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Research Article

RP-HPLC Assay Method Development and Validation for Lidocaine Ointment 5% USP

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ABSTRACT

A simple isocratic RP-HPLC method for the determination of Lidocaine was developed. Separations were performed on Xterra RP C18 column (150×4.6×3.5µm) particle column at 30°C. The mobile phase consisted of buffer and acetonitrile (70:30%v/v) pH was adjusted to 8 with 40%w/v sodium hydroxide. Lidocaine hydrochloride was internal standard. The flow rate was 1ml/min and uv detection was performed at 230nm. Lidocaine was resolved on the stationary phase and the retention time was 4.77 min with the total run time of 30min. The detector response was found linear with a correlation coefficient of 1. The calibration curve were linear in the concentration of range of 2-20 µg/mL for Lidocaine.

INTRODUCTION

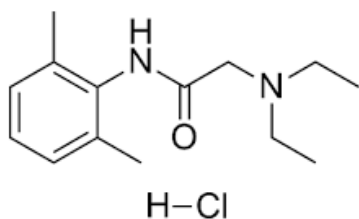


Figure 1: Structure of Lidocaine Hydrochloride

Lidocaine (LID) belongs to the family of narcotic drugs. It can be used as a topical anesthetic by stabilizing the nervous membrane which produces sensation of pain. It can be used to relieve the discomfort resulting from the virus Herpes which affects the skin as well as it is used in different types of minor surgery and dental treatment, childbirth and epidural anesthesia at birth, it is used particularly for the treatment of cardiac arrhythmias after having a heart attack.

[1]

Various formulations of Lidocaine are available in market. On basis of site of application formulations are

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classified into topical and parental. Topical formulations include gel, ointment, spray, Transdermal Patch. Parental formulations are available rather as Lidocaine-HCL or in combination with epinephrine to subside pain at site of application [2], HPLC-UV [3] thin-layer chromatography (TLC) for the Determination of Hydrocortisone Acetate and Lidocaine in a Pharmaceutical Preparation [4]

In the development of Assay method for Lidocaine Ointment USP 5% 2 trials out of 3 were performed and the 3rd trial was finalized. First trial was failed due to low sample concentration and the peak was failed to elute within the set run time of 20minutes..The second trial was failed to meet the system suitability parameters after the change in the concentration of reference and test solution. The third trial was passed with system suitability parameters and it was finalized for the assay method.

In the proposed work, Analytical Method development for Lidocaine ointment has been developed by chromatographic methods and to validate the proposed method with ICH and Pharmacopoeial guidelines for the intended analytical application.

The following methods have been reported for the estimation of lidocaine.

*Analytical methods development and validation of lidocaine in ointment formulation by *spectrometric method,.

*Stability indicating UPLC method for the determination of lidocaine and its degradation impurities.

*HPLC-DA stability indicating determination of Lidocaine hydrochloride and nitrofurazone in their combined topical dosage form.

*HPLC-MS/MS method for the simultaneous determination of Nifedipine and Lidocaine in human plasma

*Development and validation of HPLC method for the simultaneous determination of Lidocaine in topical formulation.

Review of Literature

The following methods have been reported for the estimation of Lidocaine

- Karthik.KB *et al* published analytical method development and validation of Lidocaine in ointment formulation by spectrophotometric method
- Shanmuga SP *et al* published validated and gradient stability indicating UPLC method for the determination of Lidocaine and its degradation impurities in pharmaceutical dosage form
- Shaalan RA *et al* published HPLC-DAD stability indicating determination of Lidocaine hydrochloride and nitrofurazone in their combined topical dosage form
- Grigoriev A *et al* published development of an HPLC-MS/MS method for the simultaneous determination of lidocaine and Nifedipine in human plasma
- Narendra M R *et al* published development and validation of HPLC method for the simultaneous determination of Lidocaine and prilocaine in topical formulation.

