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Estimation of Lidocaine-HCl in Pharmaceutical drugs by HPLC-UV System

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ABSTRACT

An accurate, precise and sensitive HPLC system is used to determination of Lidocaine-HCl in vial dosage form as parenteral solution (intra-muscular), to compare with two Lidocaine-HCl form; commercial formulations and standard Lidocaine-HCl high purity as a test formulation. Lidocaine-HCl concentrations were analyzed by a HPLC-UV System (λ = 254 nm) at 25 ^{o}C. The separation was achieved using the Ion Pac Ercus C18 RP-Column; 5µm, (250×4.5 mm id). The mobile phase consisted of acetonitrile/ water (20/80) with 5% acetic acid at pH 3.4. The method was found to be linearity in the range (0.1 to 0.5) µg/ml (n = 5) with $R^2 \geq 0.9987$, also, the recoveries were range within 96.0-100%. The detection limit of quantification (LLOQ) was 0.01645 µg/ml and lower limit of detection (LLOD) 0.00521 µg/ml. showing average intra assay and inter-assay coefficients of ± RSD % about 0.526 %. The standard Lidocaine-HCl drug eluted at a flow rate of 1.0 ml/min. The results of recoveries, ± RSD, and statistical parameters obtained in this study, clearly indicated that the HPLC–UV system offer a successfully and excellent method for the separation and determination of Lidocaine-HCl in the commercial drugs.

Keywords: Lidocaine-HCl as parenteral solution (intra-muscular) and Standardized, HPLC- UV System.

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INTRODUCTION

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 relive the pain, itching and inflammation caused by sunburn and hemorrhoids, skin problems and other light [1, 2]. This type of drugs was used since 1949s to relieve pain and discomfort associated with medical examinations are the input devices through the throat or urethra [3]. Lidocaine-HCl belongs to the family of narcotic drugs and can be used as a topical anesthetic by the stability of the nervous membrane which produces a feeling pain and remove it extinguished issued by the end of the nerve signals in the skin [4, 5]. It can be used to relieve the discomfort resulting from the virus Herpes which affects the skin as well as the infection in the different types of minor surgery and dental treatment, childbirth and epidural anesthesia at birth and identification of conjunctivitis and is used particularly for the treatment of cardiac arrhythmias after having a heart attack [6,7, 8].

Lidocaine-HCl liquid [2-(diethyl amino)-N-(2,6-dimethylphenyl) acetamide] ($C_{14}H_{22}N_2O$).HCl.H_2O and Molecular weight is 234.34 [9].

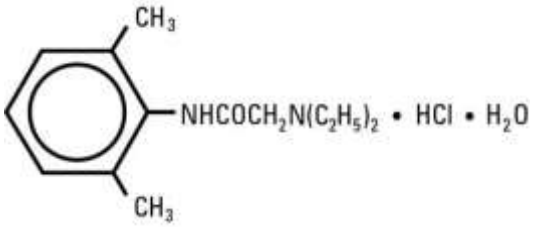

Figure 1: Structure of Lidocaine-HCl

There are a several methods were used for the quantitative determination of Lidocaine-HCl. The most interesting field of the Ion Chromatography IC application is the analysis of local anesthetic for example, to control the quality of the pharmaceutical itself and in clinical analysis to study the effects and the evolution of the pharmaceutical in the human body [10]. The analysis of clinics favor local administration of anesthetics is also important due to the wide ranging application of these drugs [11]. The fabricated plastic vial used for the keeping liquid Lidocain–HCl drug from semi-rigid polymer specially formulated polyolefin [12]. The structural similarities of some impurities with Lidocaine-HCl make the separation of the individual components within Lidocaine-HCl potentially difficult [13, 14]. However, these otherwise hydrophilic compounds can be separated by performance the liquid chromatography, reversed phase- HPLC at a wavelength, 254 nm that assist in accentuating the small hydrophobicity differences [15]. All types of liquid Lidocain–HCl drugs contain chromophores, making UV-Vis measurements effective with high sensitivity [16]. Lidocaine derivatization with hydrochloric acid can be determined by changing the wavelength that depends

on absorbance morality factors before sample derivatization measurements [17,18]. The Lidocaine-HCl are separated on a Ion pac Ercus C18 RP-Column; 5µm, (250×4.5 mm id). The mobile phase consists of a mixture of a water/ acetonitrile (80/ 20 v/v) with 5 % acetic acid in the pH 3.4, Column and quantified by UV-Vis detection. This method, although effective, is an indirect detection method, which requires additional preparation time and reagents for derivatization [19, 20, 21].

