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Extraction-Spectrophotometric Determination of Lidocaine Hydrochloride in Pharmaceuticals

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Abstract

Simple, selective and highly detectable spectrophotometric method has been developed and validated for the determination of Lidocaine hydrochloride in standard and commercial solutions .The method is based on the formation of a soluble colored Lidocaine hydrochloride-eriochrome black T ion-pair complex at pH 1.80. The colored complex was extracted quantitatively into chloroform and measured at 508 nm. Beer's law was obeyed in the concentration range of 0.10-10 mg L^{-1} with molar absorptivity of 2.3623 x10^4 L mol^{-1} cm^{-1}.The limits of detection and quantification were 0.024 mg L^{-1}, and 0.100 mg L^{-1} respectively. Using Job's continuous variations method, the stoichiometry of the ion-pair complex was found to be 1:1. Intra-day accuracy and precision of the method were estimated with a relative error (0.57%), and the relative standard deviation (0.25-1.23). This developed method has been successfully employed to determine concentration of Lidocaine hydrochloride in injection and spray without interference by the common co-formulated substances.The numerical results obtained using both proposed and official methods were in concordance with each other.

Keywords: Lidocaine hydrochloride (LD-HCL), Spectrophotometry, eriochrome black T (EBT), ion-pair

1. Introduction

Lidocaine or Xylocaine hydrochloride (LD-HCl), an amino amide is non-irritating, and cause lesser allergic reactions than esters. It acts as a local anesthetics by interfering with the propagation of peripheral nerve impulses by blocking the sensation of pain. LD-HCl is, also employed in spinal anesthesia and as an antiarrhythmic drug (Lemke, & Williams, 2008)

Lidocaine is a weak base and to be water soluble salt and an injectable form the addition of hydrochloride is required (Martindale, 2007)

LD-HCl, chemical name,2-(diethyl amino)-N-(2,6-dimethylphenyl) acetamide mono hydrochloride, $C_{14}H_{22}N_2O$,. HCl, and has a molecular weight of 270.8 g/mol. LD-HCL hydrate is a white, crystalline powder, odorless and has a slightly bitter taste. LD-HCL is highly soluble in water or in ethanol, soluble in chloroform, and practically insoluble in ether (Lemke, & Williams, 2008).

Its aqueous solution is stable because of the stability of amide functional group. It has the following structural formula as illustrated in Fig 1.1.

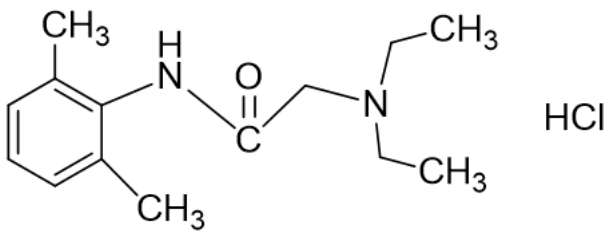


Figure 1.1. The chemical structure of Lidocaine hydrochloride

Structurally, lidocaine can be viewed as an open-chain analog of isogramine and thus is a bioisosteric analog of isogramine which is 3-(dimethyl aminomethyl)-indole. Advantages of lidocaine derivatives over procaine derivatives are that they are more stable to hydrolysis and can be sterilized by autoclaving. It is more potent, has lower side effects such as less local irritation in comparison to procaine derivatives, and can be used as alternatives for patients' sensitive to procaine type anesthetic. A local anesthetic molecule, which is composed of three moieties: 1) aromatic ring; 2) intermediate ester or amide chain; and 3) terminal chain Fig. 1.2 (Yagiela, Dowd, Johnson, Mariotti, & Neidle, 2011).

