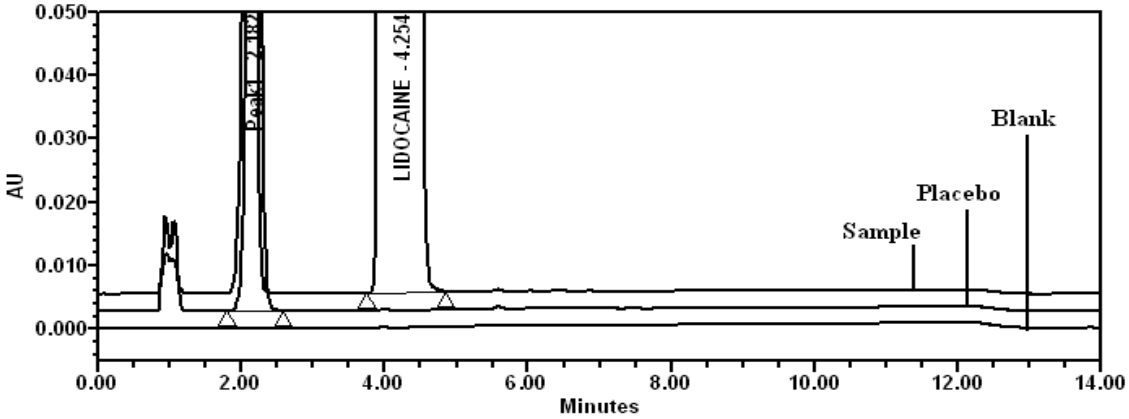


Figure 4.3: Water Stress:

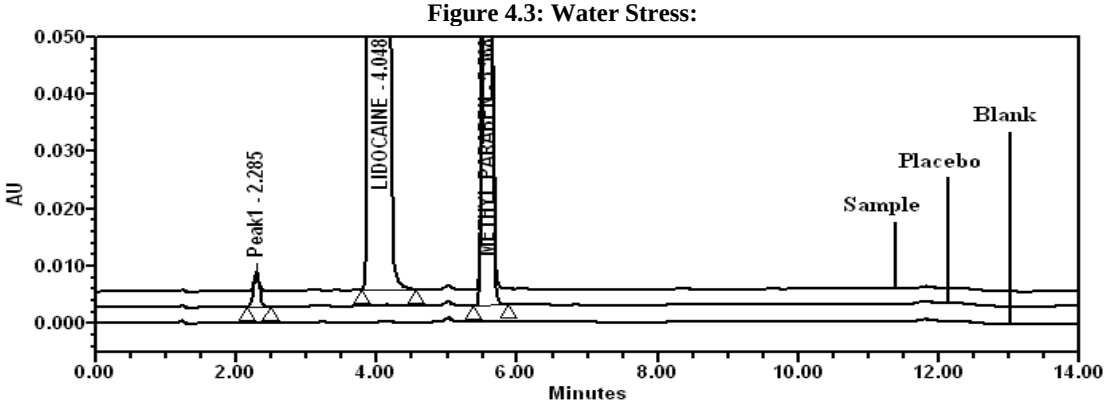


Figure 4.4: Peroxy Stress:

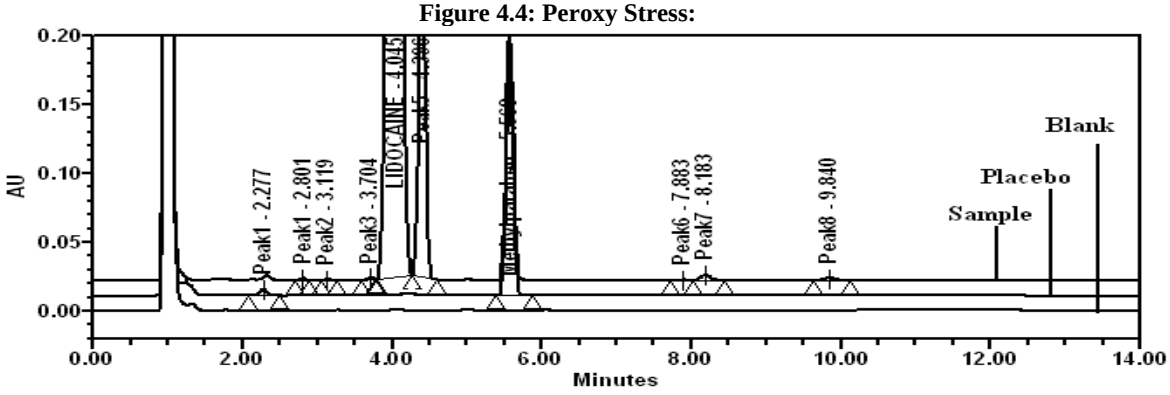


Figure 4.1.4: Purity plot for Peroxy Stress:

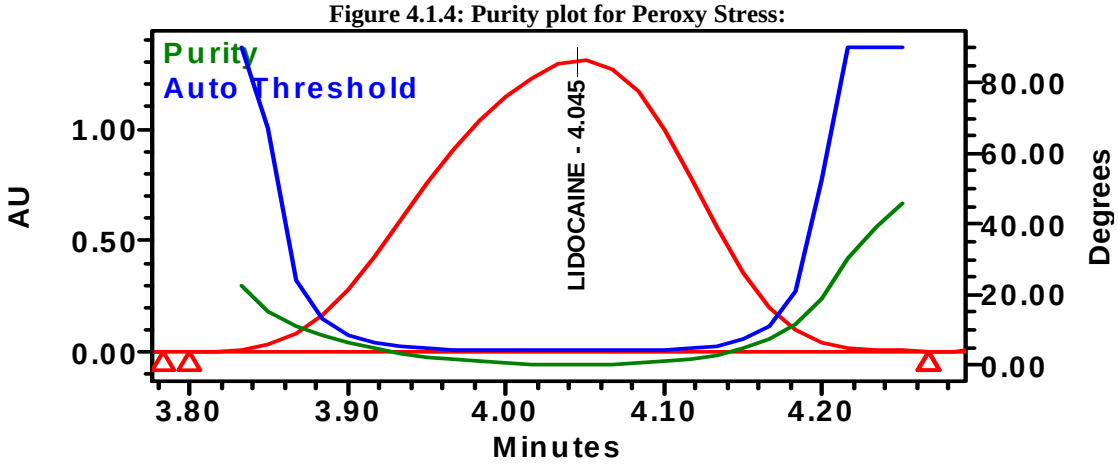


Figure 4.5: Thermal Stress:

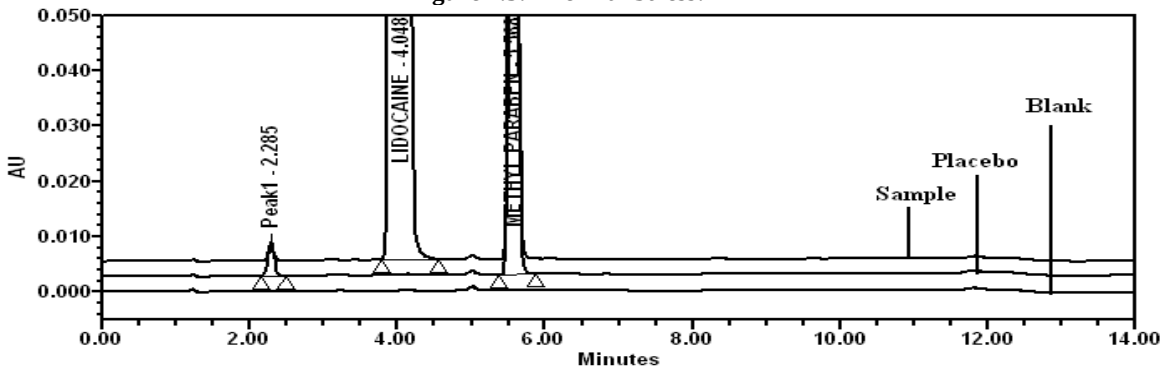


Figure 4.6: Photolytic Stress:

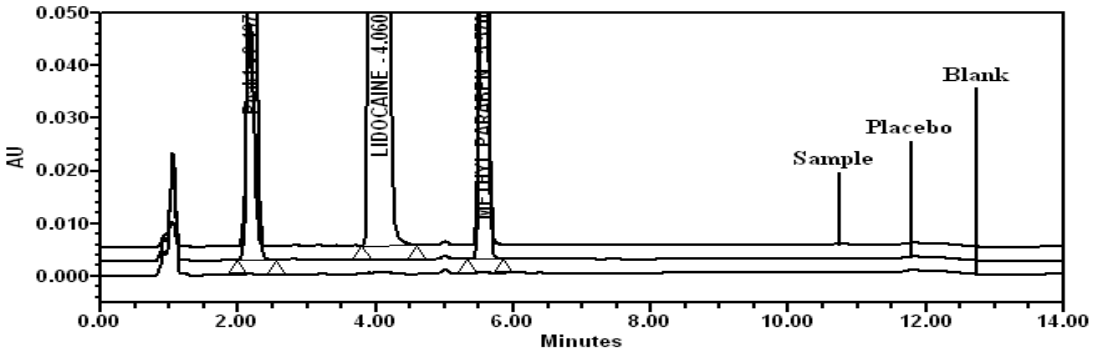
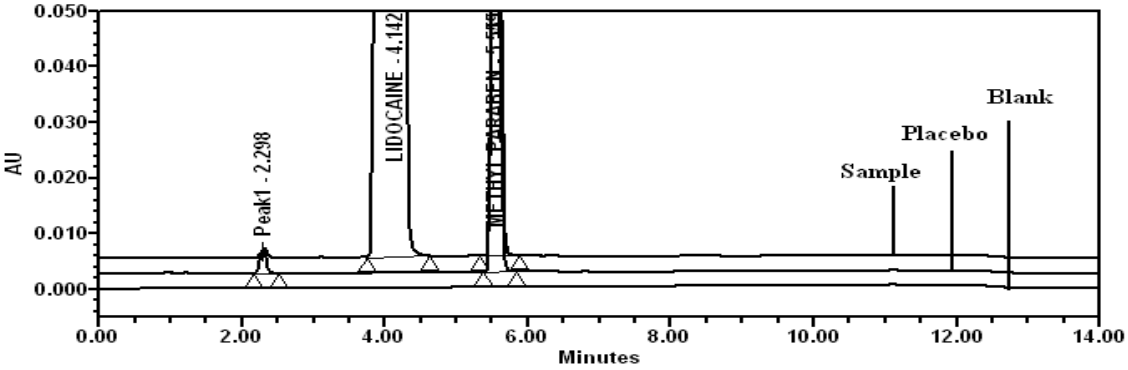


Figure 4.7: Humidity Stress:



Peak purity results from the PDA detector for the peaks produced by the degradation of Lidocaine, confirmed that all these peaks were homogenous and pure for all the stressed samples analysed (Table 3). The mass balance (% assay + % sum of all compounds + % sum of all degradants) results were calculated for all of the stressed samples and were found to be more than 98 % (Table 3). The purity and assay of Lidocaine was unaffected by the presence of its impurities and degradation products, which confirms the stability-indicating power of the developed method.

Table 3: Forced Degradation Studies of Lidocaine & its related substances:

Condition	%degradation	Purity Angle	Purity Threshold	Purity Flag	Mass Balance
Acid	0.02	3.604	5.317	No	98.2%
Base	0.03	4.819	5.5528	No	99.3%
Water	0.01	4.124	5.357	No	100.2%
Oxidation	9.25	3.730	5.485	No	97.8%
Thermal	0.01	4.128	5.826	No	99.8%
Photolytic	0.01	2.869	3.852	No	100.6%
Humidity	0.02	4.576	5.128	No	100.4%

3.3. Results of method validation studies

3.3.1. Method validation

The optimized RP-UPLC method validated according to ICH guideline Q2 (R1), with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and robustness. System suitability features were also assessed.

