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Determination of Lidocaine HCl in commercially cream and injection forms by GC-FID method

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Abstract: In the present study, GC-FID method for the determination of lidocaine HCl in commercially creams and injection forms was developed and validated. The linearity of method was observed in the concentration range of 0.1-5.0 µg/mL. The accuracy (RE%) and precision (RSD%) values of the within-day and between-day of GC-FID method are less than 10.0% and 3.0%, respectively, and also limit of detection (LOD) and the limit of quantitation (LOQ) values were observed as 0.03 and 0.11 µg/mL, respectively. The analytical recovery value of lidocaine HCl was determined as 99.47% on average. As a result, it was concluded that the developed and validated GC-FID method can be easily used in routine analyzes in quality control laboratories.

Keywords: Lidocaine HCl; local anesthesia; cream; injection; GC-FID method.

1. Introduction

Local anesthesia is the process of temporarily removing and desensitizing the nerves in the tissues of the area to be operated. Local anesthetics have the ability to temporarily block the impulse conduction in the nerve fiber. In small operations, local anesthesia is provided by administering drugs that prevent nerve conduction to the immediate surroundings of the operation area. Local anesthetics reversibly block nerves to reduce pain sensation in various regional or local treatments. Local anesthetics are widely used in minor surgery clinics, especially in dental treatments[1-3].

Lidocaine (2-(Diethylamino)-N-(2,6-dimethylphenyl)-acetamide) is one of the amino amide type local anesthetics used to block regional nerves with low toxic potential. Lidocaine is also used in other conditions such as multiple sclerosis, chronic daily headache, migraine and cluster headaches, neuropathic pains, apart from local anesthetic use [4,5]. Lidocaine is available in the form of injection or infusion solutions and pharmaceutical formulations such as many topical formulations. It is also available as a transdermal patch applied directly to the [1-5].

There are publications in the literature on the quantification of lidocaine HCl in commercial formulations. Some of the most recently published methods include spectrophotometry[5-9],

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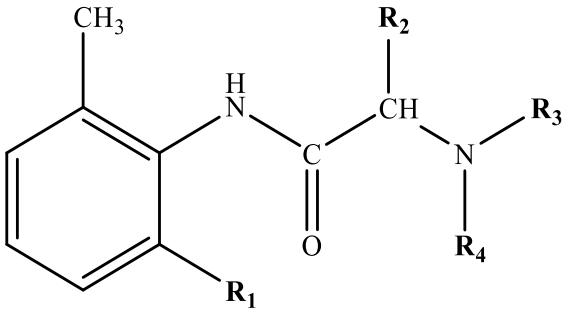
electrochemical[10-12], HPLC[13-16], and capillary electrophoresis[17-19]. The only two publications have been reach regarding the GC-FID method of lidocaine HCl in commercial formulations[20-21].

In the literature review, two publications related to the analysis of lidocaine with the GC-FID method in commercial formulations were reached. One of these publications was published by our group. In this publication, a forced degradation study of lidocaine was performed[20]. In the other study, lidocaine and prilocaine were analyzed simultaneously by gas chromatography method in a topical local anesthetic cream[21]. In this study, it is aimed to develop and validate a new GC-FID method for the determination of lidocaine HCl in commercial cream and injection forms and to show that it is applicable in real samples. The method was validated by studying specificity, linearity, stability, analytical recovery, LOD, LOQ, accuracy and precision parameters according to ICH[21]. We indicated to be possible of determination of lidocaine HCl and provided GC elution and resolution with considerable accuracy and sensitivity of analyte and easily applied to commercial cream and injections forms.

2. Experimental

2.1. Materials

The standard substances of lidocaine HCl and prilocaine HCl (IS) (Figure 1) were supplied from Novagenix Bio Analytical R&D Centre (Ankara, Turkey). The chromatographic grade methanol and analytical grade all other chemicals were supplied from Merck (Germany). The commercial cream and injection forms containing lidocaine were obtained from the local market (Erzurum); Jetokain ampoule (Adeka A.Ş.; its content: 20 mg/mL lidocaine) and Jetmonal ampoule (Adeka A.Ş.; its content: 20 mg/mL lidocaine) and Emla cream (Eczacıbaşı A.S.; its content: 25 mg lidocaine and 25 mg prilocaine). The gases used in the study were supplied from Havaş (Ankara, Turkey).



