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## New developing and Validation method to Determine Lidocaine Hydrochloride in Raw Material and Injection Forms by Using RP-HPLC

Prof. Dr. Saad Antakli <sup>1,\*</sup>

### ABSTRACT

This work was aimed to develop a rapid, simple, sensitive and precise high performance liquid chromatography (HPLC) method for the estimation of Lidocaine hydrochloride (Lido) in both injection dosage forms and raw materials.

The chromatographic separation was achieved with acetonitrile:phosphoric acid (pH = 3) in ratio of 30:70 (v/v) as mobile phase on a Shim-pack clc-C<sub>8</sub> column (25 cm x 4.6 mm i.d., 5  $\mu$ m) with UV detection at 212 nm, pump flow rate 1.0 mL/min and sample injection volume 20  $\mu$ L.

The method was validated with respect to linearity, precision, accuracy and specificity. The calibration curve showed good linearity over the concentration range of (2.5 - 250)  $\mu$ g/mL in presence of Caffeine (Caff) as internal standard with limit of detection and limit of quantification were to be 0.31  $\mu$ g/mL and 0.94  $\mu$ g/mL, respectively.

The developed method was very rapid with a run time of 4.5 min and found to be successively applied for the quality control of (Lido) in pharmaceutical formulations.

**KEYWORDS:** HPLC, Lidocaine hydrochloride, Chromatographic analysis, Validation, Internal standard.

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1 Faculty of Science, University of Aleppo, Aleppo, Syria.

\* Corresponding author. E-mail address: [saad.intakli@wpu.edu.sy](mailto:saad.intakli@wpu.edu.sy)

## 1. Introduction

### **Lidocaine hydrochloride:**

Acetamide, 2-(Diethylamino)-N-(2,6-dimethylphenyl)-, hydrochloride, monohydrate or Lidocaine hydrochloride (Lido) is a white or almost white, crystalline powder. Very soluble in water, freely soluble in ethanol (96 percent) [1]. Lidocaine-HCl is a local anesthetic material with strong and fast acting. It has a high permeability of the tissue and is suitable for external use to relieve the pain, itching and inflammation [2].

Several methods have been applied in the literature for the determination of (Lido) in dosage forms and in biological fluids. Techniques such as spectrophotometry [3,4,5,6], high performance liquid chromatography (HPLC) [1,7,8], liquid chromatography [9,10], electrochemical analysis [11] and electrophoresis analysis [12] were used to determine (Lido) in different pharmaceutical forms.

## 2. Materials and Method

### **Apparatus:**

HPLC analysis was performed on a YL 9100 HPLC system equipped with a binary pump YL9111, vacuum degasser series YL9101, YL9130 column compartment and YL9120, UV/Vis. Detector (Korea). Chromatographic separations were obtained by using Shim-pack clc-C<sub>8</sub> column (25 cm × 4.6 mm i.d., 5 µm) (Shimadzu, Japan). Ultrasonic bath (Daihan, USA), analytical balance TE64 Sartorius (Germany) sensitivity 0.1 mg. Germany digital pipettes (Isolab).

### **Chemicals:**

Ssolvents and materials were used as analytical grade: water, acetonitrile (Isolab, Germany), all were HPLC grade and Phosphoric acid (Isolab, Germany). Caffeine (Caff) pure drug substance, its purity was 99.84 % (Abbott health care, India) and (Lido) purity 99.21%, (Abbott health care, India).

### **Stock standard preparations:**

2.5 mg/mL of (Lido) was prepared as stock standard solution by dissolving an appropriate weight of this material in bi-distillation water, by taking the purity of the material into consideration.

2.5 mg/mL of (Caff) was prepared as stock standard solution by dissolving an appropriate amount of this material in bi- distillation water, by taking the purity of the material into consideration.

### **Calibration Curve:**

To construct the calibration curve, seven standard solutions (2.5, 25, 50, 100, 150, 200, 250) µg/mL for (Lido) were prepared and the area of peaks was measured of each solution five times.

