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Simultaneous estimation of lidocaine and prilocaine in topical cream by green gas chromatography

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ABSTRACT

Lidocaine (LDC) and prilocaine (PLC) are estimated in a topical local anesthetic cream using a direct, eco-friendly, stability-indicating gas chromatographic technique with flame ionization detector. The mixture of LDC and PLC was separated using Zebron DB drug column. The column temperature and flow rate were 230°C and 14 ml/minute, respectively. The retention time was found to be 5.1 minutes for PLC and 5.4 minutes for LDC. Linearity was observed in the concentration range of 20–100 µg/ml and 10–50 µg/ml for LDC and PLC, respectively. The method was validated and values of linearity, limit of detection, limit of quantification, precision, and accuracy were found to be in good accordance with the International Conference on Harmonization guideline. A direct, stability-indicating method was developed for the determination of LDC and PLC in topical dosage forms in the presence of its degradation products. The proposed method can be useful in the quality control of LDC and PLC in their topical formulation.

INTRODUCTION

Lidocaine (LDC) is a local anesthetic which acts by causing barricade of sodium channel that shows a decrease in sodium conductance and lowered rate of electrical depolarization, ultimately leading to blockade of conduction. Synthetically, LDC is 2-(diethylamino)-N-(2, 6-dimethylphenyl) acetamide (Powell and Hydrochloride, 1986). Prilocaine (PLC) is an amide local anesthetic with pharmacological properties like lignocaine. Synthetically, PLC is (RS)-N-(2-Methylphenyl)-2-(propylamino) propanamide. PLC, in contrast to other amide analgesics, is an optional amino subordinate of Toludine. It delivers less vasodilation and toxicity than LDC and is viewed as moderately free from an unfavorably susceptible response (Rishiraj *et al*., 2005; Warren *et al*., 1974). The structures of PLC and LDC are shown in Figure 1.

Many studies have been reported in the literature to determine PLC and LDC. A few spectrophotometric (Atila and Kadioglu, 2012; Karthikkumar *et al*., 2012; Rizk *et al*., 1997), chromatographic (Fijałek *et al*., 2005; Kadioglu and Atila, 2008; Klein *et al*., 1994; Liawruangrath *et al*., 2001; Malenovic *et al*., 2005; Mohammad, 2009; Pendela *et al*., 2011; Plenis *et al*., 2013; Ricci Júnior *et al*., 2002; Storms and Stewart, 2002; Wiberg and Jacobsson, 2004; Zylber-Katz *et al*., 1978), fluid chromatography-couple mass spectrometry (Dal Bo *et al*., 1999; Koehler *et al*., 2005; Ter Weijden *et al*., 2012), gas chromatography flame ionization detection (Baniceru *et al*., 2004; Culea *et al*., 1989; Keenaghan, 1968; Levine *et al*., 1983; Reynolds and Beckett, 1968), gas chromatography-mass spectrometry (Kadioglu and Atila, 2007; Watanabe *et al*., 1998; Yang *et al*., 2009), capillary electrophoresis (Siluveru and Stewart, 1997), and high performance liquid chromatography (HPLC) methods were reported to determine PLC and LDC in both pharmaceutical formulations and human plasma.

Many studies have been reported to determine PLC and LDC in pharmaceutical preparation (Atila and Kadioglu, 2012; Dal Bo *et al*., 1999; Fijałek *et al*., 2005; Karthikkumar *et al*., 2012; Liawruangrath *et al*., 2001; Malenovic *et al*., 2005; Mohammad, 2009; Narendra and Shailesh, 2017; Pendela *et al*., 2011; Plenis *et al*., 2013; Ricci Júnior *et al*., 2002; Rizk *et al*., 1997; Wiberg and Jacobsson, 2004), human plasma (Dal Bo *et al*., 1999;

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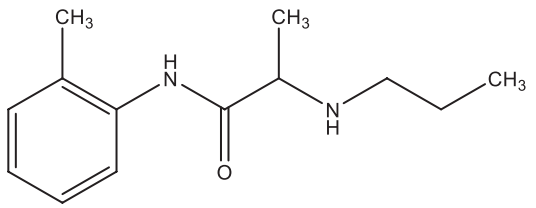
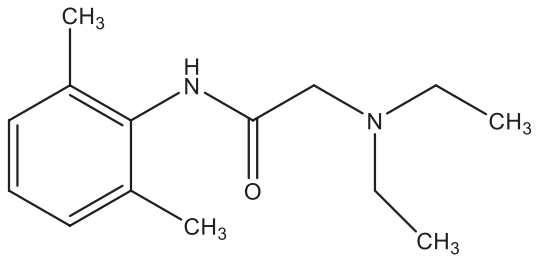


(a) Prilocaine



(b) Lidocaine

Figure 1. Chemical structures of (a) PLC and (b) LDC.

