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Development and Validation of RP-HPLC Method for Estimation of Lidocaine in Various Pharmaceutical Dosage Forms

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ABSTRACT

Objective: To develop extraction procedures for extracting Lidocaine from various pharmaceutical dosage forms (ointment, gel, injection, aerosol, transdermal patch) and to analyze them by development of accurate, precise and robust reverse phase high performance liquid chromatography method.

Method: Chromatographic procedures were developed using Chromatopak, Peerless C18 column (Column dimensions: 250 mm x 4.6 mm, 5 µm) with mobile phase comprising of Dipotassium monohydrogen phosphate buffer (10Mm): ACN in ratio 20:80 at a flow rate of 1ml/min, with detection wavelength at 263nm. The retention time of Lidocaine was found to be at 5.43±0.03.

Results: The method was validated according to ICH guidelines (Q2) R1.Linearity of LID was found in concentration range of 20-100ug/ml with r2=0.999. Limit of Detection and Limit of Quantification were found to be 1.54ug/ml and 4.68ug/ml. %RSD values for intraday and interday precision were also found to be >2%. Accuracy studies were also in range between 95%-105%. The method proved to be robust when chromatographic parameters like Ph, mobile phase ratio, flow rate, wavelength were altered.

Conclusion: The % Assay values of marketed formulation were found to be within prescribed range. Thus this proposed RP-HPLC method can be used in routine quality control analysis of LID from its various pharmaceutical dosage forms.

Keywords: Lidocaine, ointment, gel, injection, aerosol, transdermal patch, RP-HPLC.

INTRODUCTION

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 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.

Various analytical methods are available in the literature for estimation of LID in biological and pharmaceutical samples which includes GC, [2,3] Spectrophotometric determination of Lidocaine in pharmaceuticals, [4] HPLC-UV [1] method, thin-layer chromatography (TLC)for the Determination of Hydrocortisone Acetate and Lidocaine in a Pharmaceutical Preparation [5] etc. No method is reported in literature for estimation of LID from all of its available pharmaceutical preparation. This present Research work includes extraction methods of Lidocaine from various pharmaceutical

formulations along with its analysis by well developed RP-HPLC method.

EXPERIMENTAL PART

APPARTAUS AND SOFTWARE

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AT pump and Shimadzu SPD20AV absorbance detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20 µl. Data acquisition and integration was performed using Spinchrome software (Spincho biotech, Vadodara).The chromatographic elution of analyte was obtained by using CHROMATOPAK, Peerless C18 column (Column dimensions: 250 mm x 4.6 mm, 5 µm).

REAGENTS AND CHEMICALS

Lidocaine was provided as gift sample from SIDMAK LABORATORIES PVT. LIMITED INDIA. HPLC grade Acetonitrile and Ortho Phosphoric Acid were supplied from Rankem, India. Dipotassium monohydrogen phosphate AR grade was purchased from Rankem and Thermo Fisher Scientific India Pvt. Ltd. Respectively. Water used throughout the experiment was Purified HPLC grade water .The pharmaceutical samples used in the present study include Lidocaine 5% ointment; lignocaine hydrochloride injection 2%, Lidocaine spray 10%w/w, Lidocaine hydrochloride gel 2%, and Lidocaine patch 5%.

CHROMATOGRAPHIC CONDITIONS

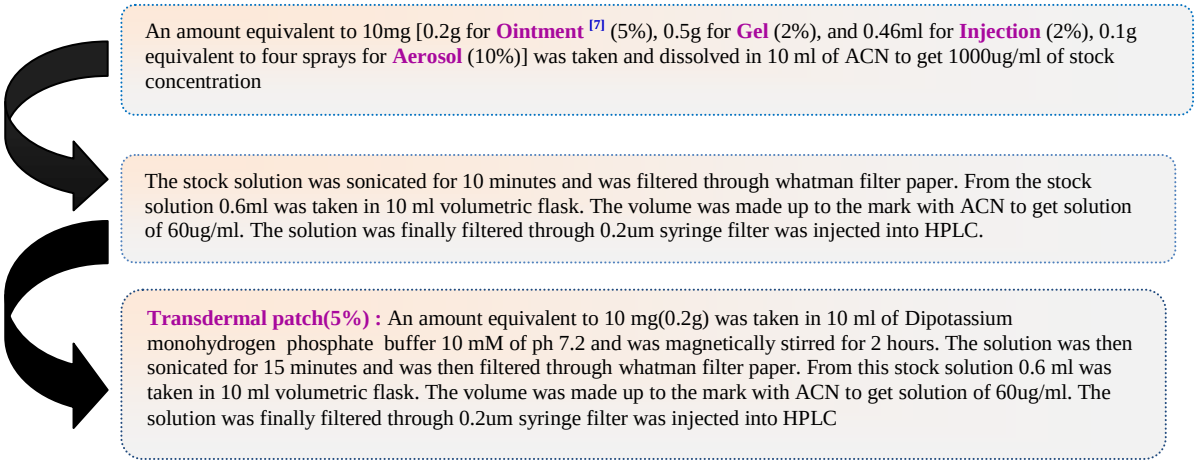
The mobile phase comprised of Acn: Dipotassium monohydrogen phosphate buffer10 mm, prepared by 0.0870g of Dipotassium monohydrogen phosphate in 50 ml of double distilled water and adjusted to pH 7.2 using Ortho Phosphoric Acid which was finally filtered with 0.2 µm Nylon membrane filter. The elution was carried out with a mixture of Acetonitrile: 10mM Dipotassium monohydrogen phosphate buffer pH 7.2 in the proportion of 80:20. Resulting solution was degassed by ultrasonication for 10 minutes.

