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Analytical Method Development and Validation for Determination of Lidocaine Using RP-HPLC Techniques

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ABSTRACT

The objective of this work was Analytical Method Development for Determination of Lidocaine Using HPLC methods which are simple, accurate, precise, specific, sensitive, reproducible and economical methods. Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug products. Lidocaine works by inhibiting sodium ion channels in nerve membranes, which prevents the initiation and conduction of nerve impulses, producing local anesthesia. The result for subjected study was found to be Linearity rang (ug/ml) 20-100, Retention time 6.49/ml, % recovery 99%-101%, correlation coefficient (r^2)0.9992, Intraday Precision (%RSD) 0.57, Interday Precision (%RSD)0.43. In summary, the study successfully developed and validated a simple, reliable, and stability-indicating HPLC method for the estimation of Lidocaine.

Keywords: stability-indicating HPLC, Lidocaine, Forced degradation, correlation coefficient, Precision

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INTRODUCTION

At its core, analytical chemistry is concerned with determining the qualitative (what is present) and quantitative (how much is present) composition of materials. In the pharmaceutical sector, analytical monitoring is critical not only during drug development but also throughout a product's shelf life—spanning manufacturing, packaging, distribution, and end-use. Analytical testing ensures that pharmaceutical products are safe, effective, and of the highest quality, thus directly impacting patient health and regulatory compliance

Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug products. As no specific set of conditions is applicable to all drug products and drug substances and the regulatory guidance does not specify about the conditions to be used, thus aim of any strategy used for forced degradation is to produce the desired amount of degradation.

Chromatography is a method of separation, where the individual components are separated and analyzed. In this technique two or more components are separated by a dynamic differential migrational process, in a system consisting of two phases, one of which moves continuously in a given direction in which the individual components exhibit different mobilities due to the difference in their adsorption or partition or molecular size etc.

HPLC is a type of chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rates, the liquid must be pressurized to several hundred pounds per square inch or more. high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography

Advantages are

- Speed (analysis can be accomplished in 20 minutes or less),
- Greater sensitivity (various detectors can be employed),
- Improved resolution (wide variety of stationary phases) Reusable columns (expensive columns but can be used for many analysis),
- Ideal for substances of low volatility,
- Easy sample recovery, handling and maintenance,
- Instrumentation lends itself to automation and quantitation (less time and labour),
- Precise and reproducible,

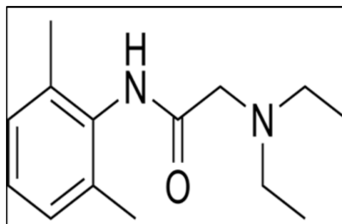
Applications of HPLC

1. Separation of plant extracts components which resembles in structures[21].
2. Degradation studies of products.

3. Complex molecules analysis by bioassay.
4. Analysis of antibiotics.
5. Quality control of lipsticks.
6. Assay of various compounds.

DRUG PROFILE:

Structure:



It is official in IP[28a].

IUPAC Name:2-(Diethylamino)-N-(2,6-dimethylphenyl) acetamide

Description: Lidocaine is a local anesthetic and antiarrhythmic drug. It blocks nerve signals in the body and is commonly used for minor surgical procedures, dental work, and the treatment of ventricular arrhythmias.

Melting Point: 66–69 °C

MATERIALS AND METHOD

Chemicals and Reagents

HPLC grade Acetonitrile, GAA, Methanol and Milli-Q water was used for dilution of stock solution.

Table 1: Reagents/ Materials

Sr. No.	Reagents/ Materials	Grade
1	Milli-Q water	HPLC grade
2	Methanol	HPLC grade
3	Acetonitrile	HPLC grade
4	Glacial Acetic acid	HPLC grade

Instrument

- Agilent- 1100 series HPLC system comprising of Quaternary gradient pumps G1311A with on line Degasser G1322A, Variable wavelength UV detector G1314A, Autosampler G1313A, Zodiac 100 C18 column (250mm x 4.6 mm i.d, 5um).
- UV Spectrophotometer (PROLAB)
- Digital Balance (RADWAG)
- Ultra-sonicator
- Digital pH meter (Prolab India).