Kadioglu and Atila, 2007; 2008; Klein *et al*., 1994; Ter Weijden *et al*., 2012; Zylber-Katz *et al*., 1978), human serum (Koehler *et al*., 2005; Siluveru and Stewart, 1997), and in human blood (Watanabe *et al*., 1998).

To the best of our insight into the study of analytical chemistry, no such green analytical method was reported for estimation of PLC and LDC in a topical cream. Researchers developing green gas chromatography follow the 3 R's rule (Reduce, Replace, and Recycle). The focus of the present work is to develop and validate green gas chromatographic (GC) method for PLC and LDC in a topical formulation. We have developed some eco-friendly GC methods for several drugs with minimum use of solvents in our laboratory (Chandan *et al*., 2013; Gurupadayya *et al*., 2010; Indupriya *et al*., 2011; Soujanya *et al*., 2011; Thejaswini *et al*., 2012; Vijayakumar *et al*., 2016). The proposed technique was in accordance with the parameters of International Conference on Harmonization (ICH) guidelines, including linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (ICH, 2005).

MATERIALS AND METHODS

Experimental

Instrument

The instrument utilized in this investigation is the GC Shimadzu 2014 model associated with a flame ionization detector controlled by GC solution software. A sample applicator for GC was equipped with l µl syringe (Hamilton Bonaduz AG, Switzerland); the column utilized was Zebron DB drug column (Length: 30 m, Diameter: 0.25 mm and Film: 0.50 µm) with a temperature range of −40 to 320 (Max. 340°C).

Materials

Pure LDC and PLC were procured from Martin and Brown Bio-Sciences (Nalagarh), Himachal Pradesh, Mumbai, India. Marketable LDC (2.5%) and PLC (2.5%) topical local analgesic cream (PRILOX 30 g) was obtained from a local pharmacy for the examination. HPLC grade 2-propanol was acquired from Merck specialities Pvt Ltd, Worli, Mumbai, India. Distilled water utilized in the trial was acquired from Milli-Q framework (Millipore). Millipore water is procured from Merck specialities Pvt. Ltd, Worli, Mumbai, India and H_2O_2 and other chemicals of analytical grade were procured from ACE Rasayan, Mysuru.

ANALYTICAL PROCEDURES

Standard solutions

By weighing 10 mg of PLC and LDC into 10 ml volumetric flask, standard stock solutions were prepared. Suitable dilutions of PLC and LDC were made using 2-propanol. The volume of injection was set to 1 µl. Calibration curve was plotted between peak area against the concentration for LDC and PLC independently. The linearity ranges were observed in the range of 20–100 µg/ml for LDC and 10–50 µg/ml PLC based on ICH guidelines.

Analysis of marketed formulation

To estimate the content of LDC and PLC in marketed topical formulation (LDC 2.5% and PLC 2.5% cream), the cream (1 g comparable to 25 mg of both LDC and PLC) was weighed and extracted into 50 ml 2-propanol with the aid of ultra-sonication for 15 minutes. On subsequent filtration into a 100 ml volumetric flask by using syringe filter, the volume was made with 2-propanol to obtain a solution of 20 µg/ml for LDC and 10 µg/ml for PLC. Furthermore, dilution was made with 2-propanol from the above solution.

Chromatographic conditions

The mobile phase is comprised of nitrogen gas as carrier gas, hydrogen and zero air as supportive gases for the formation of flame in the flame ionization detector. The rate of flow was 14 ml per minute throughout the run. The temperature of the oven was adjusted at 210°C for 1 minute then increased to 280°C (5°C/minute). The inlet temperature and detector temperature were 200°C and 280°C, respectively. The injection volume and the split ratio were 1 µl and 10:0, respectively, for determination of LDC and PLC in a topical formulation.

