Curr. Pharm. Res. **2017**, *7(2),* 2064-2073

Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

HPTLC Method Development for Simultaneous Estimation of Lidocaine and Prilocaine in Topical Formulation.

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Received 08 January 2017; received in revised form 15 March 2017; accepted 15 March 2017

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ABSTRACT

To develop a simple, specific, accurate and precise high performance thin layer chromatography method for simultaneous estimation of Lidocaine and Prilocaine in a topical local anesthetic cream. The mixture of Prilocaine and Lidocaine separated on Aluminum precoated silica gel 60 F254 plates using di-isopropyl ether: methanol: ammonia (10: 0.5: 0.1 % v/v/v) as mobile phase and detection was carried a wavelength of 225 nm The method was validated as per ICH guidelines. The Rf value was found to be 0.45 ± 0.03 for Prilocaine and 0.55 ± 0.02 for Lidocaine, respectively. Linearity was observed in the concentration range of 100-600 ng/spot for both Lidocaine and Prilocaine, respectively. HPTLC method was validated according to ICH guideline and values of linearity, precision, robustness, LOD, LOQ, selectivity, recovery were found to be in good accordance with the prescribed value.

Conclusion: The proposed method can be used successfully for routine analysis of Lidocaine and Prilocaine in their topical formulation.

KEYWORDS

HPTLC, Prilocaine and Lidocaine.

1. INTRODUCTION

Lidocaine (LDC) is an 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 is achieved which ultimately leads to blockade of conduction in case of local anesthetic. Lidocaine, 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, Prilocaine is (RS)-N-(2-Methylphenyl)-2-(propylamino) propanamide. Prilocaine, unlike other amide anesthetics, is a secondary amino derivative of toludine. It produces less vasodilation and toxicity than lidocaine and is considered relatively free from an allergic reaction [2-3]. The chemical structures of prilocaine and Lidocaine are shown in Fig. 1. In order to determine Prilocaine and Lidocaine, many studies have been reported in literature.

Several spectrophotometric [4-6], chromatographic [7-18], LC-MS/MS [19-21] and GC-MS [22-24] and capillary electrophoresis [25] techniques were developed in order to determine Prilocaine and Lidocaine 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) and high performance thin layer chromatography (HPTLC) methods for determination of Prilocaine and Lidocaine 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].

(A) **(B)**

Fig. 1: Chemical structures of Prilocaine (A) and Lidocaine (B)

In order to determine Prilocaine and Lidocaine, many studies have been reported in literature. Several spectrophotometric [4-6], chromatographic [7-18], LC-MS/MS [19-21] and GC-MS [22-24] and capillary electrophoresis [25] techniques were developed in order to determine Prilocaine and Lidocaine 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) and high performance thin layer chromatography (HPTLC) methods for determination of Prilocaine and Lidocaine 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].

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Pure Lidocaine and Prilocaine were kindly gifted by Neon Labs Pvt. Ltd (Thane), Mumbai, India. Commercial Lidocaine (2.5%) and Prilocaine (2.5 %) topical local anesthetic cream were purchased from local market for the study. Di-isopropyl ether, methanol, diethylamine and ammonia used, were of analytical grade (S.K Enterprises, Pune, India). Double distilled water used in the experiment was obtained from Milli-Q system (Millipore).

2.2. Instrumentation and Chromatographic Conditions

Chromatography was performed on 10 cm × 20 cm aluminum foil-packed silica gel 60F254 HPTLC plates (Merck, Darmstadt, Germany). Before use the plates were washed with methanolthen dried in an oven at 110°C for 5 min. Samples were applied as 6 mm bands by spraying at a rate of 0.1 µL/s by means of aCAMAG (Switzerland) Linomat V sample applicator equipped with a 100 µL syringe (Hamilton, Reno, Nevada, USA). The distance between bands was 6 mm. Ascending development of the plate, migration distance 80 mm, was performed at 25 ± 2°Cwith di-isopropyl ether: methanol: ammonia (10: 0.5: 0.1 % v/v/v), as mobile phase in a CAMAG twin-trough chamber previously saturated for 20 min. Densitometric scanning at 225 nm was then performed with a CAMAG TLC Scanner equipped with WINCATS software, using a deuterium light source; the slit dimensions were 5 mm × 0.45 mm.

2.3. Preparation of Standard Stock Solutions

Accurately weighed LDC (10 mg) and PLC (10 mg) were transferred to 10 mL volumetric flask and dissolved in, and then diluted to the mark with methanol. The stock solution was further diluted with methanol to obtain a solution of LDC (100 ng/spot)and PLC (100 ng/spot), respectively.

2.4. Analysis of Marketed Formulation

To determine the content of LDC and PLC in marketed topical formulation (Lidocaine 2.5% and Prilocaine 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 ultra-sonication for 15 min. then filtered into a 25 mL volumetric flask. The volume was made with methanol. From the above solution, further dilution was made with methanol to obtain a solution of LDC (100 ng/spot) and PLC (100 ng/spot), respectively.