- Digital Hot air oven

Preparation of Standard Solutions

Preparation of Stock standard solution of Lidocaine:

Accurately weighed quantity of 10.0 mg of Lidocaine transferred to 10.0 ml volumetric flask. & the volume was made up to 10 ml using methanol.

Working standard solution of Lidocaine

Dilute 1 ml of standard stock solution to 10 ml volumetric flask and make up the volume with mobile phase to get a concentration of 100 µg/m

Fixation of suitable parameters for the drugs Location of λmax

An aliquot portion of standard stock solution A, were appropriately diluted with mobile phase to get conc. 10µg/mL. The solutions were scanned in the range of 400nm to 200nm against solvent blank. Lidocaine shows maximum absorbance at 254 nm.

Selection of mobile phase

The mobile phase containing mixture of Solution 1: Water and Glacial acetic acid adjust pH of 3.4 with 1 N Sodium hydroxide and B) ACN (80:20) was found to be most satisfactory as it gave good resolution and sharp peaks. The detection wavelength selected was 254 nm and flow rate of 1.0 ml/min.

Sample Preparation:

10mg Lidocaine taken in 10ml volumetric flask and make up the volume with Methanol**.**

Table 2: Final Chromatographic Condition

Column	Zodiac 100 C18 Column		
Mobile phase	Solution A: Water and Glacial acetic acid (930:50) adjust pH of 3.4 with 1 N Sodium hydroxide MP= Acetonitrile and Solution (20:80)		
Wavelength	254 nm	Temperature	25°C
Flow rate	1ml/min	Run time	20 min
Retention Time	6.49	Injection volume	10µL
pH	3.4		

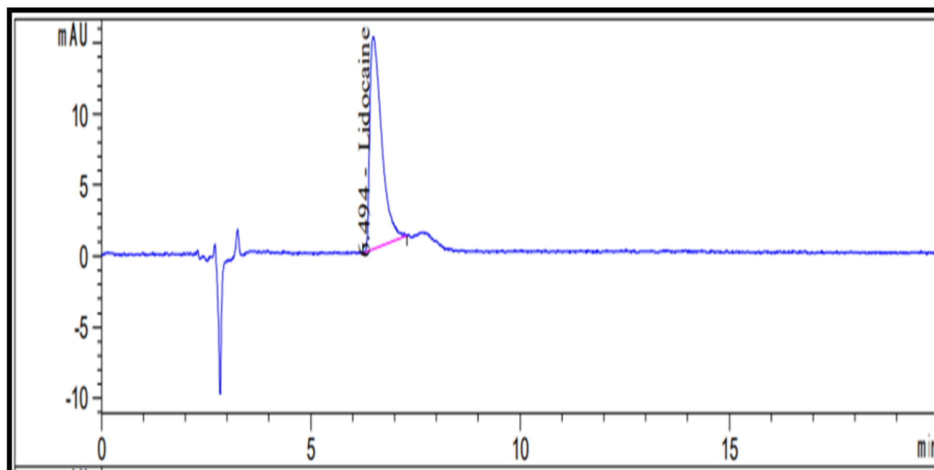


Figure 1 Chromatogram of Lidocaine

Validation of proposed method.

According to the guidelines of ICH Q2 (R1) all the parameters as discussed below were analyzed and validated accurately following the procedure of the proposed method.

System suitability test parameters of Lidocaine

The system suitability is the pharmacopeial requirement and is used to verify the resolution and reproducibility of the chromatographic system. The test was performed by collecting the data from five replicate injection of standard solution.