### **Samples preparation:**

Two Syrian products were studied:

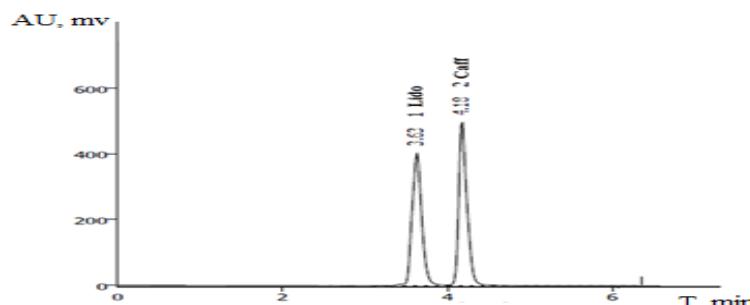
- Containing five "Obarcaine" vials (each vial contains 1000 mg Lido/50 mL) was transferred to a beaker then a 2.5 mL of it was transferred to a 10 mL volumetric

flask. The volume was completed to 10 mL using HPLC-grade water. Then 0.1 mL was taken to 10 mL volumetric flask (which equivalent to 50  $\mu\text{g/mL}$  theoretically) which contains 25  $\mu\text{g/mL}$  Caff and adjusted to volume with mobile phase and filtered through a 0.45  $\mu\text{m}$  nylon syringe filter and degassed by ultrasonication, then 20  $\mu\text{L}$  of the sample was injected into the chromatograph system.

- Containing five "Lidosol" ampule (each ampule contains 50 mg Lido/5 mL) was transferred to a beaker then a 5 mL of it was transferred to a 10 mL volumetric flask, the volume was completed to 10 mL using HPLC-grade water. Then 0.1 mL was taken to 10 mL volumetric flask (which equivalent to 50  $\mu\text{g/mL}$  theoretically) which is contains 25  $\mu\text{g/mL}$  Caff and adjusted to volume with mobile phase and filtered through a 0.45  $\mu\text{m}$  nylon syringe filter and degassed by ultrasonication, then 20  $\mu\text{L}$  of the sample was injected into the chromatograph system.

### 3. Results and Discussion

One of the chromatograms of seven different standard concentrations mixtures (Lido) and (Caff) as internal standard is presented in fig. 1. Each concentration was injected five times under optimized method conditions. The chromatogram showed that (Lido) was well separated from (Caff) with a good resolution and the time of analysis was achieved in less than 5 min.



**FIG. 1: CHROMATOGRAM OF STANDARD SOLUTION: 1- (LIDO) 50  $\mu\text{G/ML}$ , 2- (CAFF) 25  $\mu\text{G/ML}$ . CHROMATOGRAPHIC CONDITIONS: C<sub>8</sub> COLUMN; MOBILE PHASE: ACETONITRILE:PHOSPHORIC ACID (PH = 3) 30:70 (V/V) FLOW RATE 1.0 ML/MIN, TEMPERATURE 30 °C AND DETECTION AT 212 NM.**

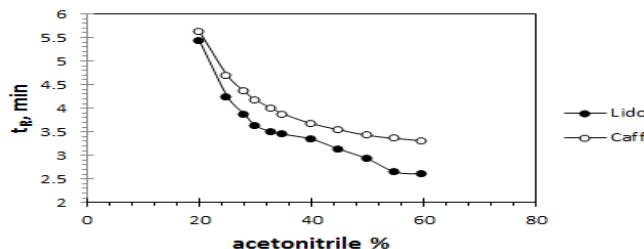
#### Optimization of the HPLC conditions:

##### Selection of $\lambda_{\text{max}}$ wavelength:

The wavelength at which the maximum absorption (212 nm) occurs is selected for further analysis. A definite concentration of Lido solution was scanned in UV range of 200 – 300 nm. Water was used as a blank. The absorbance of solutions in HPLC method was measured at 212 nm and calibration curve of Lido was built up accordingly.

### Mobile phase effect:

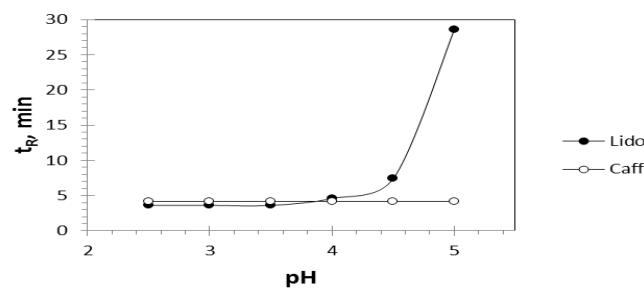
The effect of composition of the mobile phase (using C<sub>8</sub> column 25 cm × 4.6 mm i.d., 5 µm) on the retention time of (Lido) and the internal standard (Caff) was investigated in fig. 2. The percentage of Acetonitrile (30 – 60) % in the mobile phase had a significant effect on the retention behavior of the studied compounds. An increase in the percentage of acetonitrile has decreased the retention of compounds; Lido and Caff. A satisfactory separation of Lido and Caff with satisfactory resolution was obtained with a mobile phase containing 30% acetonitrile.



