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Spectrophotometric Determination of Loperamide Hydrochloride in Pure and Pharmaceutical Dosage Forms Using Oxidative Coupling Reaction

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Abstract

Loperamide hydrochloride (LopH) has been determined by oxidative - coupling reaction using phenothiazine reagent in the presence of potassium dichromate as an oxidant to create a greenish-blue soluble complex measured at 625 nm., linearity range is from0.8 to 40 µg.ml^{-1} of LopH. The molar absorptivity is 0.3799×10^{4} l.mol^{-1}.cm^{-1}, Sandell's sensitivity is 0.1351µg cm$^{-2,}$ limit of detection and limit of quantitation are 0.33918, and 1.1306 µg.ml^{-1} respectively. The method is new ,simple, sensitive and it has been applied successfully for determination of LopH in pharmaceutical preparation of three dosage forms of LopH; Loperamide 2 mg/tablets Medochemie Ltd, Limassol-Cyprus (Europe), Loperamide 2 mg/tablets Safa Co. - Diala (Iraq) and Loperamide 2 mg/capsules Janssen pharmaceuticals (Belgium).

Keywords:loperamide, phenothiazine, pharmaceutical formulation, potassium dichromate

1. Introduction

LopH is a synthetic, piperidine derivative, opioid agonist, an antidiarrheal, and an anti-coronaviral agent [1] it is considered safe enough to be sold over the counter in contrast with other opioid agonists unless uses of high doses [2,3]. The maximum doses of LopH is 12-16 mg/day [4] which is offering a rapid action and good bioavailability [5]. Chemically LopH is 1-piperidinebutanamide, 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide;hydrochloride $C_{29}H_{33}ClN_2O_2$,HCl=513.5)[6]. The chemical structure of LOPH is as shown below:

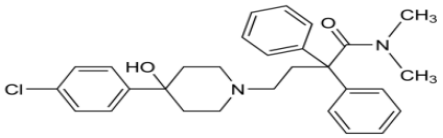

Fig. 1. The chemical structure of LopH [7]

LopH is a white or yellowish–white, amorphous or microcrystalline powder slightly soluble in water; freely soluble in chloroform and methanol, its melting point is 225° with some decomposition [8].

LopH hydrochloride as an electron donor compound was reacted with 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), tetracyanoethylene (TCNE) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) as π-acceptors in acetonitrile. The products of the reaction were isolated and characterized using FT-IR, ^{1}H NMR, elemental analysis and thermogravimetric analysis (TG) [9].

An estimation of LopH hydrochloride in tablet has been followed by formation of complexes with bromo phenol blue and thymol blue. [10,11]

A chromatographic determination of LopH using silica gel HPTLC plates with fluorescent indicator was developed for drug quality control [12]. Other developed HPLC method for determination of LopH was use an isocratic elution with flow rate of 2.0ml/min. Acetonitrile and buffered solution was used as mobile phase [13,14]. LopH in the presence of its acid degradation products has also been determined using ZORBAX Eclipse XDB C_{18} column [15].

A potentiometric membrane ion selective electrode for selective determination of LopH hydrochloride were investigated, the electrochemical behaviour of LopH using PVC membrane were followed by the modified carbon nanotubes paste sensors [16].

LopH has not been determined by oxidative-coupling procedure which is simple, the aim of the study is to offer a new simple spectrophotometric method for determination of LopH in pharmaceutical

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dosage forms by coupling with the oxidized form of phenothiazine the safe compound compared with organic reagents.

2. Experimental

2.1. Apparatus

Sartorius BL201 S balance was used for weight measurements, electromag water bath was used to provide the required temperature. pH measurements were performed using HANNA 301 pH meter, 1.0 cm quartz cells and double-beam shimadzu 1800 spectrophotometer, japan were used for spectral measurements.

2.2. Chemicals

Analytical grade chemicals were used.

Pure LopH (99.65%), was kindly provided by the state Company for Drugs Industry and Medical Appliances Samarra-Iraq)

LopH (100 µg.ml^{-1}): this solution was prepared by dissolving of 0.005 gm of pure LopH powder in 5 ml of absolute ethanol and then diluted to make 50 ml. This solution is reprepared after three days

Phenothiazine reagent (1x10^{-3}M): this solution is prepared by dissolving exactly 0.0099 gm of the solid reagent in 5 ml of absolute ethanol, then diluted to 50 ml distilled water. The solution is stay stable for not more than 4 days.

Potassium dichromate solution (5 x 10^{-4} M): is prepared by dissolving of 0.0147 gm of solid potassium dichromate in 3 ml of H_2SO_4 (1M) and dilute the solution to make 100ml.This solution is weekly prepared.