3. RESULTS AND DISCUSSION

3.1. Optimization of Procedures

The TLC procedure was optimized with a view to develop assay method.

Both the pure drugs were spotted on the TLC plates and run in different solvent systems. Initially Di-isopropyl ether: methanol in varying ratios was tried. The mobile phase Di-isopropyl ether: methanol 10: 0.5 (v/v) was tried. The developed spot was diffused. To the above mobile phase, 0.1 mL ammonia was added. Both the peaks gave good resolution, sharp and symmetrical peak when plates were scanned at 225 nm. The Rf value were found to be 0.45 ± 0.03 for PLC and

0.55 ± 0.02 for LDC, respectively (Fig. 2). Also, the spots for PLC and for LDC were compact and not diffused. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 20 min ensure good reproducibility and peak shape of PLC and for LDC.

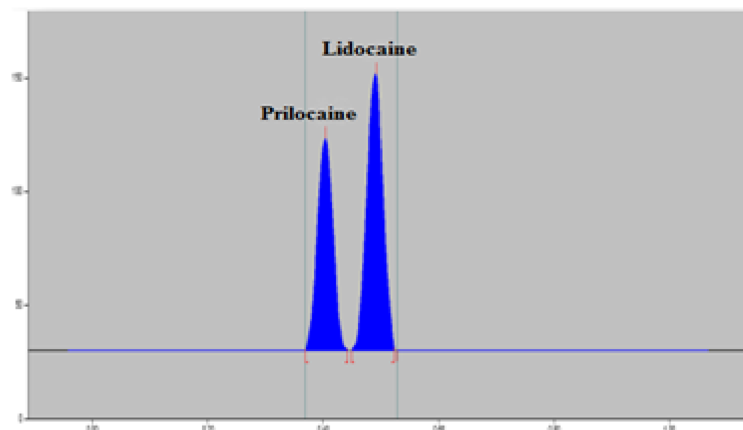

Fig. 2: Typical densitogram of PLC and LDC.

3.2. Linearity and Range

Different volumes of stock solution 1 to 6 μL were spotted in triplicate on TLC plates to obtain concentration of 100-600 ng/spot of PLC and LDC, respectively. The plate was developed on previously described mobile phase. The peak areas were plotted against corresponding concentrations to obtain the calibration graphs. Linear regression data for the calibration plots revealed good linear relationships between response and concentration over. The linear regression equations were Y= 6.6149X + 10.791 (r2= 0.9995) for PLC and Y= 10.224x + 807.42 (r2= 0.997) for LDC. The plots obtained from linear regression are given in fig.3for PLC and fig.4 for LDC, respectively.

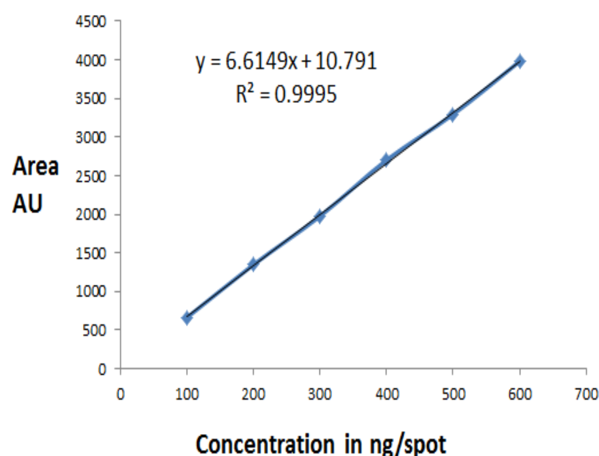

Fig. 3: Calibration curve for PLC.

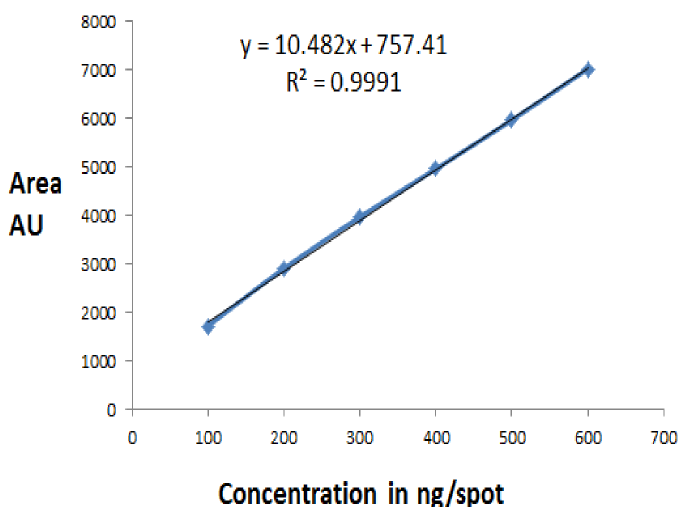

Fig. 4: Calibration curve for LDC.