**FIG. 2: MOBILE PHASE EFFECT ON THE RETENTION TIME OF: (LIDO) 50 µG/ML AND (CAFF) 25 µG/ML. CHROMATOGRAPHIC CONDITIONS: C<sub>8</sub> COLUMN; MOBILE PHASE: VARIABLE RATIO OF ACETONITRILE:PHOSPHORIC ACID (PH = 3), FLOW RATE 1.0 ML/MIN, TEMPERATURE 30 °C AND DETECTION AT 212 NM.**

### pH effect of mobile phase:

Effect of pH on the chromatographic elution of both compounds (Lido) and (Caff) was investigated by changing the pH values of the aqueous component of the mobile phase from (2.5 to 5) by H<sub>3</sub>PO<sub>4</sub>, fig. 3. It was observed that the pH of mobile phase values ranged from (2.5 to 4) permitted the elution of drugs in the following order (Lido) and (Caff), but when the pH values range became from (4 to 5) the order was (Caff) and (Lido). pH = 3 was chosen for the optimum separation of these compounds.

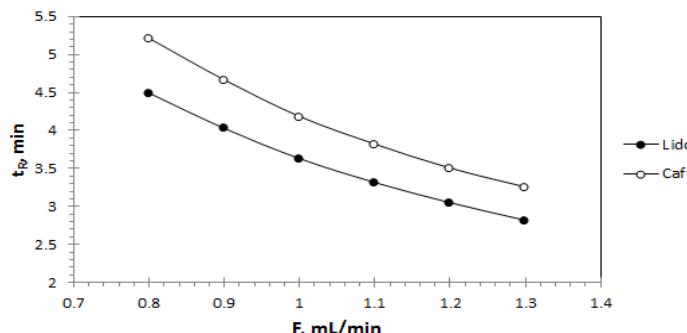


**FIG. 3: pH EFFECT OF MOBILE PHASE ON THE RETENTION TIME (T<sub>R</sub>) OF (LIDO) 50 µG/ML AND (CAFF) 25 µG/ML. CHROMATOGRAPHIC CONDITIONS: C<sub>8</sub> COLUMN; MOBILE PHASE: ACETONITRILE:PHOSPHORIC ACID (VARIABLE VALUE OF PH) 30:70 (V/V); FLOW RATE 1.0 ML/MIN, TEMPERATURE 30 °C AND DETECTION AT 212 NM.**

### Flow rate effect:

The optimum flow rate was identified by changing flow rate from 0.8 to 1.3 mL/min. It was concluded that each two peaks were completely separated, with a

fine and symmetrical aspect at flow rate (1 mL/min) which also corresponds to the deviation point as seen in fig. 4.



**FIG. 4: FLOW RATE EFFECT OF MOBILE PHASE ON THE RETENTION TIME OF (LIDO) 50  $\mu$ GML AND (CAFF) 25  $\mu$ GML. CHROMATOGRAPHIC CONDITIONS: C<sub>8</sub> COLUMN; MOBILE PHASE: ACETONITRILE:PHOSPHORIC ACID (PH = 3) 30:70 (V/V); FLOW RATE (VARIABLE VALUE), TEMPERATURE 30 °C AND DETECTION AT 212 NM.**

### Optimum chromatographic conditions:

Table 1 presents the chromatographic method conditions, which were applied for simultaneous determination of (Lido) in presence an internal standard (Caff).