PREPARATION OF STANDARD SOLUTION OF LIDOCAINE [6]

Stock solution of (1000 µg/ml) was prepared by accurately weighing 10 mg of LID in 10 ml volumetric flask. The drug was dissolved in ACN and the solution was diluted to volume. Further dilutions were made from this stock solution and the injection volume was kept 20 µL. A calibration curve was plotted between concentrations against their respective area for LID. From the calibration curve, it was found that linearity range is between 20-100ug/ml.

ANALYSIS OF MARKETED FORMULATION

EXTRACTION PROCEDURE:



RESULT AND DISCUSSION

Optimization of Chromatographic Conditions

To optimize the chromatographic conditions, the effect of chromatographic variables such as composition of mobile phase, ratio of mobile phase and flow rate were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and theoretical plates were calculated. Finally, a simple and inexpensive method was developed by using a combination of Dipotassium monohydrogen phosphate buffer and ACN in ratio 20:80. Optimized chromatographic conditions are listed in Table 1

TABLE 1

METHOD PARAMETER	OPTIMIZED VALUE
COLUMN	CHROMATOPAK, Peerless C18 column (Column dimensions: 250 mm x 4.6 mm, 5 µm).
MOBILE PHASE	Dipotassium monohydrogen phosphate buffer (10Mm): ACN (20:80)
FLOW RATE	1 ml/min
RETENTION TIME tR (MINUTES)	5.430±0.03
DETECTION WAVELENGTH(nm)	263
TEMPERATURE	Ambient
INJECTION VOLUME	20uL
TAILING FACTOR	1.3±0.019
THEORETICAL PLATES(N)	11935±88.33

METHOD VALIDATION [8]

LINEARITY

The calibration curve was constructed by plotting concentrations of LID versus peak areas, and the regression equations were calculated. The linearity of the method was investigated by using concentrations in the range 20-100µg/ml .Retention time for LID was found to be 5.43 min respectively. The linear regression equation is Y= 0.831x+1.583 (r2=0.999). The plot obtained from linear regression is given in (Fig 1)