Interfering compounds (3000 µg.ml^{-1}): is prepared singly by dissolving of 0.3 gm of each forging compound in distilled water to make 100 ml.

Surfactant solution (0.1%):0.1 gm of solid SDS and CTAB was dissolved in distilled water to make exactly 100ml.

Surfactant solution (0.5%):0.5 gm of pure Triton X-100 was dissolved in distilled water to make exactly 100ml

2.3. The principle of the determination

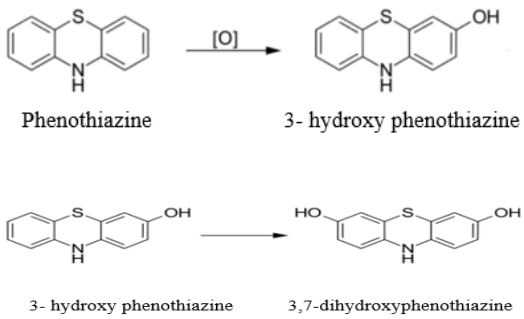
The reaction is based on an oxidation of the reagent followed by coupling with LopH to produce a greenish-blue coloured complex measured at 625 nm. the starting reaction condition include the use of 2 ml of reagent ,0.5 ml of oxidant ,6 ml of drug, and dilution to 25 ml in volumetric flask.

2.4. Chemical reactions

1-Oxidation reaction

Phenothiazine undergoes oxidation by the action of acidic potassium dichromate solution to produce the hydroxy substituted compound [17,18] as shown in scheme 1 step one and two.

Step one:

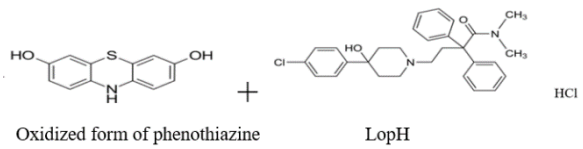


Scheme 1. Oxidation of phenothiazine

2- Coupling reaction

The hydroxy substituted form of phenothiazine is coupled with LopH to form the coloured product as shown in scheme 2.

step two:



→ Greenish-blue coloured product measured at 625nm.

Scheme 2. Coupling reaction of phenothiazine with LopH

2.5. Optimization of reaction conditions

2.5.1. Phenothiazine reagent: in order to select the suitable amount of phenothiazine 0.5 ,1,2,2.5,3,3.5 ml of phenothiazine reagent (1x10^{-3} M) has been firstly added, followed by the acidic oxidant and finally the drug, Table 1. Show that 2.5 ml of the reagent give the higher absorbance value.Note that potassium dichromate was already dissolved in 3 ml of H_2SO_4 (1M), and the reaction does not require to further acidifying step.

2.5.2. Oxidation of phenothiazine: to select the suitable oxidant and its amount, effect of (5x10^{-4}M) of each oxidant N-bromosuccenimide, ferric chloride, and potassium dichromate on the reaction sensitivity were examined. Table 2 show that potassium dichromate gives the highest absorbance and it is used in subsequent steps, while Table 3 show the optimum volume of dichromate can be used to produce optimum absorbance. The oxidation step requires five minutes to complete and this is shown in Table 4.

Table 1:Phenothiazine amount

Phenothiazine (1x10^{-3} M) ml	0.5	1	2	2.5	3	**3.5**
Absorbance	**0.148**	**0.149**	**0.150**	**0.159**	**0.156**	**0.151**

Table 2:Selection of oxidant

0.5 ml of ($5x10^{-4}$M) oxidant	NBC	$FeCl_3$	**$K_2Cr_2O_7$**
Absorbance	**0.115**	**0.149**	**0.159**

Table 3: Select the suitable amount of oxidant

V ml of $K_2Cr_2O_7$ ($5x10^{-4}$M)	0.5	1.0	1.5	2.0	2.5
Absorbance	0.159	0.171	0.168	0.157	0.143

Table 4 : The time require to complete oxidation

Time(min.)	3	5	7	10	15
Absorbance	0.160	0.171	0.162	0.157	0.148

2.5.3. Surfactant: reactions in the presence of surfactants may increases the absorption intensities of the coloured product, and this may be referring to the relation between chemical structures and surface properties (19), in fact there is no clear explanation of surfactant behaviour because of the wide range of surfactant and wide spectrum of possible environments for their molecules (20).

Table 5 Effect of surfactant

Surfactant	0.5 ml
CTAB (0.1%)	0.142
SDS (0.1%)	0.118
Triton X-100(0.5%)	0.143

In the subsequent experiment, 0.5, 1, and 2 ml of (1%) of cationic CTAB surfactant (cetyltrimethylammonium bromide), and same volumes of (1%) of an anionic surfactant SDS (sodium dodecyl sulphate), also same amount of (0.5 %) of the neutral surfactant Triton X-100 were added to the reaction component. Table 5 indicates that there are no enhancements in the absorbance value of the last optimized value 0.171.