**TABLE 1: (LIDO) DETERMINATION CHROMATOGRAPHIC CONDITIONS, IN PRESENCE (CAFF) AS INTERNAL STANDARD.**

Specification	HPLC method
Column	Shim-pack clc-C <sub>8</sub> (25 cm × 4.6 mm, 5 $\mu$ m)
Mobile phase	Phosphoric acid (pH = 3):acetonitrile 70:30 (v/v),
Internal standard	Caff
Flow rate	1 mL/min
Temperature	30 °C
Detector	UV 212 nm
Injection volume	20 $\mu$ L

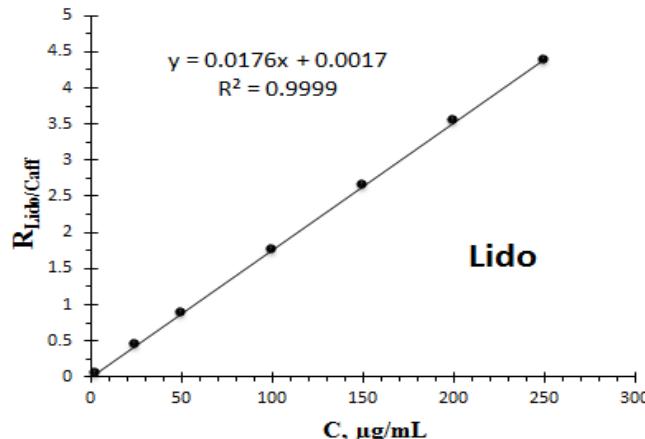
### METHOD VALIDATION:

The method was validated according to ICH guidelines [13].

The following validation characteristics were addressed:

#### Linearity:

Standard solutions containing (Lido) were prepared in a mixture of acetonitrile:phosphoric acid (pH = 3) in ratio of 30:70 (v/v) from a fresh stock solution (2.5 mg/mL) to construct the calibration curve. The least square regression analysis was carried out for the obtained data. Calibration curve consisted of seven different concentrations in the range (2.5 – 250)  $\mu$ g/mL for (Lido) with correlation coefficient of the regression equation greater than 0.999. Each concentration level was performed five times. The equation of the calibration curve attained was  $y = 0.0176x + 0.0017$ . It was obtained by plotting the peak area ratio of (Lido) to the internal standard (Caff) (y) as a function of analyte concentration (x) in  $\mu$ g/mL as seen in fig. 5.



**FIG. 5: LINEAR RELATIONSHIP FOR (LIDO):**  $C_1: 2.5 \mu\text{G}/\text{ML}$ ,  $C_2: 25 \mu\text{G}/\text{ML}$ ,  $C_3: 50 \mu\text{G}/\text{ML}$ ,  $C_4: 100 \mu\text{G}/\text{ML}$ ,  $C_5: 150 \mu\text{G}/\text{ML}$ ,  $C_6: 200 \mu\text{G}/\text{ML}$ ,  $C_7: 250 \mu\text{G}/\text{ML}$ .  $N = 5$  FOR EACH CONCENTRATION.

#### Limit of detection (LOD) and Limit of quantification (LOQ):

The (LOD) and (LOQ) were obtained from the calibration curves. The (LOD) and (LOQ) were calculated based on the standard deviation of the intercept (SD) and the slope (S) of the calibration curves using the formulae  $3.3 \text{ SD}/\text{S}$  and  $10 \text{ SD}/\text{S}$  respectively [15]. The LOD and LOQ of (Lido) were  $0.31$  and  $0.94 \mu\text{g}/\text{mL}$ , respectively.

#### Precision:

The precision of the method was established by determining the system precision including intra-day and inter-day precision, and repeatability and was expressed as %RSD. For intra-day and inter-day precision evaluations, three different concentrations ( $25, 40$  and  $50 \mu\text{g}/\text{mL}$  of Lido) were prepared and injected five times in the same day and three different days, respectively and their %RSD values were calculated. The results of precision experiments are presented in table 2. The %RSDs for intra-day and inter-day precision, and repeatability were less than 3%.

**TABLE 2: INTRA-DAY AND INTER-DAY PRECISION OF (LIDO).**

Drug	Theoretical concentration ( $\mu\text{g}/\text{mL}$ )	Intra-day					
		*Observed concentration ( $\mu\text{g}/\text{mL}$ )			Precision RSD (%)		
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
(Lido)	25	24.48	25.18	25.38	1.85	1.90	2.20
	40	39.29	40.82	40.55	1.50	1.52	1.75
	50	49.12	50.51	49.06	1.25	1.25	1.45
Inter-day							

Drug	Theoretical concentration (µg/mL)	*Observed concentration (µg/mL)			Precision RSD (%)		
		D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
(Lido)	25	24.35	24.70	25.30	1.72	1.97	2.28
	40	40.26	39.51	39.03	1.55	1.56	1.82
	50	50.38	50.34	49.33	1.27	1.30	1.51

T: three different times in the same day.