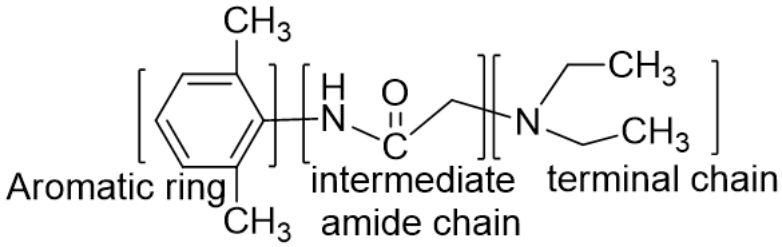


Figure 1.2. The chemical composition of Lidocaine

Depending on the type of aromatic chain, local anesthetic can be divided into two main classes: amide (e.g., articaine, bupivacaine, lidocaine, & ropivacaine) and ester (e.g., benzocaine, cocaine, proparacaine, & tetracaine). Compared to ester anesthetics, amide local anesthetics are more commonly used in clinics because of relatively lower allergic reactions of human associated to amide local anesthetics (Railan & Alster, 2007; Berkman, MacGregor, & Alster, 2012). Apart from that, amide local anesthetics have better lipid solubility, higher potency and longer duration of action than the ester type (Becker, & Reed, 2006). In this study the chromogenic reagent is Eriochrome Black T [Sodium 1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulphonate (NaH_2In)] which is acidic dye of azo (-N=N-) class that contain $–SO_3Na$ group allowing it to be soluble in water (VOGE L´ s, 1989).

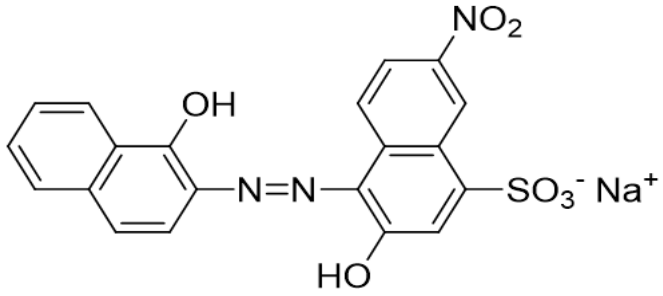


Figure 1.3. The chemical structure of Eriochrome Black T

At low pH (e.g. 1.8) EBT is a completely ionized forming anion (H_2In-) which react with positive protonated drug resulting in an ion-association complex formed by electrostatic attraction between cationic drugs and the anion of the dye. This ion-association complex which is a hydrophobic species is extracted using organic solvents. The concentration of the colored ion- pair in the organic phase is measured spectrophotometrically.

Various analytical techniques for LD-HCL determination in biological and pharmaceutical samples have been published, including the chromatographic methods,gas chromatography(GC) determination of LD-HCl in whole blood (Keenaghan, 1968), (Edhorn, 1971), (Asada, 1979) (Hattori,Yamamoto, & Yamada, 1991) (Watanabe, Namera, Yashiki, Iwasaki, & Kojima, 1998), from serum (Kruczek, 1981)(Prat , & Bruguerolle, 1986) (Baniceru, Croitoru, & Popescu, 2004), from plsma (Björk , Pettersson, & Osterlöf, 1990), in human plasma and urine (Ohshima& Takayasu, 1999), in human urine (Koster, Hofman, & Jong, 1998), to distinguish the drug from its thermal degradation produts **(**Stavchansky, Eghbali & Geary, 1987), in pharmaceutical preparations (Tarli P, Benocci & Neri, 1969), and Forced Degradation of Lidocaine HCl by NMR Spectroscopy and GC-FID Methods(Kadioglu, Atila, Serdar, Gultekin, & Alcan, 2013), thin-layer chromatography (TLC)for the Determination of Hydrocortisone Acetate and Lidocaine in a Pharmaceutical Preparation (Dołowy, Kulpińska-Kucia, & Pyka, 2014), high-performance liquid chromatography (HPLC) and TLC (Živanović, Živanov-Stakić, & Radulović, 1998) (Abdelwahab, Nouruddin, Abdelkawy, Emam, 2016), HPLC, in plasma with solid phase extraction and UV detection (Kang, Jun, & McCall, 1999), in pharmaceuticals (Smith & Nuessle, 1981) (Waraszkiewicz, Milano, & DiRubio, 1981) (Atay & Öztop, 1997) (Parissi-Poulou & Panderi, 1999) (Gebauer, McClure, & Vlahakis, 2001) (Liawruangrath, Liawruangrath, & Pibool, 2001) (Malenovic, Medenica, Ivanovic, Jancic, & Markovic, 2005) (Zivanovic, Zecevic, Markovic, Petrovic, & Ivanovic, 2005) (Salas, Talero, Rabasco, & González, 2008) (Stojanović, Malenović, Marković, Ivanović, & Medenica, 2010)(Shaalan & Belal, 2010) (Belal & Haggag, 2012)