3.3.2. Method precision

ICH (International Conference on Harmonization of technical Requirements for Registration of Pharmaceuticals for Human Use) considers ruggedness as the method reproducibility and intermediate precision. The method reproducibility was determined from the %RSD. The intermediate precision was determined from the difference in the average recoveries and the difference in the %RSD of the recoveries among the three analysts. The RSD of the area of Lidocaine related compounds were within 0.4%. The RSD of results obtained in intermediate precision studies was within 1.0%. The results for all the tested compounds were listed in Table 4 reveal that the method has good reproducibility and intermediate precision.

Table 4 Intraday – Inter day precision studies of Lidocaine Related substances

Name of impurity	Intraday precision		Inter day precision	
	*% of impurity	*%RSD	*% of impurity	*%RSD
5-HMF	0.244	0.3	0.245	0.9
4-HBA	0.221	0.1	0.225	0.2
IMP-K	0.217	0.3	0.215	0.6
IMP-E	0.223	0.0	0.220	0.9
2,6-DMA	0.215	0.2	0.221	0.8
IMP-H	0.231	0.2	0.230	0.5

*Mean of six replicates.

3.3.3. Determination of limit of quantification and detection (LOQ and LOD)

As per the guideline ICH Q2(R1), LOD is defined as the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The linearity performed above, used for the determination of limit of quantification and detection. Residual standard deviation (σ) method was applied and the LOQ and LOD values were predicted using following formulas (a) and (b) and established the precision at these predicted levels. The results are tabulated in Table 5

$$LOQ = \frac{10\,\sigma}{S} \quad \text{---------- (a)}$$

$$LOD = \frac{3.3\,\sigma}{S} \quad \text{---------- (b)}$$

Where σ = standard deviation of response and S = slope of the calibration curve

Table 5 Limit of quantification & Limit of detection

Name of impurity	Limit of Quantification			Limit of Detection		
	Conc.µg/mL	% of impurity	% RSD	Conc.µg/mL	% of impurity	% RSD
5-HMF	0.108	0.010	1.6	0.036	0.003	4.9
4-HBA	0.075	0.007	2.8	0.025	0.002	4.8
IMP-K	0.118	0.011	1.8	0.039	0.001	2.9
IMP-E	0.109	0.011	1.4	0.036	0.001	4.1
2, 6-DMA	0.105	0.010	1.9	0.035	0.001	4.7
IMP-H	0.112	0.012	2.0	0.037	0.002	3.6

3.3.4. Accuracy

Accuracy was evaluated by the simultaneous determination of analytes in solution prepared by standard addition method. The experiment was carried out by adding known amount of each related impurities corresponding to different concentration levels of LOQ, 50 %, 75 %, 100 % and 150 % of the specification level in sample solution. The samples were prepared in triplicate at each level. The quantification of added analyte was carried out by using an external standard of corresponding main drug prepared at the analytical concentration. The experimental results revealed that approximately 97–103% recoveries were obtained for all the investigated related compounds. Therefore, based on the recovery data (Table 6) the estimation of related compounds that are prescribed in this report has been demonstrated to be accurate for intended purpose and is adequate for routine analysis.

Table 6 Accuracy study of Lidocaine Related substances

	5-HMF			4-HBA			IMP-K		
	Add	Rec	% R	Add	Rec	%R	Add	Rec	%R
LOQ	0.108	0.108	100.2	0.075	0.074	98.9	0.114	0.114	99.6
50	1.083	1.111	102.6	1.052	1.090	103.6	1.035	1.006	97.2
75	1.513	1.507	99.6	1.468	1.456	99.2	1.446	1.481	102.4
100	2.146	2.099	97.8	2.073	2.042	98.5	2.071	2.098	101.3
150	3.005	3.014	100.3	3.030	3.085	101.8	3.034	2.995	98.7

Add-µg/mL added; Rec- µg/mL recovered; %R- %recovery

	IMP-E			2,6-DMA			IMP-H		
	Add	Rec	% R	Add	Rec	%R	Add	Rec	% R
LOQ	0.109	0.112	102.5	0.105	0.103	98.1	0.112	0.109	97.4
50	1.094	1.096	100.2	1.098	1.082	98.5	1.084	1.054	97.2
75	1.532	1.529	99.8	1.456	1.460	100.3	1.562	1.574	100.8
100	2.188	2.151	98.3	2.089	2.110	101.0	2.075	2.102	101.3
150	3.052	2.976	97.5	3.042	3.033	99.7	3.032	3.114	102.7