2.5.4. The sequence of additions: in spite of that the reagent must be firstly oxidized and secondly coupled, the other sequence may sometimes exhibit enhancement on the intensity of the colour and subsequent on absorbance intensity (21).

Order number	**Reaction components**	**Absorbance**
I	D+R+O	0.142
II	R+O+D	0.172
III	O+D+R	0.130
	0..147	0.133

Table 6: Study the sequence of additions

In Table 6 three different sequences are followed but the R(Reagent) followed by O(Oxidant) followed by D (Drug) sequence gives the best result which is used in pre-experiments and in post – experiments. Table 6 show the results.

2.5.5. The influence of time: at room temperature (20 ±2C˚) the absorbance of the optimum value resulted from the above experiments is followed for one hour, the result in Table 7 show that the reaction requires five minutes to go to completion and fixed at the same value for at least 30 minutes.

Table 7: Stability of coloured product

Time (min.)	Absorbance
5	0.171
10	0.170
15	0.172
20	0.172
25	0.172
30	0.172
35	0.176
40	0.176
45	0.178
50	0.177
55	0.179
60	0.179
70	0.18
80	0.182
100	0.182
110	0.182
120	0.180

2.6. Absorption spectrum

Under the selected reaction conditions, absorption spectrum of the coloured LopH product exhibit a maximum at 625 nm as it is in the starting condition without perceptible changes toward red or blue shift, the figure below. Figure .2 show the optimum wavelength of LopH also show that there was no optimum peak belong to the blank at the same wavelength.

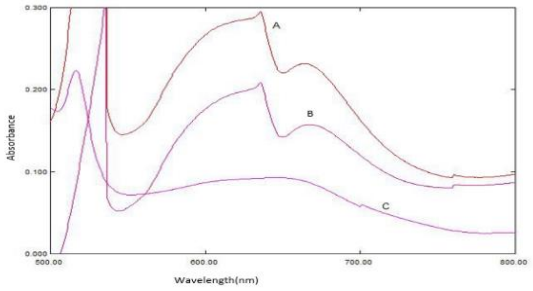


Fig. 2. Absorption spectrum of 24 µg.ml^{-1}of LopH ,A: sample vs. distilled water, B: sample vs. blank, and C: blank vs. distilled water

2.7. Calibration curve

2.5 ml of (1 x 10^{-3} M) phenothiazine reagent, 1ml of $K_2Cr_2O_7$ (5x10^{-4}M), and increasing volumes (0.2-10) ml of 100 µg.ml^{-1}standard LopH solution were added in this sequence and mixed together, the volumes were completed to 25 ml in a volumetric flask with distilled water and finally measured after 5 minutes at 625 nm against blank solution, the absorbances values exhibit clear and close relation with the real amount of LopH. Figure 3 show the calibration curve and the negative deviation from linearity at concentration of LopH higher than 40 µg.ml^{-1}. Table 8 list the information evaluated from calibration.

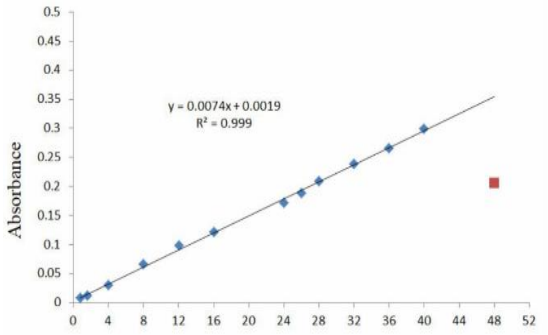


Fig.3 Calibration curve for the determination of LopH

Table 8: Information evaluated form calibration curve

Variable (unit)	Value
Linearity range (µg ml^{-1})	0. 8-40
Limit of detection LOD (µg ml^{-1})	0.33918
Limit of quantitation LOQ (µg ml^{-1})	1.1306
Molar absorptivity (l.mol^{-1}.cm^{-1})	0.3799×10^4
Sandell's sensitivity (µg cm $^{-2}$)	0.1351
Slope	0.0074
Intercept	0.0019
Correlation coefficient	0.999

2.8. Accuracy and precision

The accuracy and precision of the method are calculated for three different concentrations of LopH according to proposed procedure. Table 9 show the good accuracy and good precision of the method

Table 9:Accuracy and precision of the method

Drug	Amount added (µg/ml)	Recovery (%)	Average recovery (%)	RSD*
LopH	12	103.708	99.351	1.302
	24	97.012		1.370
	36	97.335		0.736

*Average of five determinations.