D: three different days.

Precision (RSD %) = (standard deviation/mean concentration) × 100.

\* Five separate determinations were performed and the mean was calculated.

#### recovery:

The recovery of the HPLC method was determined by applying the drug sample added with the known amount of (Lido) standard solution corresponding to 80%, 100% and 120%. All the mixtures were analyzed separately under optimized chromatographic conditions. The analysis was performed five times and % recovery was calculated. Good recovery of (Lido) was observed as shown in table 3.

TABLE 3: RECOVERIES OF (LIDO) IN "OBARCAINE" VIAL AND "LIDOSOL" AMPULE.

Formulation	Amount of sample taken* (µg/mL)	Amount of standard added (µg/mL)	Total amount found* (µg/mL)	Rec %	RSD %
Obarcaine (vial)	49.72	40.00	90.32	101.50	1.44
		50.00	99.48	99.52	1.29
		60.00	110.58	101.43	1.31
Lidosol (ampule)	50.41	40.00	90.29	99.70	1.51
		50.00	99.97	99.12	1.54
		60.00	110.03	99.37	1.37

\*n = 5

#### Robustness:

Robustness of HPLC method was determined by deliberately varying certain parameters like pH of mobile phase, percentage of organic solvent in mobile phase and column temperature. For all changes in conditions the samples were analysed in triplicate. When the effect was altering one set of conditions was tested, the other conditions were held constant at optimum values. The robustness was investigated by achieving deliberate changes in pH of mobile phase by  $\pm 0.1$ , change in acetonitrile composition of mobile phase by  $\pm 2$  and column temperature by  $\pm 5$  °C. Robustness of the method was carried out in triplicate at a concentration of 50 µg/mL. The system suitability parameters remained unaffected over deliberate small changes in the chromatographic system, illustrating that the method was robust over an acceptable working range of its HPLC operational parameters, table 4.

TABLE 4: ROBUSTNESS TEST CONDITIONS USED IN THIS STUDY.

Parameter	Level	Lido	
		% Mean assav*	% RSD of results*
pH	3 - 0.1	102.34	1.62
	3 + 0.1	100.82	2.07
CH <sub>3</sub> CN %	30 - 2	101.97	1.44
	30 + 2	97.65	1.11
Column temperature °C	30 - 5	102.66	1.84
	30 + 5	99.51	1.42

\*n = 3

### System suitability:

System suitability parameters [1] such as number of theoretical plates N, tailing factor T<sub>f</sub>, capacity factor (k'), resolution R<sub>s</sub> and relative standard deviation RSD% of the peak area were assessed by injecting five replicate injections of freshly prepared standard solution (containing 50 µg/mL of (Lido) in the presence of 25 µg/mL of internal standard (Caff)). The results of system suitability parameters were within the accepted range. (Lido) was repeatedly retained and well separated at 4.5 min with %RSD less than 5% at confidence limit 95% indicating good repeatability of replicate injections on the integral HPLC system used. In addition, it was established that k' = 0.86. In general, k' affects peak resolution. The resolution usually worsens or improves depending on whether k' decreases or increases. Better k' values are achieved by changing either the mobile phase composition or the stationary phase [14]. In contrast, changes in the column conditions such as flow rate, column length and particle size do not affect k'. Ideally, a precise and rugged method should have R<sub>s</sub> greater than 1.5[14]. The present method exhibited R<sub>s</sub> of 1.6 thus indicating an optimum k' value. The acceptance limit of T<sub>f</sub> < 1.5 was achieved with this method and T<sub>f</sub> never exceeded 1.26; indicating excellent peak symmetry. Finally, N always exceeded 2000 in all chromatographic runs, which ensured good column efficacy throughout the developed separation process.