(Drljača et al, 2016), liquid chromatography (LC) for pharmaceuticals assay (Youngvises, Liawruangrath, & Liawruangrath, 2003) (Mohammad 2009) (Pendela, Kahsay, Baekelandt, Schepdael, &Adams, 2011), electrophoretic method (Candioti, Robles, Mantovani & Goicoechea, 2006), electro analytical method (Abrutis, Radulescu, Baiulescu & Coşofreţ, 1985), (Hassan & Ahmed, 1986), (Oliveira, Salazar-Banda, Ferreira, Oliveira, & Avacaa, 2007) (Rahbar, Ramezani, & Babapour, 2015), atomic absorption spectrophotometry- indirect methods (Nerín, Garnica & Cacho, 1991) (El-Ries, Abou Attia, Abdel-Gawad, & Abu El-Wafa, 1994), Spectrophotometric methods for pharmaceuticals detection ,using cis-aconitic anhydride (Feldmann, and Koehler, 1959), bromocresol green (Ismaiel, Yassa, & Gad-El-Rub, 1975), haematoxylin reagent in the presence of boric acid (Saleh & Askal, 1995) , Ion-pair formation Oxidation by Ce (IV) (Rizk, Issa, Shoukry & Atia, 1997), bromocresol green using flow injection analysis (Němcová, Rychlovský, Tománková & Živanovič, 2001), p-benzoquinone (Amin & El-didamony, 2003), partial least - squares multivariate calibration (Aksu, Bozdoğan & Kunt, 2006), with Eosin Methylene Blue (Zong-hui, Li-jun, & Rong, 2007), **i**n binary mixture using novel mathematical methods namely amplitude subtraction and amplitude factor, in addition to ratio subtraction coupled with first derivative(Lotfy, Tawakkol, Fahmy & Shehata, 2013), liquid-liquid extraction-fluorimetry (Wen-ting; Xiao-lan, & Shu-mei, 2014), and liquid chromatographic official method for the determination of LD-HCL in pharmaceutical preparations is cited in the United States Pharmacopeia (NF-27, 2008). The objective of this study is to develop accurate, precise, sensitive, selective, reproducible, easy and low cost spectrophotometric method for the determination of LD HCl in bulk analysis and in pharmaceutical samples.

2. Experimental Part

2.1 Apparatus

A Perkin Elmer Lambda 25 double beam UV-visible spectrophotometer was used to perform spectral runs. All other absorbance measurements were made on JENWAY 6305 UV-visible spectrophotometer with 1 cm path length glass cuvette. The pH was measured with HANNA pH meter.

2.2 Material

All chemicals used in the present study were of analytical grade. LD-HCL was kindly supplied by Awamedica pharmaceutical Industries Company (Hawler, Kurdistan, Iraq). 'The pharmaceutical samples used in this study were lidocaine B (Braun) 2%(A), lidocaine HCl (Shahid Ghazi pharmaceutical Co. Tabriz, Iran) 2% (B), lidocaine injection BP (ROTEXMEDICATRITTAU, Germany)1%(C), and lidocaine HCl BP spray (Philadelphia Pharmaceutical Company, Jordan) 10 % (D).

2.3 Reagents

2.3.1 LD-HCl Stock Solution (100 mg/L)

0.01gm of LD-HCL was dissolved in water and diluted to 100ml in a volumetric flask. A series of standard solutions from stock were prepared by a suitable dilution of stock standard solution with distilled water. The stock solution remained unchanged for 1 month when kept refrigerated.