Goal of study:

The study of the differences between the Lidocaine-HCl of two commercial types formulations and standard Lidocaine-HCl high purity as a test formulation. The results were assessed by calculating peaks height.

MATERIALS AND METHOD

All solvents and reagents were of analytical grade unless indicated otherwise, and all experiments were performed with deionized water (18.2 Ω-cm) resistivity [22]at 25 ^{o}C.

Equipment:

Chromatography experiments were carried out by a HPLC-UV chromatography consisting of:

LKB Bump 2150 –HPLC, Bromma

Ion Pac Ercus C18 RP-Column; 5µm, (250×4.5 mm id) (P/N 11051194 L) from European was chosen for some drugs separation.

Metrohme Electric injection valve with 100 µL loop fitted in. A PD 303 UV detector single beam (Japan) equipped with an 18 µl flow cell (Helma. UK.) Data logger Lab JackU12 acquisitions (Ocean control/ Australia). Personal computer supplied with modify software programs / cvi programs UV. Printer (EPSON-L210 / Japan). pH meter (Hana- Italy).

Reagents and standards:

Acetonitrile and methanol, HPLC grade, BDH Chem. LTD, Acetic acid, BDH M/ 312/11 LTD 121578 Cas 56-44-2

Lidocaine-HCl hydrate liquid and analar Lidocaine-HCl as standard Sigma-Aldrach German. Water was obtained by following purification in a deionized water system.

From a stock solution containing 10.0 µg/ml Lidocaine-HCl in methanol, a standard curve was prepared at the concentration of 0.5, 1.0, 1.5, 2.0 and 2.5 µg/ml in methanol. For standardization, 25 mL of the standard solutions of Lidocaine hydrochloride in methanol were transferred to glass tubes, the evaporation of methanol was achieved under air stream at room temperature. The concentration range of standard curve was diluted five times in mobile phase and the

corresponding solution was submitted to chromatographic analysis at 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml of lidocaine hydrochloride [23, 24].

Procedure

All of chromatographic measurements were carried out using a home-made HPLC-UV chromatography at 25 ^{o}C and pressure of 90 bar [25], which consisting LKB pump 2150-HPLC pumping the eluent at 1 ml/min. Lidocaine-HCl samples or standard were manually injected with Metrohm electronic injection valve fitted with 100 µl loop in eluent of a mixture of a water/acetonitrile (80: 20 v/v) with acetic acid 5 % at pH 3.4 [26]. Ion Pac Ercus C18 RP-Column; 5µm, (250×4.5 mm id) (P/N 11051194 L) was used as a separation column [27]. APD 303 UV-Vis detector single beam spectrophotometer (Japan) equipped with 18 µl flow cell (Helma UK) was used to measure the absorbance signal at 254 nm of the separated species. A data logger lab jack-Ocean control/ Australia. Personal computer and printer were handling the data of the HPLC-UV system. The peaks height of a symmetrical peaks is corresponding to the Lidocaine-HCl concentration of standards and sample concentrations [28, 29].

Table 1: Method Parameters

Parameters	Conditions
Description Column	Ion Pac Ercus C18 RP-Column; 5µm, (250×4.5 mm id) (P/N 11051194 L)
System Suitability Requirement	USP Tailing Factor @ 5 %Peak Height 1.31 Plates 6417.77
Isocratic Mobil phase	Acetonitrile/ water (20/80) with 5% acetic acid at pH 3.4
Test sample	lidocaine-HCl diluted in the mobile phase
Detection System	UV detection
Maximum Wavelength	254 nm
Flow Rate	1.0 mL / min
Temperature	25 °C
Pressure Background	90 Bar
Retention Time	19 min
Run Time	26 min
Injection Volume	100 µL