Table 3: System suitability test parameters of Lidocaine

Sr. No	Retention time	Peak area	Theoretical plates	Tailing factor
1	6.52	198	3730	1.58
2	6.48	204	3722	1.56
3	6.55	202	3721	1.55
4	6.57	200	3713	1.53
5	6.55	199	3721	1.6
Statistics				
Mean	6.53	200.60	3721.40	1.56
±SD	0.04	2.41	6.02	0.02
%RSD	0.54	1.20	0.16	1.55

Preparation of Calibration Curves for Lidocaine

Each of the standard solution was injected separately. The aliquot portion of stock solution A mobile phase to get concentration of 20, 40, 60, 100, 100 µg/ml for Lidocaine. The chromatogram was recorded. The graph is plotted as concentration versus response area (peak area) depicted in Figure 2

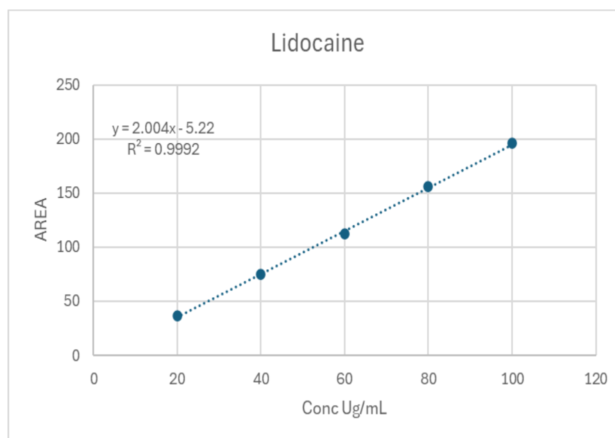


Figure 2: Calibration curve for Lidocaine

Linearity: The series of solution of curve were analyzed in 10 to 120 µg/ml.

Table 4: Calibration curve for Lidocaine

Sr. No.	Parameters	Lidocaine
1	Linear dynamic range (µg/ml)	10-100
2	Slope	2.004
3	Y-intercept	5.22
4	Correlation coefficient (R^2)	0.9992

Accuracy: It was determined on the basis of recovery study performed by standard addition method.

Table 5: Recovery study

Drug Name: Lidocaine							
Std. conc. (%)	**Std. (ppm)**	**Peak area**	**Drug (%)**	**Drug (ppm)**	**Peak area**	**Avg. peak area**	**Drug Rec. (%)**
100%	100 ppm	295	50	295	147	148	99.66
				250	148		
			100	500	298	296	100.68
				500	294		
			150	750	354	446	100.45
				750	351		
Drug recovery Range (%) as per ICH = 100±10%							99.66%-100.15 %

The stress degradation study of Lidocaine was carried out as per ICH Q1A and photostability as per ICH Q1B guidelines. Forced degradation study of Lidocaine was carried out under acidic, alkaline, neutral and thermal stress conditions. All chromatographic parameters were kept constant as in method development.

Acidic hydrolysis study: It was performed by using 0.1 Molar HCl solution at room temperature for 24 hours

Procedure:

Equivalent weight Lidocaine was first dissolved in a small portion of methanol and then mixed with 10ml of 0.1 N HCl in a volumetric flask (10 ml). This solution was then kept for 24 hours.

The samples were further diluted with mobile phase to get concentration of 100µg/ml of Lidocaine. The sample solution was injected and the chromatogram were recorded.

