DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LIDOCAINE AND PRILOCAINE IN TOPICAL FORMULATION

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ABSTRACT

Objective: A simple, specific, accurate, and precise method, namely, reverse phase high-performance liquid chromatography was to develop for simultaneous estimation of Lidocaine (LDC) and prilocaine (PLC) in a topical local anesthetic cream.

Method: The mixture of PLC and LDC was separated on Hi Q Sil C18 HS column, (250 mm × 4.6 mm, 5 μm), column temperature ambient and flow rate 1.2 mL/minutes. The mobile phase was acetonitrile: 0.01 M diethylamine solution (pH adjusts to 6.8 with orthophosphoric acid) (60:40) with detection at 225 nm.

Results: The retention time was found to be 6.075±0.12 minutes for PLC and 8.642±0.15 minutes for LDC, respectively. Linearity was observed in the concentration range of 1-6 μg/mL for both LDC and PLC, respectively. The method was validated according to International Conference on Harmonization guideline and values of linearity, precision, robustness, limit of detection, limit of quantitation, selectivity, and recovery were found to be in good accordance with the prescribed value.

Conclusion: The proposed method can be useful in the quality control of LDC and PLC in their topical formulation.

Keywords: High-performance liquid chromatography, Prilocaine, Lidocaine, Validation.

INTRODUCTION

Lidocaine (LDC) is a local anesthetic which acts by causing blockade of sodium channel that leads to decrease in sodium conductance and depresses the rate of electrical depolarization; then, threshold potential level and propagation of action potential failure are achieved which ultimately leads to blockade of conduction in case of local anesthetic. LDC, chemically is 2-(diethylamino)-*N*-(2, 6-dimethylphenyl) acetamide [1]. Prilocaine (PLC) is an amide local anesthetic with pharmacological properties similar to lignocaine. To increase the duration of action and delay uptake by the general circulation local anesthetics may be administered with a vasoconstrictor, usually adrenaline. Chemically, PLC is (RS)-N-(2-Methylphenyl)-2-(propylamino) propanamide. PLC, unlike other amide anesthetics, is a secondary amino derivative of toludine. It produces less vasodilation and toxicity than LDC and is considered relatively free from an allergic reaction [2,3]. The chemical structures of PLC and LDC are shown in (Fig. 1).

To determine PLC and LDC, many studies have been reported in literature. Several spectrophotometric [4-6], chromatographic [7-18], liquid chromatography-tandem mass spectrometry [19-21], and gas chromatography-mass spectrometry [22-24] and capillary electrophoresis [25] techniques were developed to determine PLC and LDC in both pharmaceutical preparations and human plasma. The aim of the present work is to develop and validate a new high-performance liquid chromatography (HPLC) method for determination of PLC and LDC in topical formulation. The proposed method was validated with validation parameters, which are sensitivity, specificity, linearity, precision, and accuracy in accordance with International Conference on Harmonization (ICH) guidelines [26].

METHODS

Chemicals and reagents

Pure LDC and PLC were kindly gifted by Neon Labs Pvt., Ltd., (Thane), Mumbai, India. Commercial LDC (2.5%) and PLC (2.5%) topical local anesthetic cream were purchased from local market for the study. Acetonitrile (ACN) used of HPLC grade (S.K Enterprises, Pune, India). Double distilled water used in experiment was obtained from Milli-Q system (Millipore).

Instrumentation and chromatographic conditions

The HPLC system consisted of intelligent HPLC pump model (Jasco PU 2080 Plus) with sampler programmed at 20 μL capacity per injection was used. The detector consisted of an ultraviolet (UV)-visible spectrophotometry (Jasco UV 2075 Plus). Data were integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. Chromatographic separation was carried out with HiQSilHSC18column (250 mm × 4.6 mm, 5 μm). The mobile phase used for isocratic elution was prepared by mixing ACN: 0.01M diethylamine solution (pH adjusts to 6.8 with orthophosphoric acid) (60:40 v/v). Before use, the mobile phase was filter through 0.45 μm membrane filter and degassed by ultrasonication. The flow rate was 1.2 mL/minutes, column temperature 25°C, the injection volume was 20 μL, and detection was performed at 225 nm using a UV detector.

Preparation of standard stock solutions

The standard stock solutions of LDC and PLC were prepared by accurately weighing 10 mg of each drug into a 10 mL volumetric flask. The drugs were dissolved in methanol 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 concentration against their respective area for LDC and PLC separately. From the calibration curve, it was found that linearity ranges for both drugs 1-6 μg/mL for LDC and PLC, respectively.