OBJECTIVE

In the proposed work attempts have been made

- To develop new stability indicating Analytical method for the estimation of lidocaine by chromatographic method
- To validate the proposed method in accordance with ICH and pharmacopeia guidelines intended analytical application.
- To carry out the forced degradation stability studies of lidocaine as API and in Lidocaine Ointment USP

MATERIALS AND METHODS

Formulation Details

SR.No	Ingredients	%w/w
1	Lidocaine USP	5
2	Polyethylene Glycol	38
3	Polyethylene Glycol	57

APPARATUS AND SOFTWARE



HPLC (Agilent) computer loaded Chromeleon 6.8 software was used for all the HPLC measurements. The column was a Xterra RP C18, 150 x 4.6 x 5 μ m was used. Volumetric flask with Class A, glass beakers, Measuring cylinders of Class A grade were used. Pipets of Class A grade (both bulb and graduated) were used. Balance with sensitivity of 0.01mg was used.

REAGENTS AND CHEMICALS

Lidocaine Ointment formulation was provided a from formulation department of STRIDES ACROLAB PVT.limited.

HPLC grade Acetonitrile from Spectrochem and Potassium di hydrogen, potassium Dihydrogen phosphate AR grade was purchased from Merck and HCL AR grade was purchased from SDFCL Respectively. Water used throughout the experiment was Purified HPLC grade water.

STANDARD

Lidocaine Hydrochloride (B.No/WS No-400) with a potency of 93.8% was used

SAMPLE

Lidocaine Ointment USP5% with Batch No (2004124-001) was used.

*All the materials used were within the expiry date and were stored at recommended storage condition.

STANDARD PREPARATION

PREPARATION OF LIDOCAINE HYDROCHLORIDE STANDARD SOLUTION

40.09 mg of Lidocaine reference standard were accurately weighed on weighing balance. The weighed quantity was transferred to 100ml volumetric flask. 1ml of 0.1M HCL was added and sonicated for 5mins to ensure complete solubilization and diluted to volume with mobile phase .

PREPARATION OF SAMPLE SOLUTION:

800 mg of sample was accurately weighed and was transferred to 100ml volumetric flask. 1ml of 0.1M HCL was added and sonicated for 5mins to ensure complete solubilization.

PREPARATION OF PLACEBO

800 mg of placebo was accurately weighed and was transferred to 100ml volumetric flask. 1ml of 0.1M HCL was added and sonicated for 5mins

PREPARATION OF MOBILE PHASE:

Acetonitrile HPLC (70%) & Water (30%) were mixed and sonicated for 5 minutes.

BUFFER PREPARATION

9.703gm of Potassium Dihydrogen Orthophosphate was dissolved in 2000ml of water, adjusted the pH to 8 with 40%w/v of sodium Hydroxide solution. The solution was filtered through to 0.45 μ m membrane filter & degassed for 5minutes.

Diluent Preparation:

Mobile phase was used as diluent.

OPTIMIZED METHOD

Optimized method for the Assay of Lidocaine by HPLC was finally achieved by using the following chromatographic condition

Table 1: Optimized Method

Chromatographic Condition	
Mobile Phase	70:30 Buffer : water, the mobile phase sonicated for 5minutes
Column	X Terra RPC18, 150 x 4.6 mm
Flow Rate	1.0 ml/min
Wavelength	230 nm
Injection volume	20 μ l
Run time	30min



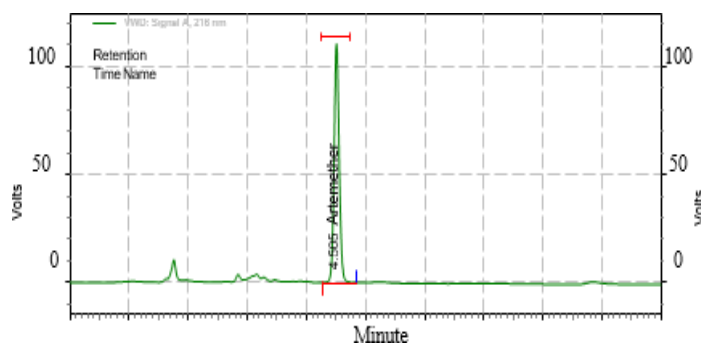


Figure 2: Chromatogram for Optimized Method

RESULTS AND DISCUSSION

1. System suitability

Standard solution of Lidocaine was injected three times into HPLC system as per test procedure. The system

suitability parameters were evaluated from standard chromatograms obtained by calculating the %RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injections.