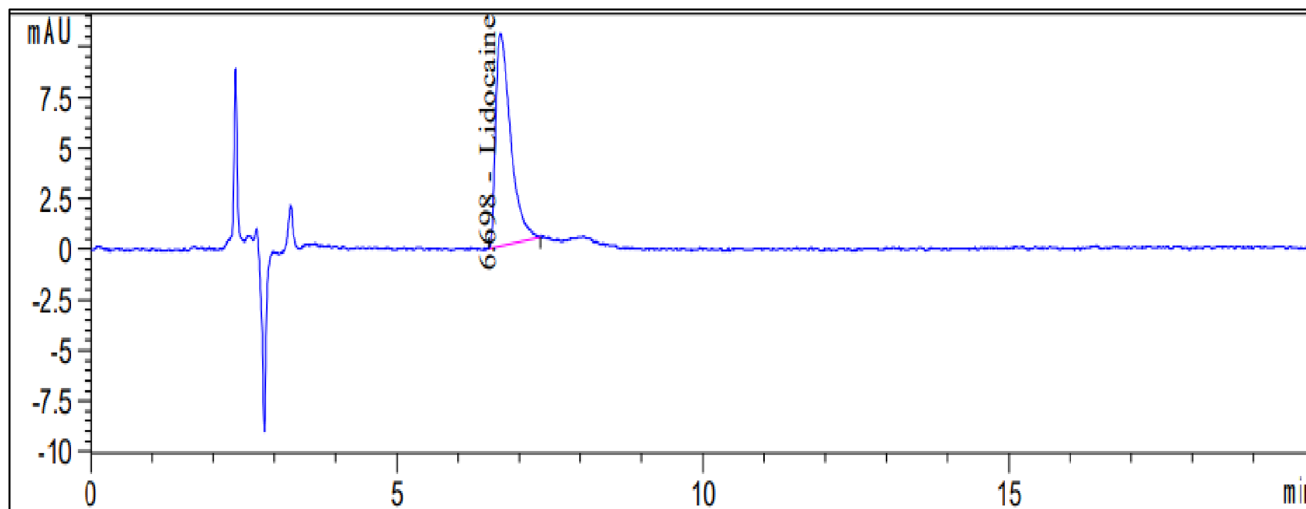


Figure 3: HPLC Chromatogram of specificity study of Lidocaine of 0.1 M HCL (At room temp. for 24hrs.)

Neutral degradation study

Procedure:

The procedure for the neutral degradation study of Lidocaine was performed by dissolving 500 µg of Lidocaine in a small amount of methanol, then mixing the solution with 2 mL of water (neutral solvent) in a 10 mL volumetric flask. The mixture was allowed to stand for 24 hours at room temperature. The chromatograms were recorded in Figure 4, and the percent content of Lidocaine remaining was calculated, with the results presented in the Table 6.

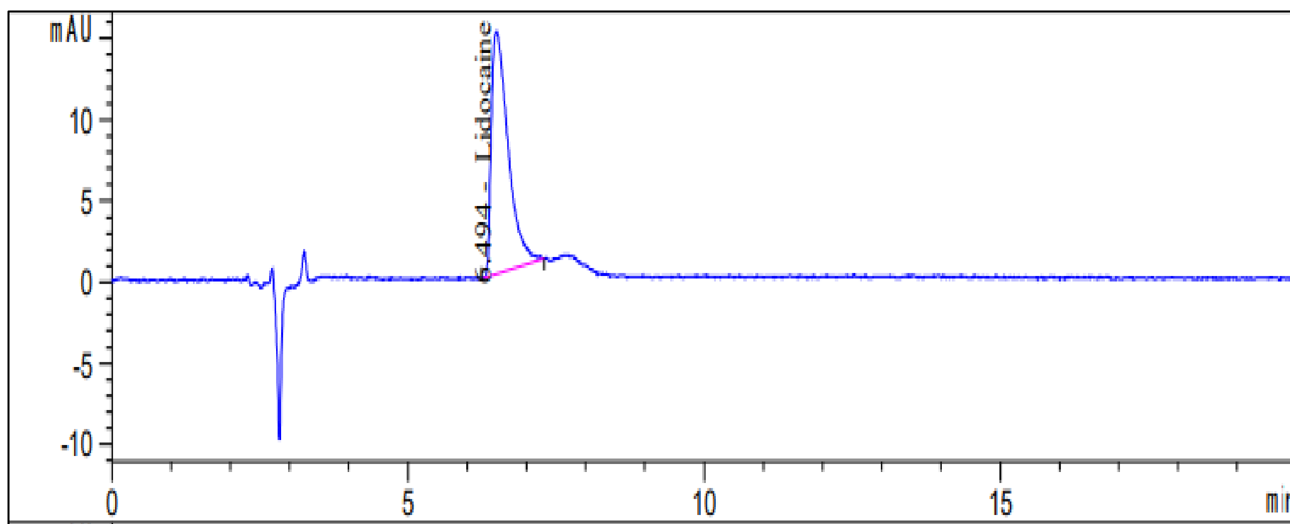


Figure 4: HPLC Chromatogram of specificity study of Lidocaine of water (neutral solvent at room temperature for 24hrs.)