Add-µg/mL added; Rec- µg/mL recovered; %R- %recovery

3.3.5. Linearity and range

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of the analyte in the sample. The linearity of the test method was established from the LOQ to 150% of the test concentration for Lidocaine and its related substances. The plots of area under the curve (AUC) of the peak responses of the analytes against their corresponding concentrations, they fitted straight lines responding to equations. The y-intercepts were close to zero with their confidence intervals containing the origin. The correlation coefficient (r) exceeds 0.98, the acceptance threshold suggested for linearity of procedures for the determination of impurity content in bulk drug and it is found to be 0.9999 in all the cases. Results were represented in Table 7.

Table 7 Linearity study of Lidocaine Related substances

	5-HMF		4-HBA		IMP-K		IMP-E		2,6-DMA		IMP-H	
	Add	Rec	Add	Rec	Add	Rec	Add	Rec	Add	Rec	Add	Rec
LOQ	0.108	0.109	0.075	0.074	0.114	0.118	0.109	0.106	0.105	0.109	0.112	0.110
50	1.083	1.100	1.052	1.070	1.035	1.080	1.094	1.132	1.098	1.112	1.084	1.082
75	1.513	1.543	1.468	1.471	1.446	1.432	1.532	1.543	1.456	1.468	1.562	1.702
100	2.146	2.195	2.073	2.095	2.071	2.079	2.188	2.190	2.089	2.096	2.075	2.087
150	3.005	3.025	3.030	3.030	3.034	3.039	3.052	3.048	3.042	3.056	3.032	3.032
r	0.999914		0.999958		0.999902		0.999935		0.999925		0.999919	

Add-µg/mL added; Rec- µg/mL recovered; r= correlation coefficient.

3.3.6. Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate change in the chromatographic conditions, i.e., change in the flow rate by ±0.2mL/min, change in column oven temperature by ±5°C and change in organic composition of mobile phase by ±2% absolute. The sample spiked with all known impurities at impurity tolerance level was injected and the resolution among the impurities was monitored. The method was demonstrated to be robust over an acceptable working range of its UPLC operational conditions. The results are tabulated in Table 8.

Table-8: Robustness study of Lidocaine Related substances

Parameter	RRT of Impurity					
	5-HMF	4-HBA	IMP-K	IMP-E	2, 6-DMA	IMP-H
Column Temp(°C)						
35	0.46	0.57	1.65	1.79	2.01	2.28
40	0.46	0.56	1.65	1.79	2.01	2.27
45	0.47	0.57	1.66	1.80	2.00	2.29
pH of buffer						
4.3	0.47	0.54	1.65	1.81	2.04	2.26
4.5	0.46	0.56	1.64	1.80	2.02	2.28
4.7	0.49	0.55	1.66	1.80	2.00	2.28
Flow rate (mL min-1)						
0.8	0.46	0.56	1.64	1.78	2.01	2.27
1.0	0.46	0.57	1.64	1.79	2.01	2.27
1.2	0.48	0.59	1.65	1.78	2.02	2.28

3.3.7. Solution stability and mobile phase stability

The RSD (%) values of all the six impurities during solution stability and mobile phase stability experiments was within 1.0%. No significant change was observed in the content of impurities during solution stability and mobile phase stability experiments confirm that sample solutions and mobile phase used during the study were stable up to 48 hours.

4. Conclusion

A stability study was carried out and an efficient UPLC method for the quantification of related substances of Lidocaine in drug product was developed and validated. The results of the stress testing of the drug, undertaken according to the ICH guidelines, revealed the stability indicating nature of the Method.

Validation experiments provided proof that the UPLC analytical method is linear in the proposed working range as well as accurate, precise (repeatability and intermediate precision levels) and specific, being able to separate the main drug from its degradation products. The proposed method was also found to be robust with respect to flow rate, column oven temperature and composition of mobile phase. Due to these characteristics, the method has stability indicating properties being fit for its intended purpose; it may find application for the routine analysis of the related substances of Lidocaine formulations.

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Conflict of Interest

The authors declare no conflict of interest.

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