Table 2: System Suitability Parameter of Lidocaine

	Retention Time	Mean Peak area
1	4.874	4081652.637
2	4.875	4081798.438
3	4.874	4084310.151
4	4.877	4084824.975
5	4.877	4086987.932
Mean	4.876	4083914.827
SD	0.0022	
%RSD	0.1	1.93

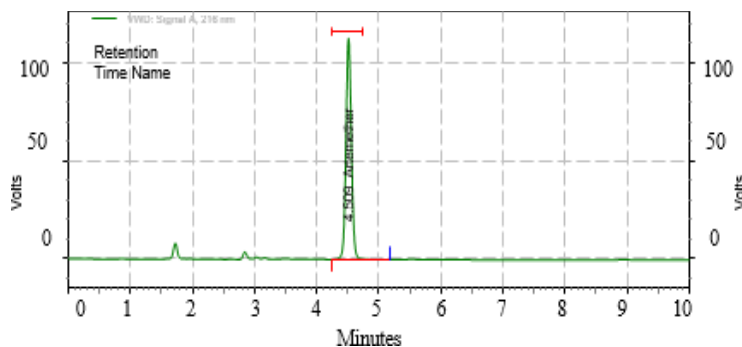


Figure 3: Chromatogram for System Suitability

2. Specificity by forced degradation studies

Specificity is the ability to assess unequivocally the analytes in the presence of components which may be expected to be present. Forced degradation of lidocaine ointment shall be carried out to confirm that during

stability study or through out the shelf life, any degradation product if found should not interfere with the main peak of lidocaine. The forced degradation pathway helps to identify the type of degradation pathway (oxidative, alkali hydrolysis, acid hydrolysis, water hydrolysis) for each of the degradates.

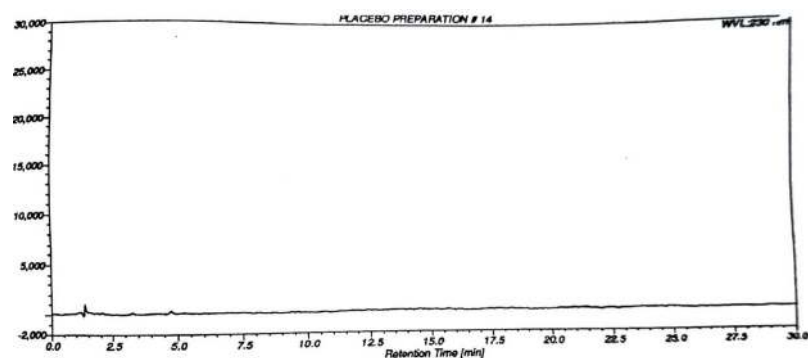


Figure 4: Chromatogram for Placebo

Figure 4: Chromatogram for Acid Stressed Sample

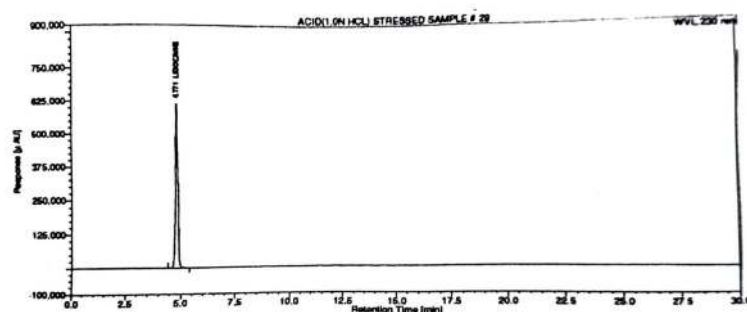


Figure 5: Chromatogram for Alkali Stressed

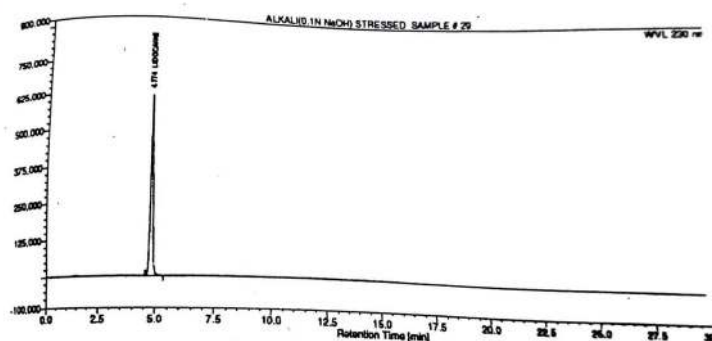


Table 3: Forced Degradation Studies

Stressed condition	Lidocaine		Assay in %
	Peak purity	Purity match	
Sample as such	1000	990	97.6
N HCL	1000	990	95.9
1.0HCL	1000	990	95.9
0.1N NaoH	1000	990	96.1
1.0N NaoH	1000	990	96.2
0.3%Hydrogen Peroxide	1000	990	83.9
Neutral	1000	990	96.6
Sunlight	1000	990	95.8
thermal	1000	990	95.8