Forced degradation studies

Forced degradation studies were performed on formulations containing LDC and PLC in liquid states as indicated by the following conditions: 5 ml of different fractions of LDC and PLC standard stock solutions were moved into 25 ml volumetric flask and treated as expressed in Table 1. After the predetermined time, all the solutions, except that of photolytic degradation were left to cool. According to ICH guidelines, neutralization is done by adding the same amount and concentration of the alkaline solution to the acidic one and vice versa. The solutions of LDC and PLC were diluted to the required volume to obtain the final concentration of 200 µg/ml.

Table 1. PLC and LDC stress testing parameters.

Degradation condition	Concentration and volume of reagent	Stress conditions	Percentage of degradation (PLC)	Percentage of degradation (LDC)
Liquid state				
Acid	1 M, HCl (5 ml)	Water bath adjusted at 70°C for 30 minutes	25.1	22.8
Base	1 M, NaOH (5 ml)		31.3	30.1
Neutral	Purified water (5 ml)		18.7	28.5
Oxidation	30% H_2O_2 (5 ml)		28.8	22.2
Photolysis	Purified water (5 ml)	UV light at 254 and 366 nm at a separation of 15 cm from the light for 4 hours, revealed to glow of a 1.6 million lux hours.	57.2	68.7

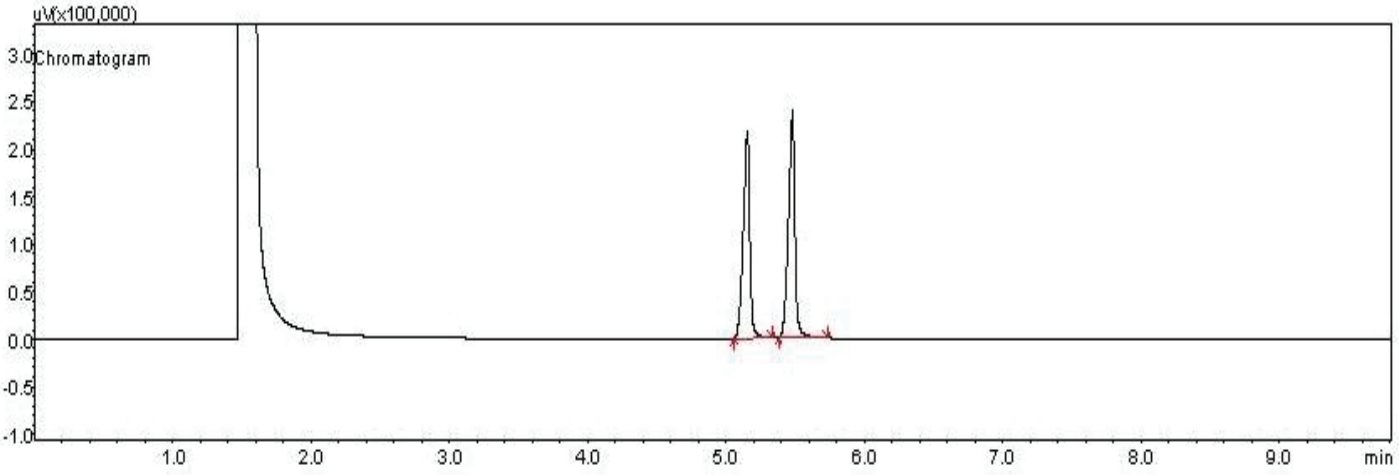


Figure 2. Chromatogram of PLC and LDC.

RESULTS AND DISCUSSIONS

Optimization of procedures

The GC methodology was used for simultaneous determination of LDC and PLC. The resolution of both drugs was more than 1.5, obtained for the separation of two drugs using 2-propanol as a solvent. Therefore, 2-propanol was selected as an ideal solvent for the separation of two drugs. The stream rate of 14 ml/minute was optimum. The retention times for LDC and PLC were observed to be 5.153 and 5.442 minutes, respectively, as depicted in Figure 2, and the overlay chromatogram of PLC and LDC was mentioned in Figure 3. The system suitability parameters for GC chromatogram are expressed in Table 2 (ICH, 2005). The comparison of the existing method parameters with reference HPLC method (Narendra and Shailesh, 2017) was highlighted in Table 3. Accordingly, the current method does not require any costly organic solvent during the process of method development.