2.9. Study of LopH reaction in the presence foreign compounds.

Starch, lactose, and sucrose as expected inactive ingredient may present in the LopH dosage forms, were added as 100-fold excess than LopH concentration, Table 10 show no significant interferences caused by these compounds.

Table 10: Study effect of starch, lactose, and sucrose on LOPH reaction

Foreign Compound (10000 µg ml $^{-1}$)	Recovery % of 100 µg of LopH in the presence of 10000µg foreign compound added			
Starch	102.325	104.069	100.581	95.348
Lactose	97.674	98.255	101.744	97.674
Sucrose	103.488	101.744	98.255	96.511

2.10 The conditional stability constant

A study on the composition of chemical reaction exhibit that the reaction undergo in two drug to one reagent as shown in figure 3 ; subsequently the conditional stability constant of the formed blue LopH complex in aqueous solution is estimated by measure the absorbances of reaction mixture contain the same amount of sample and reagent (A_s) and the absorbances of reaction mixture contain the same amount of sample and maximum amount of reagent (A_m), then calculate the ratio of the dissociation ($\alpha = A_m-A_s/A_s$) found to be $1.795x10^8$ $l^2.mol^{-2}$. The results of estimation are given in Table 11.

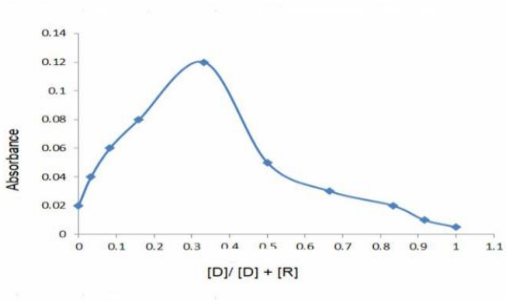


Fig. 4. Drug to Reagent composition

2.11 Application of the method

To test the applicability of the method for determination of LopH in tablets and capsule, Loperamide 2 mg/tablets Medochemie Ltd, Limassol-Cyprus (Europe):10 tablets were weighed (1.2988 gm) and a weight of drug powder equivalent to 0.01 gm of active constituents loperamide (0.6494 gm) was dissolved in 25 ml of absolute ethanol with gentle hating, then the solution was cooled, filtered, and diluted to made 100 ml.

Loperamide 2 mg/tablets Safa Co. Diala -Iraq: 10 tablets were weighed (1.1402 gm) and a weight of drug powder equivalent to 0.01 gm of active constituents loperamide (0.5701 gm) was dissolved in 25 ml of absolute ethanol with gentle hating, then the solution was cooled, filtered, and diluted to made 100 ml.

Loperamide 2 mg/Capsules Janssen pharmaceuticals (Belgium): 10 capsules were weighed (1.728 gm) and a weight of drug powder equivalent to 0.01 gm of active constituents loperamide (0.864 gm) was dissolved in 25 ml of absolute ethanol with gentle hating, then the solution was cooled, filtered, and diluted to made 100 ml.

The results are listed in Table (12) indicating a good applicability of the method with an error % not less than -3.662 and not more than -3.662.

Table 11: Ratio of the dissociation and stability constant

Concentration of LopH mol. l^{-1}	Absorbance		Ratio of dissociation α	Stability constant K (l^2mol^{-2})
	A_s	A_m		
8×10^{-6}	0.015	0.007	0.5333	1.202×10^7
16×10^{-6}	0.063	0.01	0.8412	2.64×10^8
24×10^{-6}	0.103	0.033	0.6761	2.628×10^8

Table 12: Application of the method for determination of LopH in pharmaceutical preparations

Pharmaceutical preparation	Amount taken µg ml $^{-1}$	Amount measured µg ml $^{-1}$	Recovery* (%)	Relative error* (%)	Relative standard deviation* %
Loperamide	12	12.229	101.908	1.908	**0.934**
2 mg/tablets	24	23.121	96.338	-3.662	**1.081**
Medochemie Ltd, Limassol-Cyprus (Europe)	36	35.283	98.009	-1.991	**0.850**
Loperamide	12	12.283	102.358	2.358	**1.404**
2mg/tablets	24	23.256	96.9	-3.1	**0.703**
Safa Co. Diala -Iraq	36	35.770	99.362	-0.638	**0.427**
Loperamide	12	12.202	101.683	1.683	**1.414**
2mg/Capsules	24	23.041	96.005	-3.662	**2.703**
Janssen pharmaceuticals Belgium	36	35.824	99.512	-0.488	**0.374**

*Average of five determinations.

3. Conclusion

From the study on the quantitative assay of LopH we can conclude that the method is sensitive, applicable, the reaction was taking place at room temperature, it doesn't require to addition to either acid, or base, or adjustment of pH.

4. Acknowledgment

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