	Prilocaine HCl	Lidocaine HCl
R_1	-H	$-CH_3$
R_2	$-CH_3$	-H
R_3	-H	$-C_2H_5$
R_4	$-C_3H_7$	$-C_2H_5$

Figure 1. Chemical formulations of lidocaine HCl and prilocaine HCl (IS).

2.2. Instrument and Method Conditions

The chromatographic analysis was carried out on an Agilent 6890N Network gas chromatography system equipped with a flame ionization detector, an Agilent 7683 series auto sampler, an Agilent Chemstation. HP-5 capillary column packed [(5%-Phenyl)-methylpolysiloxane 30 m (0.320mm x 25 μm) (USA)] was used for separation. The split mode (10:1) was used with nitrogen carrier gas and the flow rate of carrier gas was kept constant during run at 1.6 mL/min. Hydrogen and synthetic air were used as auxiliary gases for the detector (FID). The injector volume was 2.0 μL. The injector and detector temperatures were 300°C. The oven temperature programs: initial temperature

95°C, hold 1.7 min, hold 2 min at 220ºC, ramp rate 15°C/min, final temperature 260°C where the temperature was held for 1.0 min.

2.3. Solutions

The stock solution of lidocaine HCl (100 µg/mL) was prepared by dissolving appropriate amount of the lidocaine standard compound in methanol. The standard working (0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 µg/mL) and the quality control solutions (0.2, 1.25 and 4.0 µg/mL and 0.3, 1.5 and 4.5 µg/mL) was prepared from the stock solution. In study, prilocaine HCl (2.0 µg/mL) was used as the internal standard (IS). All solutions were stored at +4ºC.

2.4. Preparations of Commercial Cream and Injection Forms

The commercially available two injection samples (Jetokain and Jetmonal ampoule), each containing 20 mg of lidocaine per mL, and cream samples (5% Emla) containing 25 mg lidocaine and 25 mg prilocaine were purchased from the local market in Erzurum-Turkey.

For both injections samples (Jetokain and Jetmonal ampoule), the contents of 10 vials, each containing 20 mg lidocaine per mL, were completely transferred into volumetric flask and vortexed about 5 min. An aliquot of this solution, equivalent of 20 mg of lidocaine, was transferred into a 100 mL volumetric flask and diluted to the volume with the methanol, after which the solution was filtered through paper. The filtrate was diluted with methanol to obtain a 0.5 µg/mL concentration of injection samples.

An accurately weighed quantity of Emla cream equivalent to 25 mg lidocaine and 25 mg prilocaine HCl was transferred into 100 mL beaker and 60 mL of methanol was added in beaker; sonicated for 15 min and then cooled. The solution in beaker was filtered into a 100 mL volumetric flask and the filtrate was adjusted to volume with the methanol to obtain a 0.5 µg/mL concentration of cream samples.

IS (2.0 µg/mL prilocaine) was added in the solutions prepared from commercial cream and injections forms and also bulk solutions. Then these solutions were again filtered through a 0.22 µm Millipore filter. The filtered solutions were analyzed with proposed method. The mean peak area ratios (lidocaine peak area/IS peak area) were calculated from the obtained chromatograms. The amount of lidocaine HCl in commercial cream and injection forms was determined from the calibration curve.

2.5. Simulated Sample

Simulated sample containing 20 mg of lidocaine, 0.0125 mg of epinephrine, 4.5 mg of NaCl and 1.0 mg of $Na_2S_2O_5$ (sodium metabisulfite) for Jetokain injection samples and 20 mg of lidocaine and 4.6 mg of NaCl for other injection samples (Jetmonal) were prepared and also simulated sample containing 25 mg of lidocaine, 25 mg prilocaine, poloxamer188 and poloxomer407 for cream samples were prepared. The simulated solutions prepared were analyzed by the same GC-FID procedure.