Analysis of marketed formulation

To determine, the content of LDC and PLC in marketed topical formulation (LDC 2.5% and PLC 2.5% cream). A portion of the cream preparation (1 gm equivalent to 25 mg LDC and 25 mg PLC) was weighed

and extracted into 15 mL methanol with the aid of ultrasonication for 15 minutes then filtered into a 25 mL volumetric flask. The volume was made with methanol. From the above solution, further dilution was made with mobile phase to obtain a solution of LDC (1 μg/mL) and PLC (1 μg/mL), respectively.

RESULT AND DISCUSSION

Optimization of procedures

The HPLC procedure was optimized for simultaneous determination of PLC and LDC. Good resolution of both components was obtained with ACN: 0.01 M diethylamine solution (pH adjusts to 6.8 with orthophosphoric acid) at ratio 60:40 v/v. The flow rate of 1.2 mL/minutes was optimum. UV detection was made at 225 nm. At this wavelength, PLC and LDC can be quantified. Hence, 225 nm determined empirically has been found to be optimum. The average retention times for PLC and LDC was found to be 6.075 and 8.642 minutes, respectively (Fig. 2). The system suitability parameters for HPLC chromatogram are as follows (Table 1).

Linearity and range

Linearity is generally evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. For determining linearity, calibration curves were plotted over a concentration range of 1-6 μg/mL for PLC and 1-6 μg/mL for LDC, respectively. A 20 μL of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration.

Linear regression data for the calibration plots revealed good linear relationships between response and concentration. The linear regression equations were $Y=24047X+24844$ ($r^2=0.9994$) for PLC and $Y=31596X+13687$ ($r^2=0.9991$) for LDC. The plots obtained from linear regression are given in (Fig. 3) for PLC and (Fig. 4) for LDC, respectively.

Limits of detection and quantitation

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. The LOD and the LOQ for HPLC were found to be 0.2 μg/mL and 0.6 μg/mL for PLC and 0.3 μg/mL and 0.8 μg/mL for LDC, respectively.

Precision

The precision of the proposed method was assessed as intraday and interday precision by preparing three different sample solutions at low, medium, and high concentrations, which were freshly prepared and analyzed. The intraday precision % relative standard deviation (% RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Interday precision RSD% was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week. The precision of the method was expressed as RSD%. The results showed in (Table 2) shows the high precision of the method.

Accuracy

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. To check the
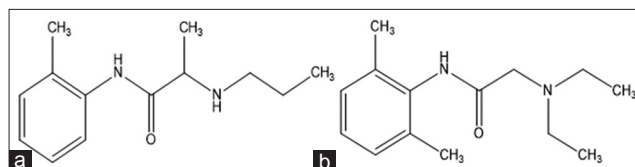

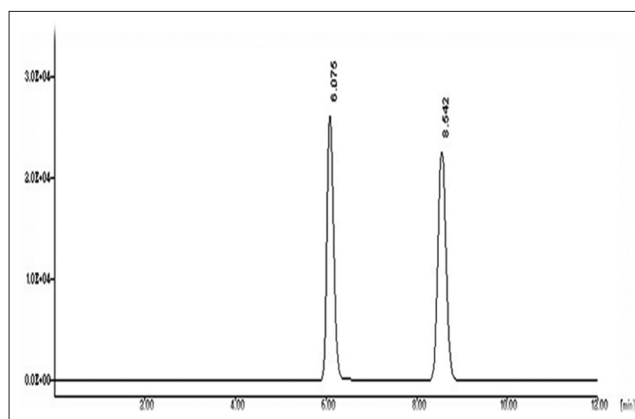**Fig. 1: Chemical structures of (a) Prilocaine, and (b) Lidocaine**

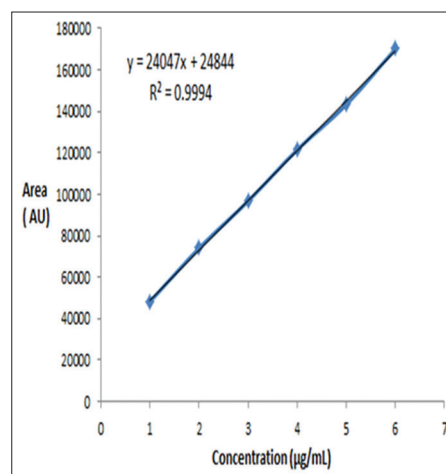**Fig. 2: Chromatogram of Prilocaine and Lidocaine**