Alkaline hydrolysis study:

It was performed by using 1 M NaOH solution at room temperature for 24 hours

Procedure:

Equivalent weight of Lidocaine (500 µg each) was first dissolved in a small portion of methanol and then mixed with 10ml of 0.1 M NaOH in a volumetric flask (10 ml). This solution was then kept for 24 hours. The solution was injected and the chromatogram were recorded. The percent content of Lidocaine remained were calculated. The chromatogram are depicted in Figure 5 Result are shown in Table 6.

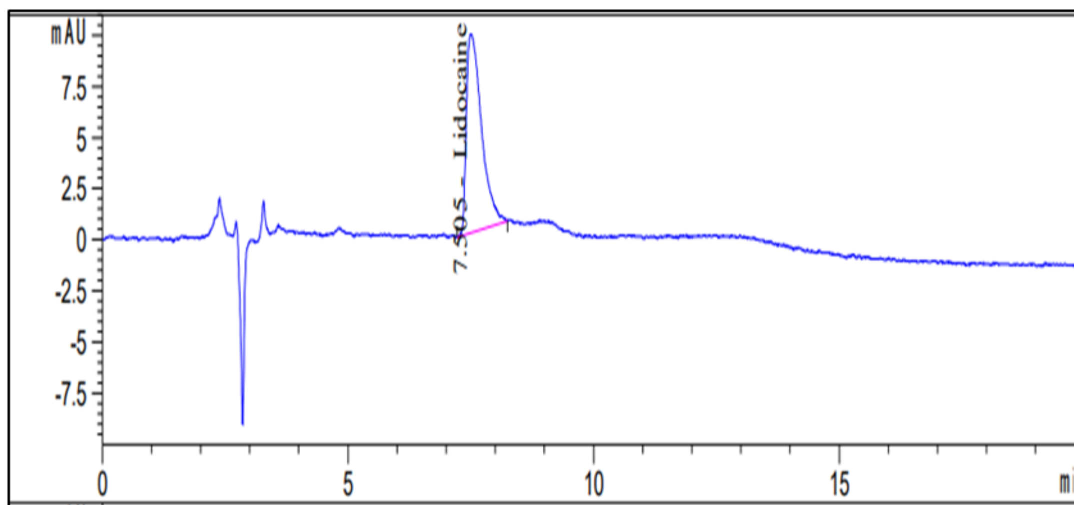


Figure 5: HPLC Chromatogram of specificity study of Lidocaine of 1 M NaOH (At room temp. for 24hrs.)

Thermal Degradation study:

Carried out by exposing the solid form of drug to the temperature of 60ºC for 24 hrs and the samples was analyzed after 24 hours. The absorbance was taken and % labelled claim was calculated. The chromatogram are depicted in Figure 6 Results are shown in Table 6