2.3.2 EBT Solution (0.01%)

A stock solution of EBT was prepared by dissolving the appropriate weight in distilled water and was diluted to 100 ml with distilled water. This solution was prepared fresh in minimal quantities on the day of testing.

2.3.3 Buffer Solution

Buffer solutions of potassium chloride- hydrochloric acid (pH 1.5-pH 4.2), sodium acetate-hydrochloric acid (pH 1.99-pH 4.92), sodium acetate-acetic acid (pH 2.8- pH 6.0) and potassium hydrogen phthalate-hydrochloric acid (pH 2.0-pH 6.0) were prepared fresh using standard methods.

2.4 Preliminary Tests

An appropriate volume 2.5 mL of 100 mg/L standard drug solution was pipetted into a 25 mL volumetric flask. Subsequently 0.5 ml of 0.1% EBT solution and 3 mL buffer solution pH 2.2 were added. The mixture was diluted to the mark with distilled water, mixed well, and transferred to a separating funnel. The funnel was shaked with 2x5 mL organic solvent for 2 mints, then allowed to stand for clear separation of the two phases. The combined chloroform layers were transferred into a cuvette.). The reagent blank was prepared in a similar way without drug solution.

3. Results and Discussion

3.1 Absorption Spectra

Under the conditions of preliminary tests of the proposed method, the absorbance/wave length spectra of the colored ion- pair complex was scanned between the ranges of 400-800 nm against the blank solution. The colored ion-pair complex showed maximum absorbance at 508 nm. In the blank experiment using blank, the color of chloroform layer

remained the same, thus the reagent blank did not show absorption at 508 nm, as seen in Fig. 2.1.

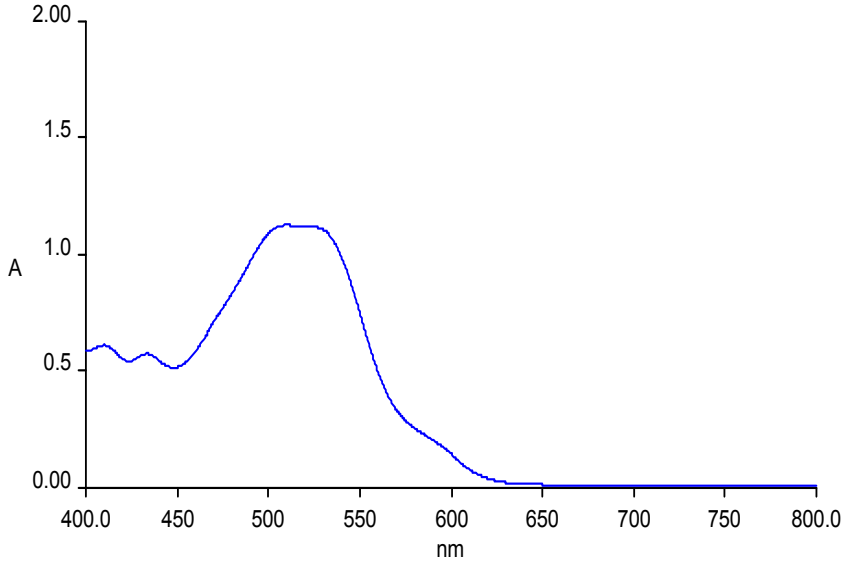
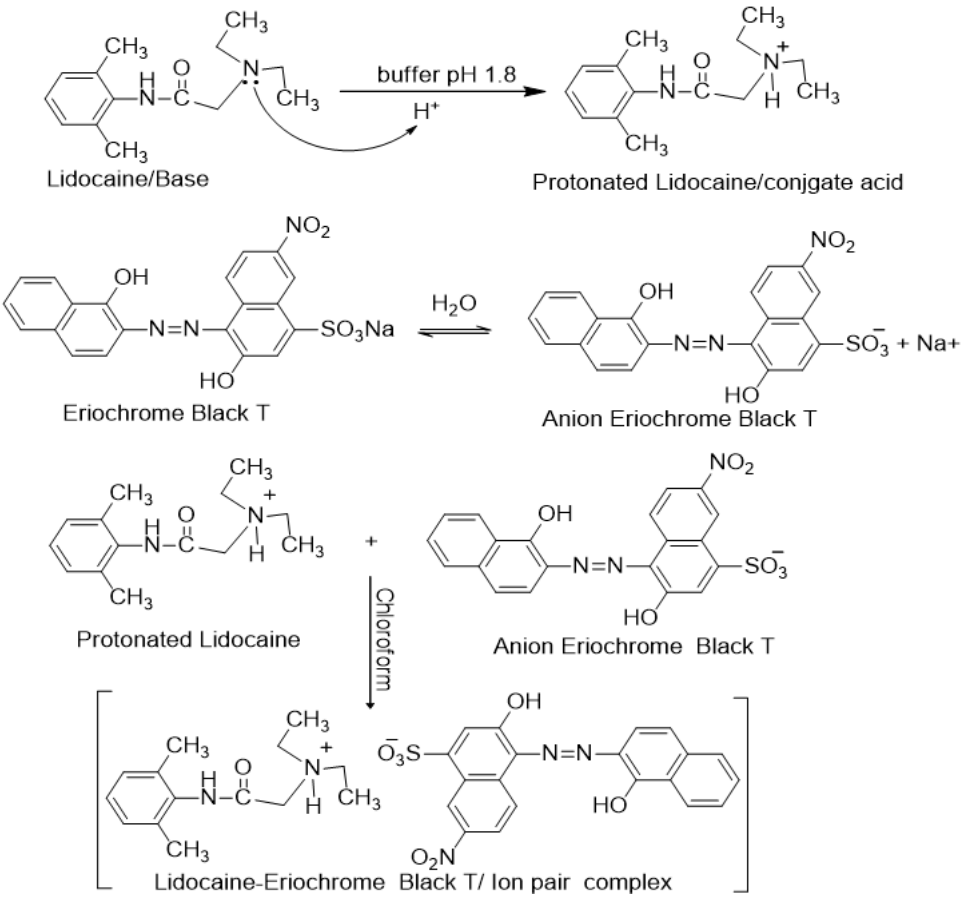