3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of the same homogeneous sample under the prescribed conditions

System precision

The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area response of six determinations should be measured and calculated the relative standard deviation.

Table 4: System Precision

Injection	Retention Time	Mean Peak area
1	4.874	4081652.637
2	4.875	4081798.438
3	4.874	4084310.151
4	4.877	4084824.975
5	4.877	4086987.932
6	4.878	4084857.353
Mean	4.876	4084071.914
%RSD	0.0%	0.0%

4. Method Precision

In method precision a homogeneous sample of single batch should be analysed six times. This indicates a method is giving consistent results of single batch.

Table 5: Method Precision

Set	Assay in %
1	97.8
2	97.9
3	97.2
4	97.9
5	97.0
6	98.6
Mean	97.7
%RSD	0.0%

5. Intermediate Precision

The intermediate precision should be carried out to ensure that the analytical results will remain unaffected with the change in instrument, analyst, column and day.

Table 6: Intermediate Precision

Set	Assay in %
1	96.2
2	96.5
3	95.7
4	97.6
5	97.8
6	97.9
Mean	96.8
%RSD	1.1%

6. Stability in Analytical Solution

Evaluated the stability in analytical solution by injecting the standard preparation and sample preparation at regular interval.

standard solution at room temperature

Table 7: Stability in Analytical Solution

Lidocaine standard preparation		
Time (Hrs)	Area	%Difference
Initial	4081652.637	--
8hrs	4092162.271	0.3
9hrs	4096913.382	0.4
12hrs	4103463.055	0.5
13hrs	4103612.506	0.5



Lidocaine standard preparation		
19hrs	4100645.846	0.5
22hrs	4106576.726	0.6
26hrs	4111607.756	0.7
28hrs	4060600.688	-0.5
29hrs	4063228.011	-0.5
31hrs	4078570.074	-0.1
32hrs	4071826.861	-0.2
33hrs	4075413.206	-0.2
34hrs	4083076.019	0.
35hrs	4075508.154	-0.2
36hrs	4068000.828	-0.3
37hrs	4071620.425	-0.2

7. Mobile phase hold time study

Evaluated the stability of mobile phase by injecting the standard standard solution in

conjunctive (like day1,day2)days and checked the system suitability parameters.

Table 8: Mobile Phase Hold Time Study

Sr.No	Days	%RSD lidocaine peak from standard solution should not be more than 2.0	Tailing factor for lidocaine peak from standard solution should not be more than 2.0	Theoretical plates for lidocaine peak from standard solution should not be less than 2000
1	Day-1	0.1%	1.18	8828
2	Day-2	0.1%	1.21	8828
3	Day-3	0.1%	1.19	8809

8. Linearity and range

Six different concentration ranging 50% to 150% of lidocaine labelled claim for linearity standard solution

ranging 203µg/ml to 596µg/ml. The calibration curve obtained by plotting peak area against concentration and injected under optimized chromatographic condition.

Table 9: Concentration and Peak Area of Linearity Study

Sr. No.	Description	Concentration in µg/ml	Area response
1.	Linearity 50%	203.6534	2209736.995
2.	Linearity 80%	323.4496	3441818.610
3.	Linearity 90%	403.317	4239278.265
	Linearity 100%	443.2457	4649363.387
4.	Linearity 110%	443.2457	4649363.387
5.	Linearity 120%	477.1880	5028432.442
6.	Linearity 150%	596.9841	6278506.854
	Regression Equation	$y=10353.055x+76115.262$	
	Correlation Coefficient(R ²)		R ² =1.000