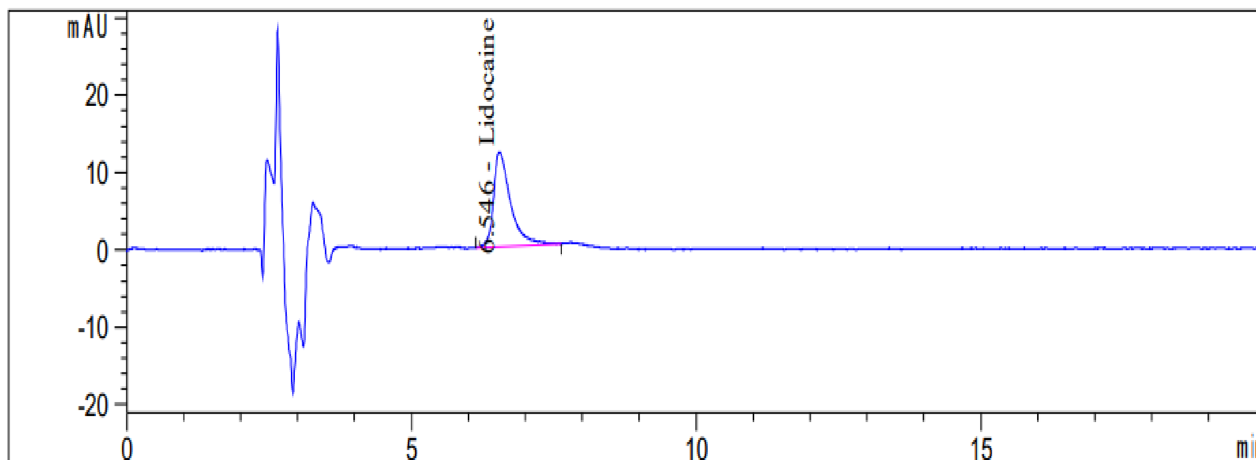


Figure 6: HPLC Chromatogram of specificity study of Lidocaine of Thermolysis Table

Table 6: Specificity study of Lidocaine by HPLC

Medium	Area	% Area	Percent Recovery (%)
Standard	295	NA	NA
Heat (at 60ºC))	268	90.8	9.2
Alkaline Hydrolysis (0.1 M NaOH)	256	86.8	13.2
Acid Hydrolysis (0.1 M HCl	271	91.9	8.1
Neutral	281	95.3	4.7

Precision Study

As displayed in Table 7; for intermediate variability for precision studies, this method is significantly precise over selected tested range of Lidocaine. Moreover, the peak area of the studied samples was also correlated with selected concentration; where the % RSDs were <2%. The RSDs were observed well below 2% that reflects an acceptable precision with minimum variations of the proposed method.

Table 7: Data of intraday study

Conc. of Standard(µg/ml)	Weight of tablet powder taken (mg)	AUC of standard solution	AUC of Sample	% Drug estimated
Drug Name: Lidocaine				
100	238.1	295	291	98.64
			289	97.97
			287	97.29

Table 8: Data of Interday Day

Conc. of Standard (µg/ml)	Weight of tablet powder taken (mg)	AUC of standard solution	AUC of Sample	% Drug estimated
Drug Name: Lidocaine				
100	237.1	295	287	97.29
			290	98.31
			288	97.63

Table 9: Data of different analyst

Conc. of Standard (µg/ml)	Weight of tablet powder taken (mg)	AUC of standard solution	AUC of Sample	% Drug estimated
Drug Name: Lidocaine				
100	237.3	295	287	97.29
			290	98.31
			288	97.63

Table10: Statistics

Sr. No.	Parameter	% of labeled claim		
		Intermediate precision		
		Intraday	Interday	Different analyst
		Drug Name: Lidocaine		
1	Mean	97.97	97.74	97.74
2	± S.D	0.55	0.42	0.42
3	%RSD	0.57	0.43	0.43

Table 11: Summarized Chromatographic Parameters

Parameter	Result (Lidocaine)
Linearity range (µg/mL)	20–100
Correlation coefficient (r^2)	0.9992
Retention time (min)	6.49
Tailing factor	1.54
Theoretical plates	3712
% Recovery	99.00% – 101.00%
Specificity	86.8% – 95.3%
Intraday Precision (%RSD)	0.57
Interday Precision (%RSD)	0.43
Different Analyst (%RSD)	0.43

Stress Degradation Study By HPLC

To assess the stability and degradation profile of Lidocaine, stress degradation studies were conducted in accordance with ICH Q1A (R2) guidelines. These studies are essential to determine the drug's behavior under various environmental and chemical stress conditions, ensuring the method can differentiate between the active pharmaceutical ingredient and its degradation products. Lidocaine was subjected to the following stress conditions:

Acidic Hydrolysis (0.1 N HCl)

Alkaline Hydrolysis (0. 1 N NaOH)

Neutral Hydrolysis (Water)

Thermal Degradation (Elevated temperatures).

CONCLUSION OF DISCUSSION

In summary, the study successfully developed and validated a simple, reliable, and stability-indicating HPLC method for the estimation of Lidocaine. The method demonstrates high linearity, precision, specificity, and robustness, and is compliant with ICH validation requirements. The stress degradation study further confirms the applicability of the method for stability studies, making it ideal for routine quality control testing, formulation analysis, and regulatory submission purposes.

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