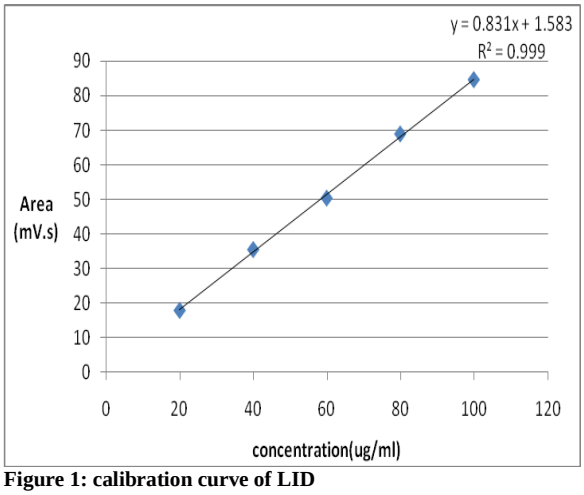


Figure 1: calibration curve of LID

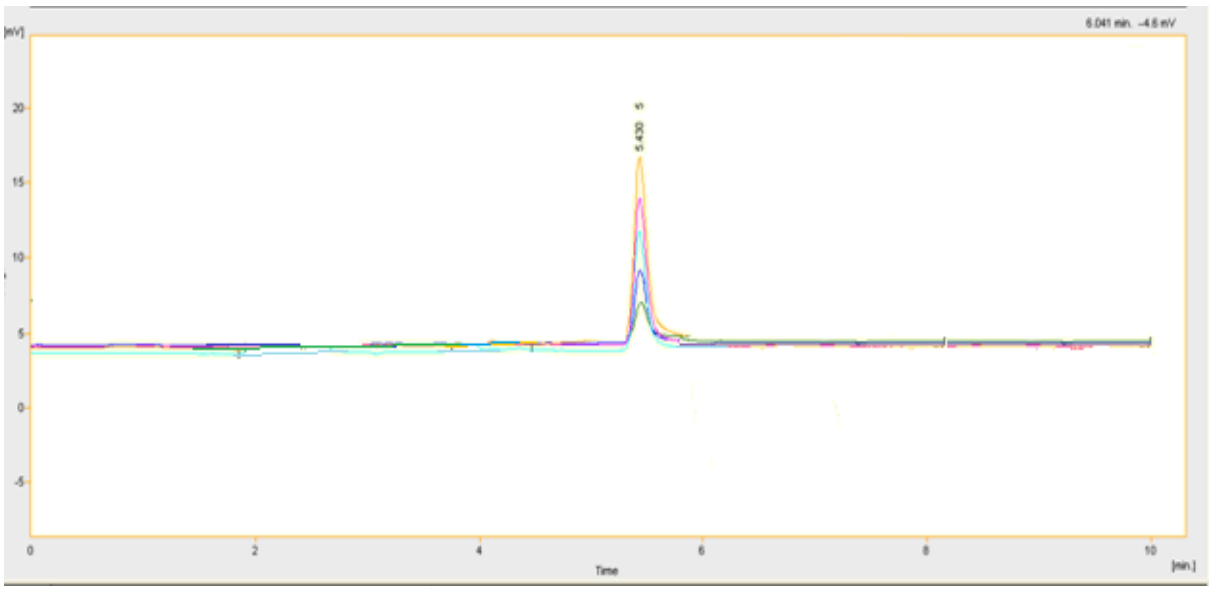


Figure 2: Chromatogram of Lidocaine showing linearity in range 20-100ug/ml at tR 5.43±0.03

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively, where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve. [6] The LOD and the LOQ for HPLC were found to be 1.54ug/ml and 4.68ug/ml.

PRECISION

The precision of the proposed method was assessed as intraday and interday precision. Three replicate injections of specific standard at various time intervals on the same day were injected into system for intraday precision and were repeated on three different days for Interday precision. The % RSD (Relative Standard Deviation) of the results was calculated.

TABLE 2: Intraday precision of LID

CONC. (µg/ml)	MEAN AREA (mV.s)	SD	%RSD
40	35.31	0.312	0.88
60	50.71	0.466	0.92
80	68.92	0.451	0.65

TABLE 3: Interday precision of LID

CONC. (µg/ml)	MEAN AREA (mV.s)	SD	%RSD
40	35.23	0.351	0.99
60	50.63	0.611	1.20
80	68.63	0.650	0.94

ACCURACY

Accuracy of the method was studied using standard addition method at three different levels (80, 100, and 120%) by recovery experiments. Known amounts of standard solutions containing LID (48, 60,72ug/ml) were added to one of the marketed formulation of concentration 60 ug/ml to reach 80%, 100% and 120% levels. Percentage Recovery was the mean of three determinations at each standard addition level

TABLE 4: Accuracy data of LID

% SPIKING	CONC TEST(ug/ml)	CONC ADDED (ug/ml)	CONC RECOVERED (ug/ml)	% RECOVERY ± STANDARD DEVIATION
80	60	48	48.8	101.3±0.55
100	60	60	59.6	99.3±0.42
120	30	72	72.9	101.6±0.42

ROBUSTNESS

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions like pH, mobile phase ratio, Wavelength and flow rate. The average value of % RSD for determination of LID was less than 2 % which revealed the robustness of the method.

TABLE 5: Robustness study for proposed HPLC method

Sr.no	Factors		tR(min)	Peak Area (mV.s)
1	PH	7	5.5	50.12
		7.2	5.43	50.39
		7.4	5.32	51.25
		Mean± SD	5.41±0.09	50.58±0.58
		%RSD	1.67	1.16
2	%Organic	78	5.56	50.5
		80	5.44	50.9
		82	5.36	51.68
		Mean± SD	5.45±0.100	51.02±0.600
		%RSD	1.84	1.17
3	Flow Rate	0.9	5.5	50.5
		1.0	5.43	51.2
		1.1	5.3	51.5
		Mean± SD	5.41±0.101	51.06±0.51
		%RSD	1.87	1.00
4	Wavelength	261	5.41	50.2
		263	5.43	50.9
		265	5.48	50.5
		Mean± SD	5.44±0.03	50.53±0.35
		%RSD	0.66	0.69

ANALYSIS OF MARKETED FORMULATION [6]

When the LID marketed formulation was analyzed by these proposed HPLC method, sharp peaks was obtained at tR 5.43 minutes, when scanned at 263nm. The amount of the label claim measured is given in table 6, all the formulations are within the limits (95%-105%), for patch the limits are (90%-110%)

TABLE 6: Assay results of marketed formulation

Sr.no	Formulation	% Assay
1	Ointment	102.1
2	Gel	99.1
3	Injection	100.1
4	Aerosol	99.6
5	Patch	95

CONCLUSION

The proposed reverse phase high performance liquid chromatography method has been developed for the analysis of LID in their marketed formulation. The method was validated as per ICH guideline. % Assay values of marketed formulation were

found to be in the prescribed range. Thus the proposed HPLC method can be used for routine quality control analysis of LID from its various Pharmaceutical dosage forms.

Authors' Contribution Statement

Dr. Rajashree Mashru has constantly guided this work and Margi Gandhi has prepared the manuscript.

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