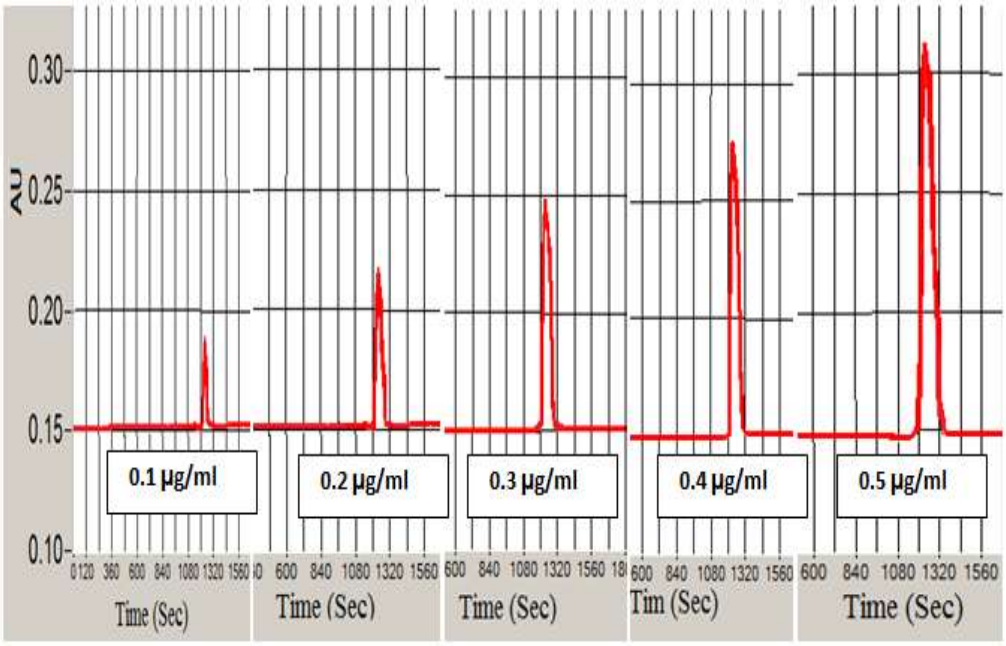

Figure 2: Chromatograms Calibration curve of Lidocaine-HCl

RESULTS AND DISCUSSION

Effect of Column type, eluent concentration and Retention Time on Lidocaine-HCl separation

Ion Pac Ercus C18 RP-Column; 5µm, (250 × 4.5 mm id) column was recommended as a suitable and efficient separation column for Lidocaine-HCl [30]. which can be detect by using UV-Vis detector at λ_{max} 254 nm with mixture of eluent consist acetonitrile/ water (20/80) with 5% acetic acid in the pH 3.4, which can be freshly prepared [31].

Figure 2 shows that the column has high efficiency to separate Lidocaine-HCl, the linear gradient is 19 minutes for each injection and one peak appearance in chromatogram. The distinct peak cause of good method sensitivity to determination Lidocaine-HCl. But some ringing peaks refer to very small concentration of CO_2 dissolve in eluent [32].

Effected Column Temperature on the separation: The Metrohm 690 IC system supply with column temperature evaluating in the range 25-45 ^oC in five degree steps. As expected, increasing the column temperature decreased retention time and led to good baseline for the separation chromatogram of the standards and samples [33].

Method performance (linearity, Reproducibility and Detection Limits):

Under the established conditions listed in Table 1, a method of the standard calibration was used to obtain the calibration curve for Lidocaine-HCl, by plotting the concentration versus the peak

height of asymmetrical peaks. It is linear over the range (0.1-0.5) µg/ml Lidocaine-HCl. Table 2 lists the R^2 and slope of the curve, which are 0.9987 and 96.0 respectively.

The reproducibility of the method was estimated by injection of a 0.2, 0.3 and 0.4 µg/ml represented standard Lidocaine-HCl and two commercial Lidocaine-HCl drugs into eluent. Excellent RSD% for retention time (t_R) and peaks height were obtained as shown in Table 2 and 3. Lower limit of detection (LLOD) and quantitation (LLOQ), LLOD=3.3 SD/S and LLOQ=10 SD/S are the concentrations that give the signal to noise ratio of 3:1 or 10:1 respectively. This can be detected and verified by the divided of standard deviation of response (SD) by the slope of calibration curves (S) [34,35] By using the single-sided student's test method (at the 95% confidence limit) for five consecutive injections of 0.4 µg/ml of Lidocaine-HCl sample and standard [36,] the values of LLOD and LLOQ were 0.00521 µg/ml and 0.01645 µg/ml respectively.