2.6. Placebo Sample

Placebo sample containing 0.0125 mg of epinephrine, 4.5 mg of NaCl and 1.0 mg of $Na_2S_2O_5$ per mL for jetokain injection samples and 4.6 mg of NaCl per mL for jetmonal injection samples were prepared and also placebo sample containing 25 mg prilocaine, poloxamer188 and poloxomer407 for cream samples were prepared and then IS solution was added in these placebo samples. The placebo solutions and placebo+IS solutions prepared were analyzed by same GC-FID procedure

3. Results

3.1. System Suitability

System suitability tests were performed before conducting the determination for linearity. The system suitability was assessed by six replicate analyses of the drugs at a concentration of 2.5 µg/mL. The acceptance criterion was ±2% for RSD % for the peak area and retention times for lidocaine and prilocaine and also, capacity factor (k), resolution factor (Rs), tailing factor (T) and theoretical plates (N) were calculated (Table 1).

Table 1. System performance parameters of lidocaine HCl and IS

Parameter	Lidocaine HCl	IS (prilocaine HCl)
Retention time (min), tr	8.58	8.38
Capacity factor, k'	7.33	7.14
Tailing factor, T	1.11	1.11
Theoretical plates, N	47114.4	43890.3
Resolution, Rs	4.56	4.56
HETP (the height of the theoretical plate; mm)	0.63	0.68

3.2. Linearity/ Range

In the linearity study of the method, a serial concentration of lidocaine solution (0.1, 0.25, 0.5, 1.0, 2.5 ve 5.0 µg/mL) was prepared and chromatograms were taken and then the peak areas of lidocaine and IS were determined. The calibration curve was derived by plotting the peak area ratios (lidocaine HCl peak area/IS peak area, y) versus the lidocaine solution concentration (x). The linear regression equation [with standard error of intercept (Sa: 7.52x10-7) and slope (Sb: 2.96x10-2); y = 1.726x + 0.0004] and the correlation coefficient (0.9995) were detected. The linear range of the method was determined as 0.1 to 5.0 concentration range (Figure 2 and Figure 3).

A series of standard solutions were prepared at concentrations smaller than 0.1 µg/mL, which is the lowest value of the calibration curve. Chromatograms of these solutions were taken and the signal/noise (S/N) ratio of 3 was determined as the limit of detection (LOD) and the concentration of 10 was determined as the limit of determination (LOQ). The LOD value for Lidocaine HCl was found to be 0.03 µg/mL and the LOQ value was 0.11 µg/mL. RSD values were observed to be less than 20% within the acceptance limits.

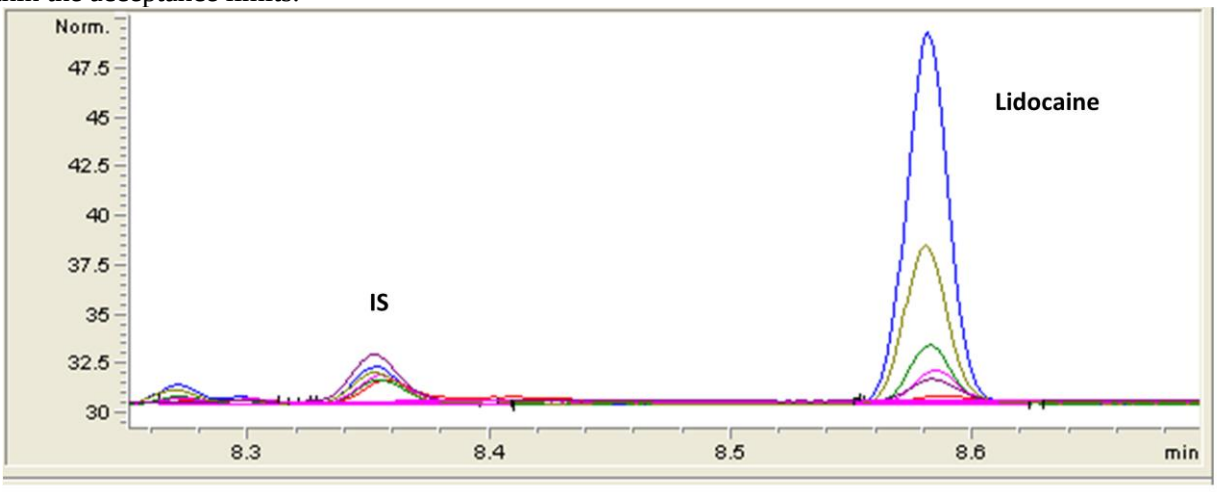


Figure 2. GC-FID Chromatograms of standard solutions of lidocaine and IS.