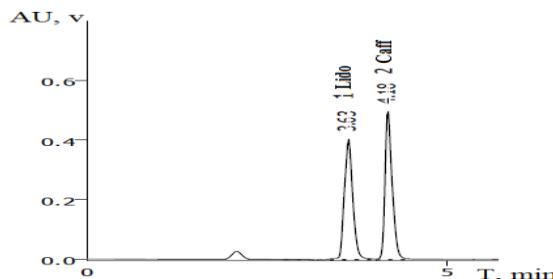
### Specificity:

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The specificity of the chromatographic method was determined to ensure separation of Lido and Caff as illustrated in fig. 6 where complete separation of Lido was noticed. The figure shows that Lido is clearly separated and the peak of analyte was well defined and excipients in the formulation did not interfere with the analyte, where the recovery was bordering to 100%.

### Application of the proposed method for estimation (Lido) in Syrian pharmaceutical formulations:

The applicability of HPLC-UV method was investigated for pharmaceutical preparations fig.6. In all the preparations. The amount of (Lido) was obtained by direct measurement using the linear relationship curve. The total analysis time was

less than 5 min with good resolution, good peak shapes and minimal tailing. Two injection formulations containing (Lido) were analyzed. Fig. 6 illustrates typical chromatogram of (Lido) in "Obarcaine" vial. The dosages of (Lido) were conformed to USP legislation<sup>1</sup>. Table 5 presents the determination results of (Lido) in the studied products for five different batches for each.



**FIG. 6: CHROMATOGRAM OF "OBCARCAINE" VIAL: 1-(LIDO), 2- (CAFF) AS INTERNAL STANDARD 25  $\mu$ G/ML. CHROMATOGRAPHIC CONDITIONS: C<sub>8</sub> COLUMN; MOBILE PHASE: ACETONITRILE:PHOSPHORIC ACID (PH = 3) 30:70 (V/V); FLOW RATE 1.0 ML/MIN, TEMPERATURE 30 °C AND DETECTION AT 212 NM.**

**TABLE 5: RECOVERIES AND AMOUNT OF (LIDO) IN SYRIAN INJECTION FORMULATIONS.**

<u>Obarcaine (vial) Oubari, (Lido) 1000 mg/50 mL</u>					
No. of batches	Result dose $\bar{x}^*$ mg/vial	SD mg/vial	RSD %	Percentage %	LC = $\bar{x} \pm t.SD/(n)^{1/2}$ mg/vial
1	1007.23	12.34	1.23	100.72	1007.23 $\pm$ 15.220
2	1005.11	11.55	1.15	100.51	1005.11 $\pm$ 14.220
3	991.85	15.86	1.6	99.19	991.85 $\pm$ 19.690
4	1007.72	12.64	1.25	100.77	1007.72 $\pm$ 15.600
5	987.64	20.56	2.08	98.76	987.64 $\pm$ 25.525
Range mg/vial	(987.64 – 1007.72) mg/vial				
<u>Lidosol (ampule) Rama Pharma, (Lido) 50 mg/5 mL</u>					
No. of batches	Result dose $\bar{x}^*$ mg/ampule	SD mg/ampule	RSD %	Percentage %	LC = $\bar{x} \pm t.SD/(n)^{1/2}$ mg/ampule
1	50.87	0.89	1.75	101.74	50.87 $\pm$ 1.105
2	49.07	0.95	1.94	98.14	49.07 $\pm$ 1.179
3	51.22	1.09	2.13	102.44	51.22 $\pm$ 1.353
4	49.71	1.34	2.7	99.42	49.71 $\pm$ 1.664
5	49.19	0.92	1.87	98.38	49.19 $\pm$ 1.142
Range mg/ampule	(49.07 – 51.22) mg/ampule				

\*n = 5

Theoretical value for (t) at four degree of freedom and 95% confidence limit are  $t = 2.776$ .

#### 4. Conclusion

A direct, specific, accurate and precise HPLC method for determination of Lidocaine hydrochloride in raw material and pharmaceutical formulations was successfully developed as per the ICH guidelines. The good analytical performance with regards to validation parameters was achieved. The developed method exhibits high sensitivity in which the LOD and LOQ were  $0.31 \mu\text{g/mL}$  and  $0.94 \mu\text{g/mL}$ , respectively. Good recoveries of (Lido) were obtained in the range of (99.12 – 101.50) %. Table 3 in different samples confirms the accuracy of developed method. The presence of active substance in actual quantities in pharmaceutical products was conformed to the Lidocaine hydrochloride formulations. Hence, this method can be conveniently used for routine quality control analysis of Lido in its pharmaceutical formulations by using C8 column instead of  $\text{L}_1 = \text{C}18$  column as presented in USP [1].

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