Table 2: The reproducibility of peak height and t_R of Lidocaine-HCl

Representative samples and drugs (µg mL^{-1})	**Peaks height (mm)**	*** ±RSD%**	**Retention Time (t_R) minutes**	*** ±RSD%**
0.2	22	±0.526	19	±0.186
0.3	31	±0.531	19	±0.124
0.4	40	±0.530	19	±0.155
0.5µg mL^{-1} for Drugs (1)	50	±0.500	19	±0.130
0.5µg mL^{-1} for Drugs (2)	50	±0.521	19	± 0.192

Table 3: Regression statistics of the proposed method with LLOD, LLOQ, Intercept and Slope

R^2	0.9987
Standard Error	0.663
Standard Error Estimate	0.632
Intercept	2
Slope	96
LLOD µg mL^{-1}	0.00521
LLOQ µg mL^{-1}	0.01645
MDL(standard) µg mL^{-1} (SD×$t_{95\%}$) at n= 5-1	0.00438
MDL(sample) µg mL^{-1} (SD×$t_{95\%}$) at n= 5-1	0.00491

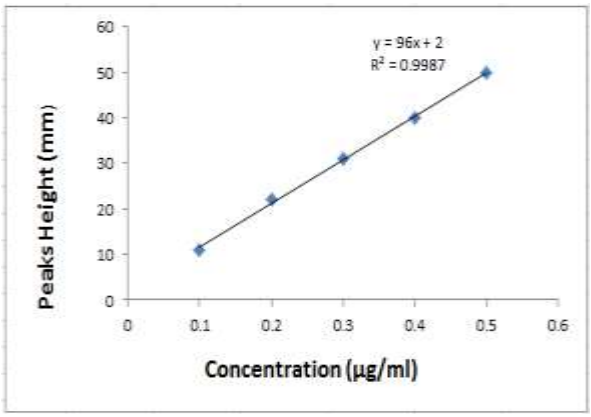

Figure 4: Standard Calibration graph of Lidocaine-HCl Lidocaine-HCl Standard

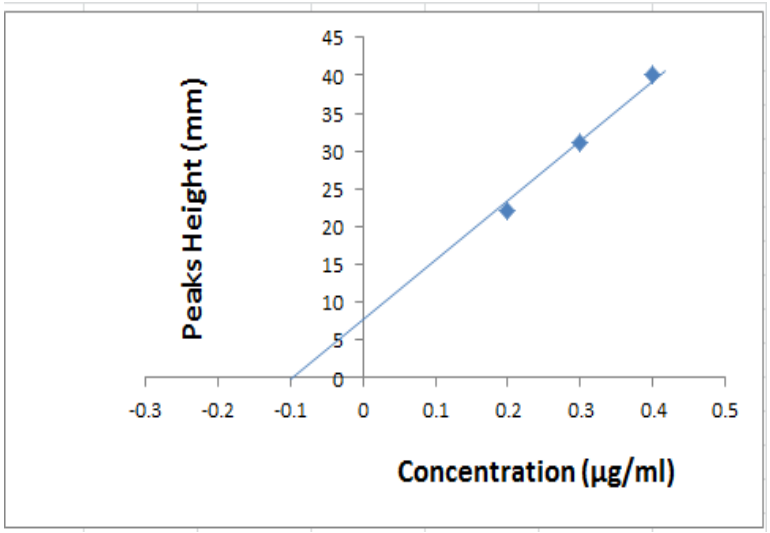

Figure 5: Standard additions for Lidocaine-HCl determination

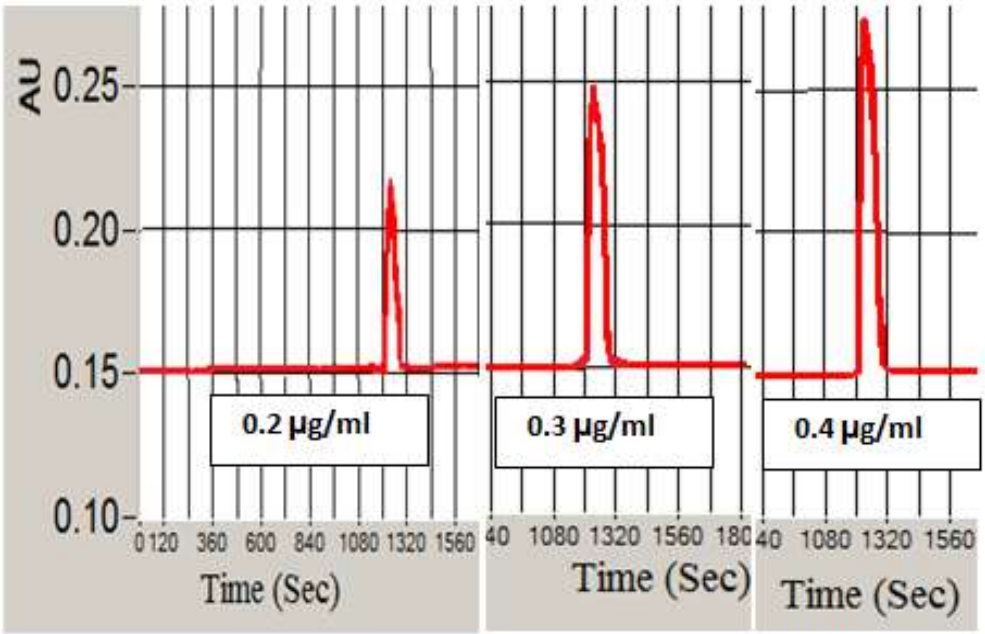

Figure 6: peaks of Standard addition Method

Accuracy:

To evaluate the accuracy of the home-made HPLC-UV System. A recovery experiments were performed on three representative standards and two commercial drug samples. Standard additions method (Fig. 5) was used for all of these determinations in order to avoid all the possible interferences [37, 38]. Table 4 summarized all of these studies. A good agreement between the results was obtained which clearly indicated that Metrohm 690 system can be used for several applications.

Table 4: Lidocane-HCl recoveries obtained by Metrohm 690 HPLC-UV system

Claimed Conc. (µg mL^{-1})	Found conc. (µg mL^{-1})	Recovery ± RSD
0.2	0.20	100 ± 0.526
0.3	0.29	96 ± 0.531