Method Validation

Linearity and range

Calibration curves were plotted to determine the linearity over a concentration range of 10–50 µg/ml for PLC and 20–100 µg/ml for LDC. A 1 µl of test solution was injected using septum injector. Chromatograms were noted. All measurements were repeated three times, by plotting a graph of relative drug concentration versus peak areas of component. The linear regression equations were $Y = 14{,}432X - 5{,}489.1$ ($r^2 = 0.9972$) for PLC and $Y = 16{,}280X - 15{,}697$ ($r^2 = 0.9933$) for LDC. The plots obtained from linear regression are given in Figure 4 for PLC and Figure 5 for LDC, respectively.

Limits of detection and limit of quantitation

The criteria used to ascertain LOD and LOQ are the 3.3 and 10 σ/s, individually.

Where σ = standard deviation of the peak area.

s = slope of the comparing calibration curve.

The values of LOD and the LOQ for PLC and LDC were mentioned in Table 4.

Precision

Three different samples were prepared at a concentration of low, medium, and high, and analysis was performed to determine the precision of the proposed method as intraday and interday precision. By analyzing standard drug solution within calibration range, both intraday precision, % relative standard deviation (% RSD) (three times on the same day) and interday precision, % RSD (three different days

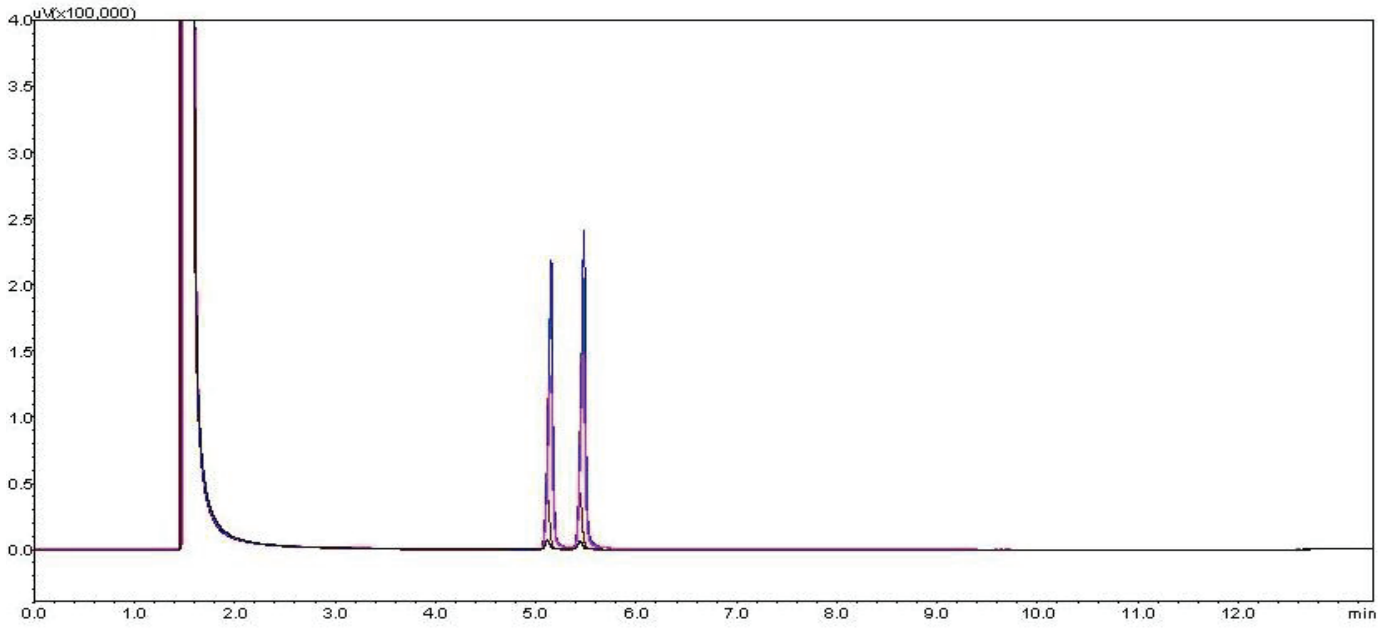


Figure 3. Overlay chromatogram of PLC and LDC.

Table 2. System suitability parameters of LDC and PLC.

Parameters	PLC (n = 6)	LDC (n = 6)	Limits
Retention time (t_R) (minutes)	5.13	5.45	–
Resolution (Rs)	-	3.920	≥ 1.5
Theoretical plates (N)	59,883.03	71,133.82	N > 2,000
Tailing factor (T)	0.963	1.003	T of ≤ 2

Table 3. Comparison of the reported method with the current method.