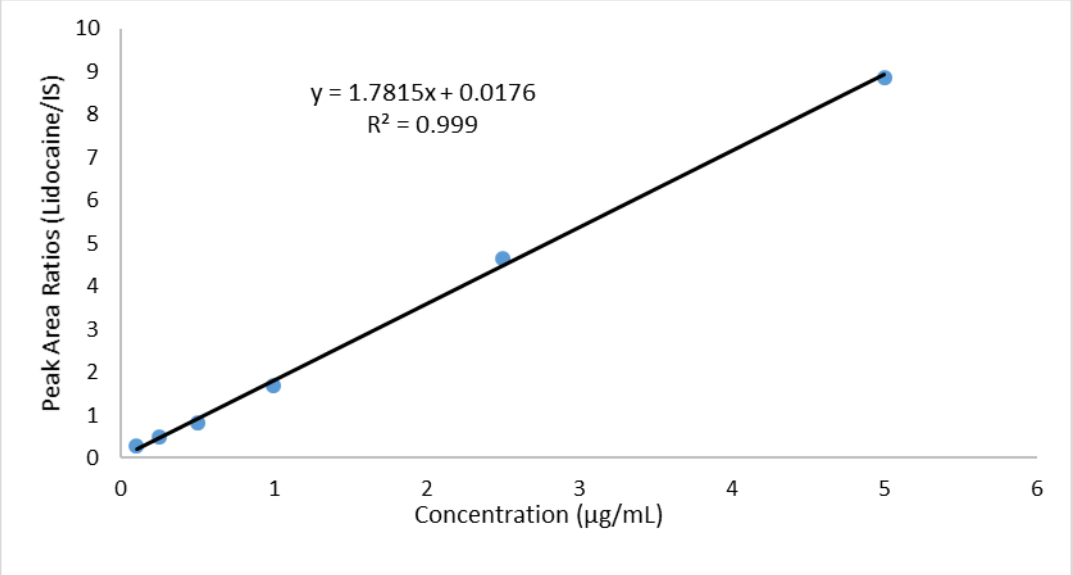


Figure 3. Calibration curve of the GC-FID method

3.3. Specificity

Specificity is the ability of the analyte to detect within the limits determined in the presence of all substances present in the sample medium. In the study to determine the specificity of the method, placebo and Simulated sample solutions were prepared with the substances found in commercial formulations. Chromatograms of these solutions were taken. No interactions were observed in the chromatograms (Figure 5). It has not been determined any interference of these substances at the levels found in dosage forms.

After chromatograms of the standard lidocaine HCl solutions are taken, placebo and simulated solutions with same properties as the compositions of commercial creams and injections were prepared and chromatograms of these solutions were taken. When all chromatograms were compared with each other, it was observed that there were no other substances in retention time of lidocaine HCl. In Figure 5 and Figure 6, it is observed that all the compounds of the injected sample separated at the different retention times and do not interfere between them. There appears to be no interaction between the excipients in the formulations in the placebo and simulated chromatograms.