Figure 2.1. Absorption spectra of LD -EBT against reagent blank treated in the preliminary tests.

3.2 Reaction Mechanism

The chemical structure of LD-HCl (colorless solution) molecule consists of three parts the third part is a tertiary amine (R_3N:)/ hydrophilic basic group and proton acceptor. At low pH, nitrogen is able to share a nonbonding pair of electrons to form a bond with the acidic proton. Acid base reaction forming protonated LD (conjugate acid /charged positively) attracts anion of acidic dye present in the solution forming ion- pair neutral species. As the extraction was performed, this ion- pair neutral species is extracted into chloroform phase while the chloroform phase changes color from clear to red. In the blank experiment, the color of the chloroform layer remains unchanged. The mechanism of ion- pair formation and extraction is described in scheme 1.



Scheme 1. Probable mechanism for the formation of 1:1 LD-EBT

3.3 Ion-Pair Composition

The ion-pair complex composition was studied by continuous variations Job's method using variable LD-HCl and dye concentrations. The result in Fig 3.1 indicates 1:1 (LD-HCl: EBT) ion- pair complex formation through the electrostatic attraction between the positive protonated lidocaine and the anion of EBT.

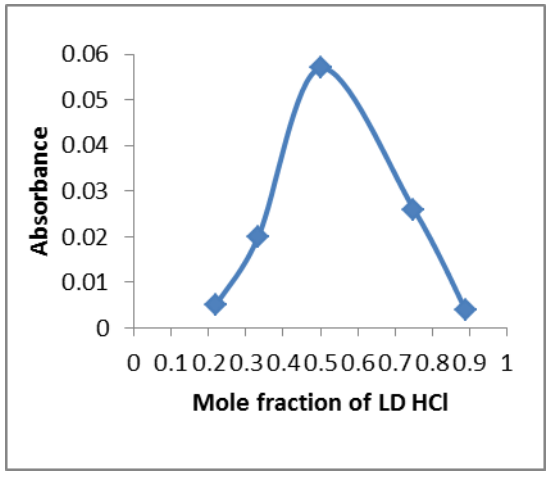


Figure 3.1. Job plot of LD-EBT ion-pair in chloroform at 508 nm

3.4 Optimum Conditions

To achieve the optimum conditions for this method, the following parameters were studied**.**