Method	Drugs	Linearity (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	RSD	[a]Rs	[b]R_t
HPLC (Reference)	LDC	1–6	0.3	0.8	99.70	7.06	8.64
	PLC	1–6	0.2	0.6	99.77	--	6.07
Current method	LDC	20–100	3.22	9.77	99.93	3.920	5.13
	PLC	10–50	1.33	4.01	99.72	---	5.4

[a]Rs: resolution; [b]R_t: retention time.

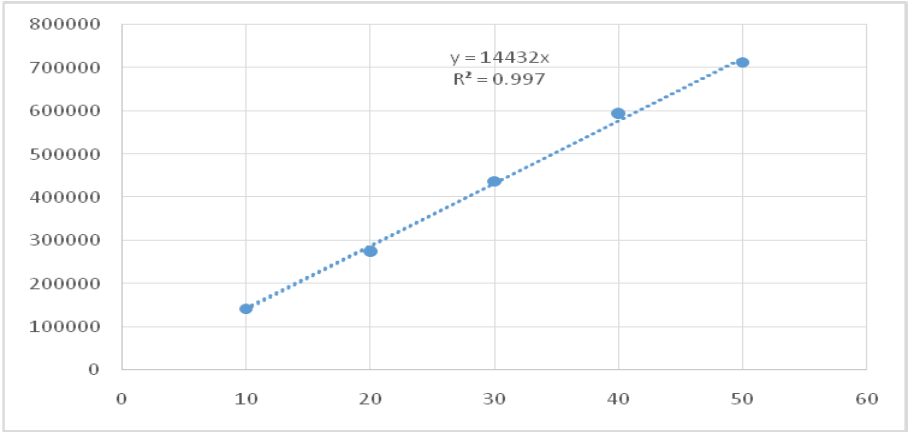


Figure 4. Calibration curve for PLC.

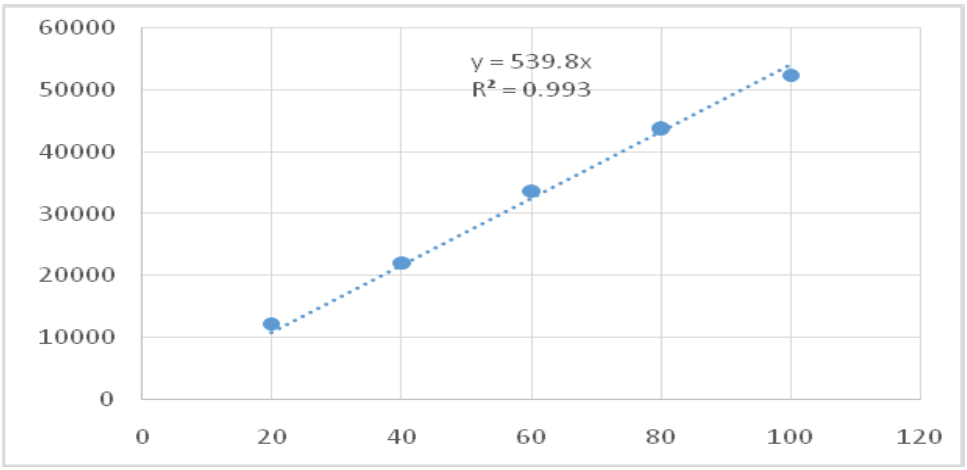


Figure 5. Calibration curve for LDC.

Table 4. LOD and LOQ for the proposed GC method.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
LDC	3.22	9.77
PLC	1.33	4.01

Table 5. Intraday and interday precision study for the proposed GC method (n = 6).

Drugs	Concentration (µg/ml)	Intraday precision			Interday precision		
		Peak area	% RSD	Mean % recovery ± SD	Peak area	% RSD	Mean % recovery ± SD
PLC	30	419,543 410,245 419,568 410,215 419,452 419,214	1.0435	99.66 ± 108	410,216 419,546 410,213 419,654 419,546 419,621	1.0614	99.72 ± 124
LDC	60	33,804 34,333 34,046 34,548 35,214 34,015	1.3484	99.79 ± 132	33,415 34,954 34,541 34,015 35,013 34,215	1.6139	99.93 ± 145

RSD < 5%.

over a period of a week) have been assessed. The precision of the method was expressed as RSD%. The precision results shown in Table 5 were found within the limit and prove that the method was highly precise.