Determination of Lidocaine HCl by GC-FID

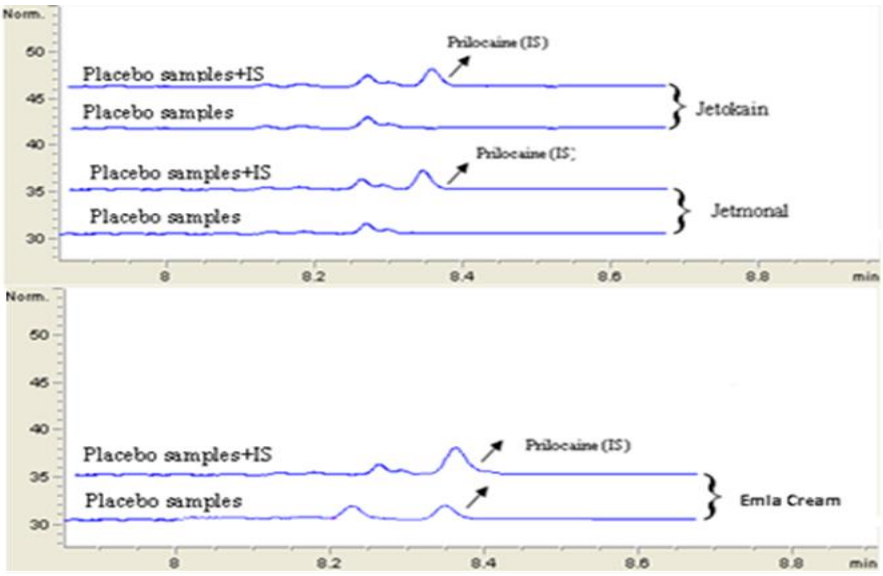


Figure 4. GC-FID chromatograms of placebo samples of injections and cream

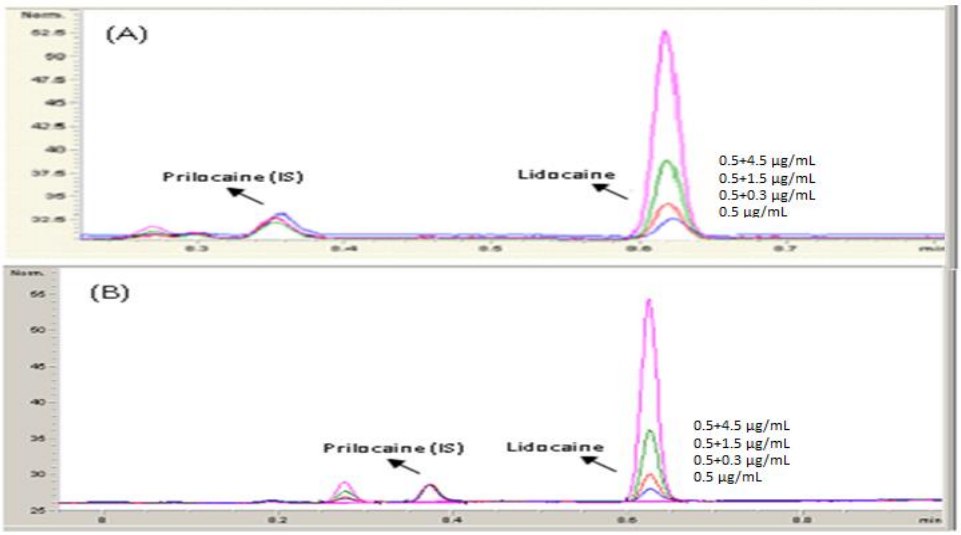


Figure 5. GC-FID chromatograms of Jetokain (A) and Jetmonal (B) injection samples