3.4.1 Effects of pH on the Ion-Pair Formation

The effect of pH was studied by extracting the colored ion pair in the presence of various buffers such as potassium chloride- hydrochloric acid (pH 1.5-pH4.2), sodium acetate-hydrochloric acid (pH 1.99-ph4.92), sodium acetate – acetic acid (pH 2.8-pH6.0) and potassium hydrogen phthalate- hydrochloric acid (pH 2.0-pH6.0). It was observed that the maximum color intensity and highest absorbance values were recorded in potassium chloride- hydrochloric acid (pH 1.8). The results are shown in Table 3.1.

Table 3.1. Effects of pH on the ion-pair formation

pH	Absorbance	pH	Absorbance
1.0	0.744	5.6	0.088
1.8	0.898	6.0	0.043
2.2	0.438	6.4	-
3.6	0.428	6.8	0.029
4.0	0.422	7.3	-
4.5	0.133	7.6	0.021
5.0	0.574	8	0.025

3.4.2 Effect of Volume of Buffer Solution

The maximum color intensity and highest absorbance values were observed in potassium chloride- hydrochloric acid buffer solution at pH 1.8. The effect of different volumes of this buffer solution to obtain maximum sensitivity was investigated. The volume of the buffer solution required to obtain maximum absorption was 2.0 mL, as shown in table (3.2).

Table 3.2. Effect of volume of buffer solution on the ion-pair formation

Volume (mL)	Absorbance
0.5	0.016
1.0	0.053
1.5	0.018
2.0	0.880
2.5	0.740
3.0	0.750
3.5	0.770
4.0	0.790

3.4.3 Solvent Selection for Extraction.

The effect of extraction solvents (2-Propanol, ethyl acetate anhydrous, butanol, chloromethane, ether and carbon tetrachloride) on LD-EBT ion-pair complex as examined. The highest absorbance of the LD-EBT ion-pair was recorded in chloroform .The ion-pair complex failed to be extracted in carbon tetrachloride, and ethyl acetate. The absorbance values of each solvent are recorded in, Table 3.3. Chloroform was selected as the most appropriate solvent for the extraction of the ion-pair.

Table 3.3. Absorbance values of LD-EBT ion-pair in different solvents.

Solvent	Absorbance
2-Propanol	0.029
Butanol	0.682
Ethyl acetate anhydrous	-0.039
Chloromethane	0.532
Ether	0.023
Chloroform	0.886
Carbon tetrachloride	-

3.4.4 Effect of EBT (0.01%) Volume

The effect of EBT volume on the color intensity of LD-EBT ion-pair was investigated in the range of 0.1mL-1.1mL. As

outlined in Table (3.4), the absorbance of LD EBT ion- pair was directly proportional to the volume EBT up to 0.3 mL. Above 0.3 mL, the absorbance remained approximately constant. At higher concentrations, the colored solution became opaque, therefore 0.3 mL of 0.01% EBT was taken as the optimum volume for the formation of the clear colored LD -EBT ion- pair complex.

Table 3.4. Effect of 0.01% EBT volume on the ion-pair formation

Volume of 0.01% EBT (mL)	Absorbance
0.1	0.1435
0.3	0.889
0.5	0.890
0.7	0.870
0.9	0.877
1.1	0.892

3.4.5 Effect of Reaction Time

The effect of the reaction time on the development of LD-EBT ion-pair and its stability was investigated. The ion- pair was stable immediately after mixing LD with EBT at room temperature, remained stable for at least 1.5 hours. Table 3.5 shows the absorbance of the ion- pair complex over different time period.