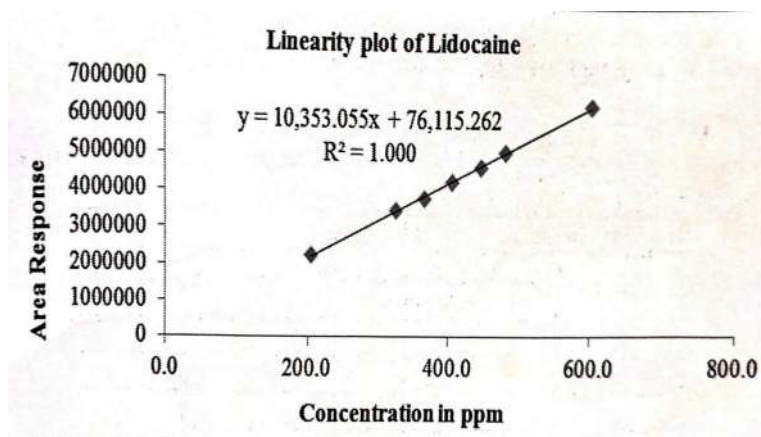


Figure 6: Linearity

9. RANGE

demonstrated to be determined with suitable accuracy and linearity

The range of analytical method is the interval between the upper and lower level if analyte that has been

Table 10: Range Studies

Lidocaine		
Level	Mean Area of linearity	Mean area of accuracy
Linearity 50%	2209736.995	2116407.746
Linearity 100%	4239278.265	4172029.322
Linearity 150%	6278506.854	6026720.931
Correlation Coefficient(R²)	1.000	1.000
RSD for accuracy	1.3%	

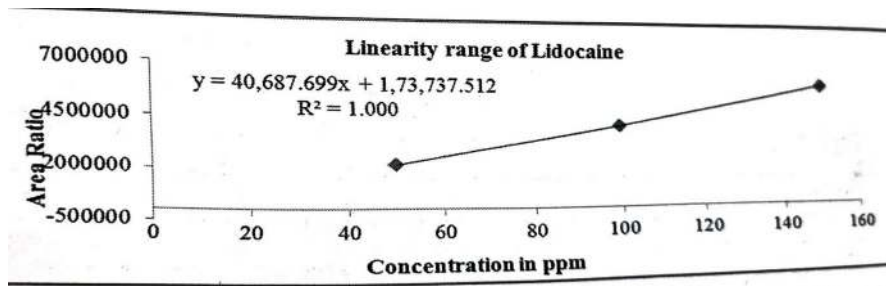


Figure 7: Range Study

10. Accuracy

accepted as either accepted value or an accepted reference value .This is sometimes termed trueness.

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is

Table 10: Range Studies

Sr.No	Levels	Area Response	*mg added(Actual)	*mg recovered	%Recovery	Mean%	%RSD
1	50%	2116491.551	20.56	20.4637	101.4	100.9%	0.6%
2		2115663.966	20.78	20.4557	100.2		
3		2117067.721	20.62	20.4693	101.1		



Sr.No	Levels	Area Response	*mg addedActual)	*mg recovered	%Recovery	Mean%	%RSD
1	100%	4171173.909	40.87	40.3299	100.5	100.7%	0.3%
2		4174996.625	40.69	40.3668	101.0		
3		4169917.432	40.82	40.3177	100.6		
1	150%	6021804.494	60.38	58.2231	98.2	98.4%	0.2%
2		6022821.071	60.18	58.2329	98.5		
3		6035537.228	60.32	58.3558	98.5		

Figure 9: Accuracy Study**11. Robustness**

Robustness studies were carried out using slight modifications in chromatographic such as:

1) Change in column temperature $\pm 5^{\circ}\text{C}$

2) Change in flow rate $\pm 0.2\text{mL/min}$

3) Change in pH ± 0.2 unit

4) Change in organic phase $\pm 5\%$

Table 11: Robustness Studies

Sr.No	%RSD lidocaine peak from standard should not be more than 2	The tailing factor for lidocaine peak from standard should not be more than 2	The theoretical plates for lidocaine peak from standard should not be less than 2000
1	0.0%	1.18	8828
2	0.1%	1.17	8501
3	0.1%	1.21	9093
4	0.0%	1.18	9701
5	0.0%	1.19	8109
6	0.1%	1.15	8109
7	0.0%	1.14	9480
8	0.1%	1.21	9356
9	0.1%	1.18	8914

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