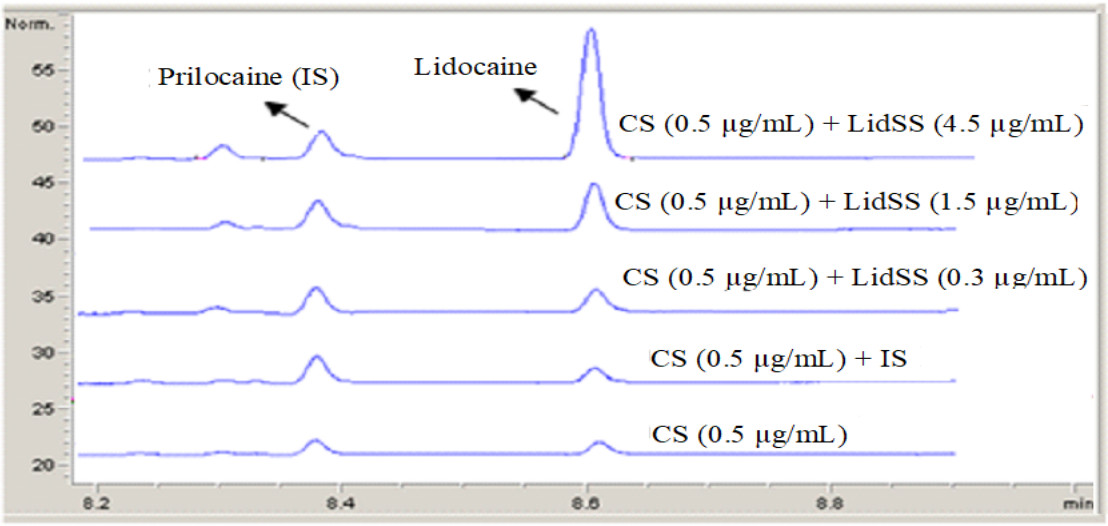


Figure 6. GC-FID chromatograms of cream samples

3.4. Precision and Accuracy

In determining the within-day and between-day accuracy and precision of the method, quality control solutions at three different concentrations falling within the calibration curve are prepared and analyzed. The within-day studies are analyzed of the same concentrations on the same day, and in the between-day studies are analyzed of the same concentrations on different days (3 days).

In this study, quality control solutions (lidocaine + 2 µg/mL IS) at three different concentrations (0.2, 1.25 and 4.0 µg/mL) were prepared, analyzed (n=6). The mean, standard deviation, relative standard deviation and relative error values of the obtained data were calculated. The precision of the method was given with the relative standard deviation and the accuracy of the method was given with the relative error.

The RSD values (relative standard deviation) for within-day and between-day in precision studies of proposed method were found as <3.0 % and <1.0 %, respectively (n=6). The RE (relative errors= found-added)/addedx100) for both the within-day and between-day for accuracy were found to be <10 %. In precision and accuracy studies of GC-FID method, it showed acceptable RSD and RE values for both the within-day and between-day were found to be less 10 % on average. These results were given in Table 2.

Table 2. Precision and accuracy of method

Added (µg/mL)	Within-day			Between-day		
	Found±SD (µg/mL)	Precision RSD%	Accuracy RE%	Found±SD (µg/mL)	Precision RSD%	Accuracy RE%
0.20	0.22±0.002	1.0	10.0	0.22±0.002	0.87	10.0
1.25	1.26±0.03	2.7	0.8	1.27±0.01	0.96	2.2
4.00	4.25±0.04	0.9	6.3	4.24±0.04	0.92	6.1

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, RE: Relative error

3.5. Analytical Recovery

Recovery studies of the method in commercial formulations (injections and cream) were performed by standard addition method. The solutions of the commercial cream and injection forms

were prepared according to the procedure described at Section "Preparations of Commercial Cream and Injection Forms". The solutions of lidocaine HCl (0.3, 1.5 and 4.5 µg/mL) and of IS (2.0 µg/mL) were spiked into solutions with a concentrations of 0.5 µg/mL of injections and cream. The solutions were analyzed with proposed method. Analytical recovery values were calculated using the formula [(peak area of total solution-peak area of added standard solution)/peak area of solution of commercial cream or injection forms) x100]. The analytical recoveries values were found ranged from 97 to 102% for commercial dosage forms (Table 3). The RSD values of recovery studies were obtained ranged from 1.5 to 2.5%. No interference from the common excipients was detected in this study.

Table 3. Analytical recovery values of lidocaine HCl in cream and injection forms

Commercial Dosage Forms (0.5 µg/mL)	**Added (µg/mL)**	**Recovery% ±SD**	**RSD %**
Jetokain ampoule	0.3	97.1±2.4	2.5
	1.5	101.2±2.1	2.1
	4.5	99.3±1.9	1.9
Jetmonal ampoule	0.3	99.7±2.4	2.4
	1.5	98.4±1.9	1.9
	4.5	98.9±2.1	2.1
Emla Cream	0.3	98.7±1.9	1.9
	1.5	99.9±2.5	2.5
	4.5	102.0±1.5	1.5

3.6. Stability

In order to determine the duration without decomposition of the solutions (standard, stock and formulation), the analyzes were made by keeping the solutions in the refrigerator (+4) and room temperature for certain periods. The recovery values were determined by comparing the obtained data with the data determined before the storage of these solutions. Recovery values were used to evaluate stability.