0.4	0.40	100 ± 0.530
0.5µg mL^{-1} for Drugs (1)	0.49	98 ± 0.500
0.5µg mL^{-1} for Drugs (2)	0.50	100 ± 0.521

Precision:

Precision of method, reported as % RSD, was estimated by measuring repeatability (intra-day assay) for five replicate injections for all concentrations of Lidocaine-HCl. The intermediate precision (inter-day variation) were also studied for two days using an intermediate concentration solution of Lidocaine-HCl. The Intra-day average recoveries were in the range (96-100) and Inter-day average recoveries (93-100) which thought to be an acceptable result [39]. The obtained results are summarized in Table 5

Table 5: Intra and inter-day precision and accuracy of standard analysis (n = 5).

	Intra-day		**Inter-day**	
Claimed conc. (µg mL^{-1})	**Found (µg mL^{-1})**	**±Recovery % RSD**	**Found (µg /ml)**	**± Recovery % RSD**
0.1	0.10	10 ± 0.567	0.10	100 ± 0.901
0.2	0.20	100 ± 0.526	0.20	100 ± 0.992
0.3	0.29	96 ± 0.531	0.28	93 ± 0.879
0.4	0.40	100 ± 0.530	0.39	97.5 ± 0.886
0.5	0.49	98 ± 0.510	0.48	96 ± 0.847
0.5 µg/ml Drug (1)	0.49	98 ± 0.500	0.49	98 ± 0.900
0.5 µg/ml Drug (2)	0.50	100 ± 0.521	0.48	96 ± 0.888

CONCLUSION

This work described a Metrohm 690 semi –automated HPLC System equipped with UV detector for Lidocaine-HCl determination in pharmaceutical drugs. This developed method offer simple, inexpensive and needs only a very small volume of the sample and using a UV detector makes this

system very specific due to one peak in the chromatogram. In this application there is no need for high sensitivity since the pharmaceutical drugs of Lidocaine-HCl have a very high concentration.

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