Fig. 3: Calibration curve for Prilocaine
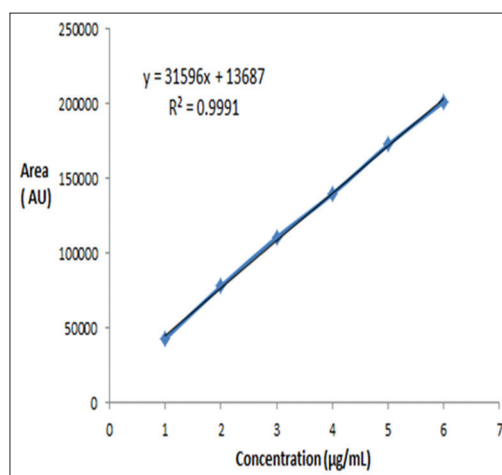
Fig. 4: Calibration curve for Lidocaine

Table 1: System suitability parameters

Parameter	PLC	LDC
Retention time (minutes)	6.075	8.642
Resolution (Rs)	-	7.068
Theoretical plates number (N)	6246	5689
Tailing factor	1.21	1.19

PLC: Prilocaine, LDC: Lidocaine

Table 2: Precision study for proposed HPLC method (n=3)

Drugs	Concentrations (µg/mL)	Intraday precision		Interday precision	
		Amount found (%)	% RSD	Amount found (%)	% RSD
PLC	2	99.57	0.75	99.23	1.12
	4	99.10	1.11	99.00	1.16
	6	99.29	1.03	99.18	1.07
LDC	2	99.39	1.19	99.08	1.10
	4	99.60	1.21	99.27	1.13
	6	99.53	1.17	99.21	1.22

PLC: Prilocaine, LDC: Lidocaine, HPLC: High-performance liquid chromatography, RSD: Relative standard deviation

Table 3: Accuracy study for proposed HPLC method (n=3)

Label claim (per gm cream)	Amount added (%)	Total amount (mg)	Amount recovered (mg)	(%) Recovery	Mean % recovery±SD
PLC 25 mg	80	45	44.89	99.76	99.77±0.105
	100	50	49.94	99.88	
	120	55	54.82	99.67	
LDC 25 mg	80	45	44.92	99.82	99.71±0.147
	100	50	49.88	99.76	
	120	55	54.75	99.54	

PLC: Prilocaine, LDC: Lidocaine, HPLC: High-performance liquid chromatography, SD: Standard deviation

Table 4: Robustness study for proposed HPLC method (n=3)

Chromatographic factors for HPLC	Rt (minutes)		% RSD	
	PLC	LDC	PLC	LDC
Flow rate				
1.1 mL	6.105	8.758	1.20	1.18
1.3 mL	5.998	8.429	1.17	1.15
Mobile Phase composition (% v/v)				
ACN: 0.01M diethylamine solution (62:38)	6.100	8.674	1.01	1.10
ACN: 0.01M diethylamine solution (58:42)	6.017	8.510	1.14	1.05
pH				
6.7	6.089	8.758	1.21	1.10
6.9	6.101	8.617	1.19	1.12

PLC: Prilocaine, LDC: Lidocaine, HPLC: High-performance liquid chromatography, ACN: Acetonitrile

degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100%, and 120%. Known amount of standard PLC and LDC were added to pre-analyzed samples and were subjected to the proposed method. Result of recovery study of HPLC method is shown in (Table 3).

Robustness

Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained. The factor chosen for study were the flow rate, mobile phase composition, and pH. In the above-changed conditions, results of robustness studies were expressed in term of % RSD of peak areas in each changed condition and were compared with similar results obtained in unchanged experimental conditions. The method was found to be unaffected by small changes with % RSD for all the parameters <2% indicating that method is robust. Result of robustness study is shown in Table 4.

Analysis of marketed formulation

When the LDC (2.5%) and PLC (2.5%) cream was analyzed by HPLC, sharp and well-defined peaks for PLC and LDC were obtained at Rt 6.075 and 8.642 minutes, respectively, when scanned at 225 nm. The amount of the label claim measured was 99.89% for PLC and 99.78% for LDC, respectively.

CONCLUSION

The proposed reverse phase (RP)-HPLC method has been developed for the simultaneous analysis of PLC and LDC in their topical formulation. The method was validated as per ICH guidelines. The validation results reveal that the methods are precise, linear, robust, and accurate, which proves the reliability of the proposed method. The HPLC method can be used for routine quality control analysis of PLC and LDC in their topical cream formulation.

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