% Recovery Value of Analyte = (peak area of analyte in solution on hold /peak area of analyte in freshly prepared solution) × 100

It was observed that all three solutions were stable without significant change in their concentrations (recovery = 100 ± 1%) when kept for one day at room temperature and six days in the refrigerator. Average values of percent recovery values obtained for all three solutions are given in Table 4.

Table 4. Stability of lidocaine HCl in solutions (stock, standard and formulation)

Concentrations (µg/mL)	**Room temperature**		**Refrigerated**			
	1 Day	**2 Days**	**1 Day**	**5 Days**	**6 Days**	**7 Days**
0.30	98.8	75.7	99.4	99.6	98.9	85.9
1.50	99.5	74.5	100.7	98.4	97.9	85.5
4.50	99.3	77.9	100.1	99.3	97.6	86.3

3.7. Application of the Method for Analysis of Commercial Cream and Injection Forms

The determination of lidocaine HCl in two brands of injections (Jetokain and Jetmonal : containing 20 mg lidocaine per mL) and cream (5% Emla : containing 20 mg lidocaine and 20 mg prilocaine) which are available in the local market were made with proposed method. Evaluation was

performed using the calibration curve method since no significance difference between the slopes of the calibration curves for standards and commercial formulation solutions was observed.

The solutions of cream and injections were prepared according to section **"***Preparations of Commercially Cream and Injection Forms"* and then analyzed using GC-FID method (Figure 5 and 6) according to the general procedures using the pre constructed calibration curve. In the analysis performed with six repetitions, the recovery value and RSD value determined for all three formulations was determined as 99.5% and 2.09% on average, respectively.

4. Discussion

Today, HPLC and GC methods are important and widely used as analytical techniques of quantitative and qualitative analysis. GC method from these methods can be considered to be a very appropriate method for analysis of lidocaine from local anaesthetic substances that this is very volatile substances. Therefore we were selected GC method with a flame ionization detector (FID) for analysis of lidocaine HCl in commercially cream and injection forms.

In this study, a highly selective and simple GC-FID method without any derivatization is reported for the analysis of lidocaine HCl in commercial cream and injection forms. Local anaesthetics are widely used for various local or regional treatments. In these cases, forensic intervention may be necessary. In forensic examinations, fast and simple analytical methods are needed . The first aim of such studies is to introduce the methods developed for the analysis of an analyte in different environments into routine use in quality control laboratories. Before the methods can be used routinely, a comprehensive examination is required. Lidocaine HCl is one of local anaesthetic substances. In such cases, analysis of lidocaine HCl in quality control laboratories is important.

Also the developed and validated method was statistically compared to two reference methods in the literature. In the reference method, two spectrophotometric methods had been developed for determination of lignocaine in pure raw material and in formulated dosage form by Rizk et al. 1997. One of these methods (method I) is based on the extraction of the ion-associate formed with reineckate salt by nitrobenzene or chloroform/acetone and other method (method II) is oxidation of lignocaine by Ce(IV). The concentration ranges of method I and II had found as 0.5-2.5 µg/mL and 1.1-3.4 µg/mL, respectively. The proposed method was linear over the concentration range 0.1–5.0 µg/mL. In this respect, it has a wide concentration range of our method. The average recovery value ranged from 97.1 to 102.0% and the RSD values obtained within- and between-day assay of quality control samples ranged from 0.87 to 2.7% for the proposed method, which indicated high accuracy and precision. In the both reference method, the average recovery value had found between 99.28 and 101.87% and also the RSD values ranged from 0.22 to 1.1%.

In conclusion, this analytical method for the determination of lidocaine HCl in cream and injection forms was validated according to ICH[22]. The validation parameters studied are sensitivity, stability, specificity, linearity, accuracy and precision. The developed GC-FID method has high recovery, excellent reproducibility and high selectivity. The values obtained from the developed method were compared with the two methods available in the literature. The linearity range of the current method is quite wide compared to other methods. Paired t test was also used to compare the values obtained from cream and two injections forms. According to the data obtained from the pair t test, no significant difference was observed between the formulations. For these reasons we can suggest that this method will be useful for studies concerning the routine quality control analysis of lidocaine HCl in pharmaceutical industry.

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