Table 3.5. Stability of LD-EBT ion-pair

Time (min)	Absorbance	Time (min)	Absorbance
1.00	0.885	25.0	0.800
2.00	0.897	30.0	0.795
3.00	0.898	40.0	0.789
4.00	0.889	50.0	0.790
5.00	0.881	60.0	0.794
10.0	0.871	75.0	0.801
15.0	0.836	90	0.741
20.0	0.832		

3.4.6 Effect of Shaking Time and Temperature

In two different sets of experiments, the effect of shaking time during extraction of the ion- pair complex in chloroform was studied between the time ranges of 0.5mints - 3.5 mints. In the first set of experiments, the maximum absorbance of the ion- pair was obtained between 1.0 min shaking and above, up to 3.5 mints. Between 1.0 mins-3.5 mints, the absorbance remained fairly constant. In the second sets of experiments, the effect of temperature on the absorbance was examined in the range of 20^0C–80 ^{0}C. The results showed that temperature had little influence on the absorbance of the LD-EBT ion-pair complex. Therefore the absorbance was measured at room temperature ($\pm 25^0$C) for subsequent experiments.

3.4.7 Extraction Efficiency

Extraction efficiency to measure how readily LD-EBT ion-pair species was extracted to chloroform layer was calculated. After the first extraction, the aqueous layer was re-extracted with another portion of chloroform. Table 3.6 shows molecular absorbance values for the extracted LD-EBT ion- pair species with chloroform after the first and second extractions of the aqueous phase. The extraction efficiency (E %) was found to be 97.78%.

Table 3.6. Absorbance of LD-EBT ion- pair after the first and second extractions

LD-HCL (mg/L)	%EBT	pH	A_1	A_2	Blank	Extraction efficiency (%E)
10	0.1	1.8	0.921	0.065	0.007	97.78

3.4.8 Final Absorption Spectra

After obtaining optimum conditions for the formation of the LD-EBT ion- pair, the final absorption spectra was checked. It was found that the color system had the same spectra as shown in Fig. (2.1).

3.4.9 Recommended Procedure

A known volume of an aqueous sample of LD-HCl was transferred to a 25mL volumetric flask. Subsequently, 2 mL of buffer pH 1.8 was added to the mixture and shaked well. Then 0.3 mL of 0.01% EBT was added to the mixture and diluted to the mark with distilled water. The diluted mixture was transferred to the separating funnel, and 5mL of chloroform was added to the mixture. The mixture was shaked for 1 min. afterwards; it was allowed to stand for clear separation of two layers of the two phases. The red chloroform layer was transferred into a glass cuvette .Absorbance of the red color ion -pair complex was measured at 508 nm. The reagent blank was prepared in a similar way without LD-HCl.

3.5 Validation of Proposed Method

3.5.1 Linearity

Under the optimum experimental conditions, the absorbance vs. concentration plot was found to be a linear plot as shown in Fig 3.2.

The calibration curve shows that the extracted color system is obeyed Beer's Law within the concentration range of 0.1mg /L - 10 mg/L. The linearity of calibration curves was proved by the high correlation coefficient value (r^2=0.9981). The interception of the calibration curve was very low, thus indicating that there occurred no systematic difference between determined and expected concentration within the investigated range using the proposed method. The apparent molar absorptivity (ε) and Sandell's sensitivity were calculated and found to be 2.3623 x 10^4 L/mol.cm and0.0122 μg/cm^2 respectively. The limits of detection and quantitation were found to be 0.024 mg/L and 0.100 mg/L respectively. These low values confirmed the good sensitivity of the proposed method. The statistical data of the calibration curve for spectrophotometric determination of LD-HCl are shown in Table 3.7