3.3. Limits of Detection and Quantitation

The sensitivity of the proposed method was estimated in terms of the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and 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 limit of detection and the limit of quantification were found to be 50 ng/spot and 80 ng/spot for PLC and 40 ng/spot and 75ng/spot for LDC, respectively.

3.4. Precision

Intra-day and inter-day precision were determined by performing replicate (n=3) analyses by preparing three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed. The intra-day precision % Relative Standard Deviation (% RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter- day 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 relative standard deviation (RSD, %). The results showed in Table 2 shows the high precision of the method.

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

Conc. (ng/spot)	Intra-day precision (n=3)			Inter-day precision (n=3)		
	Measured Conc. ± SD	(%) RSD	Recovery (%)	Measured Conc. ±SD	(%) RSD	Recovery (%)
LDC						

200	200.24 ±	0.75	100.06	198.72 ± 0.76	0.19	99.68
400	3.00	0.17	99.72	296.48 ± 1.99	0.25	99.56
600	397.76 ±	0.13	99.88	600.72 ± 14.65	1.22	100.06
	1.36					
	598.56 ±					
	1.56					
Mean		0.35			0.55	
PLC						
200	198.56 ±	0.6	99.28	199.34 ± 2.27	1.14	99.67
400	1.27	4	100.03	399.6 ± 4.08	1.02	99.90
600	400.12 ±	0.8	99.47	599.88 ± 8.34	1.39	99.98
	3.56	9				
	596.82 ±	0.7				
	4.24	1				
Mean		0.7			1.18	
		5				

3.5. Accuracy

Accuracy was done in terms of recovery studies. Recovery studies were carried out by standard addition method. The analyzed samples were spiked with extra 80 %, 100 % and 120 % of standard drugs. The mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. Result of recovery study of both methods are shown in (Table 3).

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

Label claim (per mg cream)	Amount Added (%)	Total amount added (mg)	Amount recovered (mg)	Recovery (%)	Mean (%) Recovery (± SD)
PLC	80	20	19.93	99.65	99.95
25	100	25	24.93	99.73	± 0.18
	120	30	29.88	99.6	
LDC	80	20	19.93	99.69	99.67
25	100	25	24.90	99.63	± 0.68
	120	30	29.84	99.48	

3.6. 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 mobile phase composition, saturation time, time from spotting to chromatography and time from chromatography to scanning. The 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 less than 2% indicating that methods are robust. Result of robustness study are shown in (Table 4).

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

Chromatographic factors for HPLC	Rf Value		% RSD	
Mobile Phase composition				
di-isopropyl ether: methanol: ammonia (9: 0.6: 0.1 % v/v/v)	0.42	0.53	1.12	1.03
di-isopropyl ether: methanol: ammonia (10.1: 0.5: 0.1 % v/v/v)	0.47	0.56	1.17	1.15
Saturation time				
25 min.	0.448	0.55	1.02	1.11
30 min.	0.451	0.54	1.07	1.10
Time from spotting to chromatography (+ 10)	0.451	0.553	1.15	1.07
Time from chromatography to scanning (+ 10)	0.452	0.551	1.04	1.12

3.7. Analysis of marketed formulation

Three replicate determinations were performed on the commercially available cream. When the Lidocaine (2.5%) and Prilocaine (2.5 %) cream was analyzed by HPTLC, sharp and well defined peaks for PLC and LDC were obtained at Rf values were found to be 0.45 for PLC and 0.55 for LDC, when scanned at 225 nm. The amount of the label claim measured by HPTLC % content were found to be 99.75 % for LDC and 99.67 % for PLC, respectively. There was no interference was observed from the excipients commonly present in the cream.

Cream formulation	Label claim mg	Amount found mg	% Content found*	S.D	% R.S.D.
Prilocaine	2.5	2.4910	99.67	0.5719	0.57
Lidocaine	2.5	2.4937	99.75	0.34044	0.34

4. CONCLUSION

In the present study a HPTLC method has been developed for simultaneous estimation of PLC and LDC in their topical formulation. The proposed method was sufficiently sensitive and reproducible for the analysis of the PLC and LDC in cream formulation. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with the

determination of PLC and LDC. The method is simple, precise, specific, and accurate and can be used for the routine simultaneous analysis of the PLC and LDC in pharmaceutical preparations.

5. ACKNOWLEDGEMENTS

The authors are grateful to Neon Labs Pvt. Ltd (Thane), Mumbai, India, for providing a gift sample of Prilocaine and Lidocaine. The authors are also thankful to Dr. S. G.Gattani, Professor and HOD, School of Pharmacy SRTM University Vishnupuri, Nanded, India, for encouraging and motivating for this research work.

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