Accuracy

Accuracy in the analytical method is the difference between the theoretically added amount and practically achieved amount. Recovery studies were performed in triplicate by standard addition method at 80%, 100%, and 120% to check the degree of accuracy of the method. A known amount of standard LDC and PLC were added to pre-analyzed samples, which were then exposed to the proposed method. Results of recovery study of GC method were shown in Table 6, which were obtained in accordance with ICH guidelines.

Forced degradation studies

In the GC method, forced degradation was done on topical cream. The results showed that both PLC and LDC undergo degradation after subjecting the standard drug combination to the above stress condition with the appearance of specific retention time and peak area less than that of standard drug. GC chromatograms of LDC and PLC mixture degradation condition in acid, base, neutral, oxidation, and

Table 6. Accuracy study for proposed GC (n = 3).

Label claim (per gram cream)	Amount added (%)	Total amount (µg/ml)	%Recovery	Mean % recovery ± SD
	80	30	99.55	
PLC (25 mg)	100	40	98.98	99.10 ± 0.109
	120	50	98.77	
	80	30	99.96	
LDC (25 mg)	100	40	100.81	99.62 ± 0.138
	120	50	98.09	

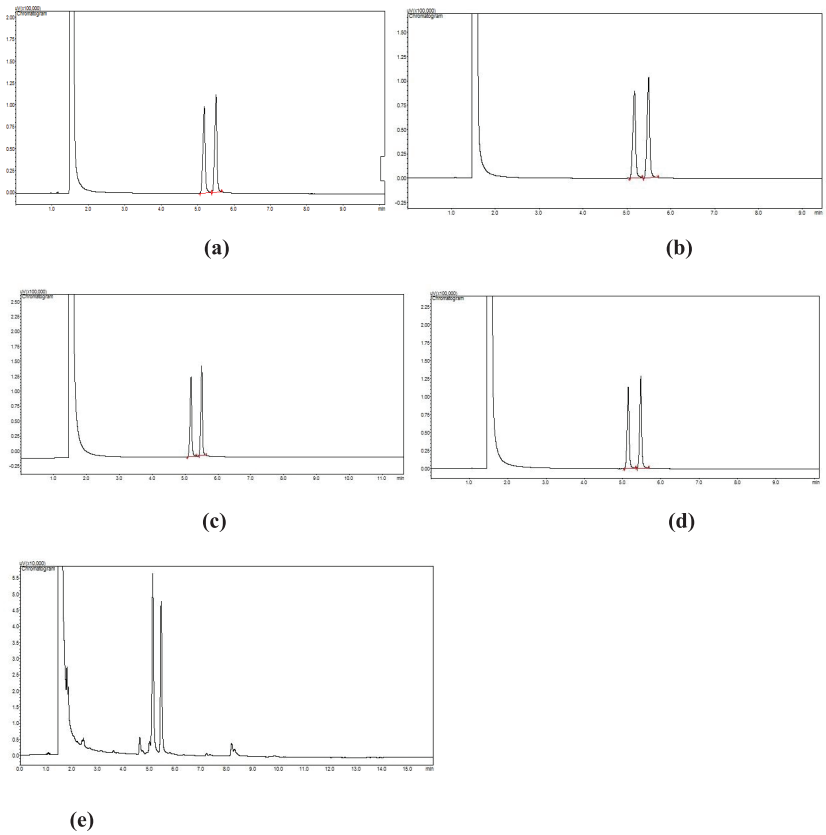


Figure 6. GC chromatograms of LDC and PLC mixture degradation condition (liquid state). (a) Basic state, (b) acidic state, (c) neutral state, (d) oxidative state, and (e) photolytic state.

photolytic state are shown in Figure 6. Hence, it proves that the method is stable for force degradation study. The results are shown in Table 1.

CONCLUSION

The proposed green GC technique has been developed for the simultaneous analysis of LDC and PLC in their topical formulation. The technique was validated according to ICH guidelines. The technique was found to be linear, precise, and accurate, which demonstrates the reliability of the proposed technique. The developed method proves superiority over other reported methods due to the minimum use of solvents. Thus, the developed green GC technique can be utilized for routine quality control examination of LDC and PLC in their topical cream formulation.

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CONFLICT OF INTEREST

None.

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