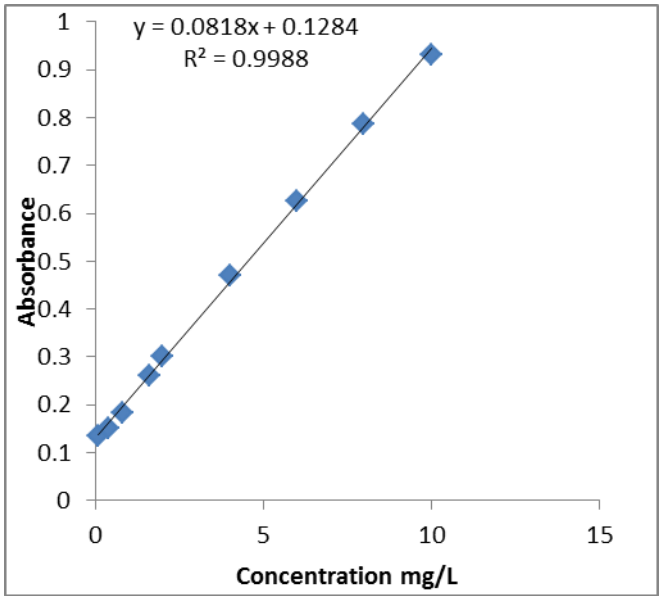


Figure 3.2. Calibration curve for spectrophotometric determination of LD-HCl

Table 3.7 Statistical data of the calibration curve for spectrophotometric determination of LD-HCl

λ nm	508
Linearity (mg.L^{-1})	0.100 - 10.00
Detection Limit (mg.L^{-1}),n=6	0.024
Quantitation limit (mg.L^{-1})	0.100
Correlation coefficient,R2	0.9988
Sandell's sensitivity, μg/cm2	0.0122
Molar absorptivity, L/mol. Cm	2.3623 x10^4

3.5.2 Accuracy and Precision

Analysis was repeated at 3 different concentration levels 2 mg/L, 6mg/L, and 10 mg/L of LD-HCl using the

recommended procedure. The percentage recovery was calculated using the following equation:

Er%=[(founded –true)/true] × 100. The percentage recovery obtained was between 98%-102%. The mean recovery value lies within 98%-100% indicating that the method was accurate. The precision was evaluated under the same operating conditions over a short interval of time. RSD% was found to be between 0.25%-1.23%. The value is below the 2%, indicating that the method was repeatable. The results of accuracy and precision data are shown in table 3.8

Table 3.8. Accuracy data for LD-HCl obtained using the proposed method.

Concentration of LD-HCl (mg/L)	Concentration of LD-HCl Found ±SD (mg/L)	Accuracy		Precision
		%Recovery	%Relative Error	%RSD
2	2.04±0.026	98	2.0	1.23
6	6.09±0.015	98.5	1.5	0.25
10	9.82 ±0.082	101.8	-1.8	0.84
Mean		99.43	0.57	

3.5.3 Selectivity

To assess the role of the inactive ingredients on the assay of LD-HCl sample, a synthetic mixture containing (NaCl, benzyl alcohol, methyl paraben, and propyl paraben) was prepared and a known amount of LD- HCL was added. The drug was extracted and analyzed using the recommended procedures. The absorbance resulting from this solution was nearly the same as those obtained for pure LD-HCl solution was identical concentration. These results indicated that there is no interference from the inactive ingredients.

3.6 Application

The proposed method was applied for the quantification of LD-HCl in four different commercial samples. The results were compared with the official LD-HCl quantification method (The US Pharmacopoeia, 2005), which uses HPLC - UV with TRACER EXTRASIL ODS-2 (5 µm, 4.6-mm and 15-cm) column. The results from the assay are given in Table 3.10. The concentrations of LD-HCl samples determined with proposed methods was close to the concentration analyzed using official method.

Table 3.10. Determination of LD-HCl concentration in pharmaceutical samples

Sample	Label claim (%)	Official method (%)		Proposed method (%)	
	(mg)	Found (mg)	Recovery (%)	Found (mg)	Recovery (%)
A	2	1.86	93.00	1.84	92.00
B	2	1.94	97.00	1.95	97.75
C	1	0.89	89.30	0.90	90.00
D	10	9.21	92.10	9.13	91.30

4. Conclusion

The proposed method is accurate, precise, sensitive, simple, and cheap and can be used in both pure and pharmaceuticals samples determination without interference. Furthermore, the proposed method could be applied for the assay of LD HCl in biological samples due to its low limit of detection. Finally the method provides advantages of stability even with slight variations in experimental conditions such as time, reagent concentration or temperature.

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