RESEARCH ARTICLE

Spectrophotometric Determination of Propranolol Hydrochloride *via* Oxidative Coupling Reaction with 2, 4-Dinitrophenyl Hydrazine

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ABSTRACT

A simple, sensitive, and accurate spectrophotometric method was developed for determining the propranolol hydrochloride (PPH) in a pharmaceutical formulation. This approach is based on the oxidation of 2,4-dinitrophenylhydrazine (2,4-DNPHz) with potassium periodate and coupling with PPH in an alkaline medium in order to from a stable greenish brown colored water-soluble dye with maximum absorption at 481 nm. The curve of the calibration is linear up to 1-35 µg/mL with molar absorptivity of (6224.16) L/mol.cm. The limit of detection (LoD), as well as the limit of quantitation (LoQ), have been respectively (0.1375 µg/mL) and (0.4166 µg/mL). The proposed approach was applied successfully to determining propranolol hydrochloride (PPH) in its dosage forms.

Keywords: Oxidative coupling, Pharmaceutical formulation, Propranolol hydrochloride, Spectrophotometric.

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INTRODUCTION

Propranolol hydrochloride (PPH), having the IUPAC name (1)-1-(Isopropropylamino)-3(1-naphthylox-2-propanol hydrochloride) is the pure beta-adrenergic blocking compound prototype with no intrinsic activity.[1,2] It is β-blocker drug commonly used to treat several diseases like myocardial infarction, hypertension, angina pectoris, arrhythmias, and hyperthyroidism.[3] As well antagonism of the β-adrenergic receptors have an impact on regulating the circulation with several mechanisms, which include reductions in the myocardial contractility as well as the reduction in the cardiac output in rennin secretion with a resultant fall in angiotensin II levels, significantly contributing to anti-hypertensive actions of that drug class.[4] Numerous approaches were used for determining medication, including spectrophotomery.[5-7] Voltammetry,[8] Spectro fluorimetry[9,10] gas chromatography-mas-spectrometry (GCMS),[11,12] high-performance liquid chromatography (HPLC),[13-15] liquid chromatography-mass spectrometry (LCMS),[16] chemiluminescence.[17] The work aims to suggest procedures for determining the Propranolol hydrochloride in dosage forms. The technique was based upon oxidation of 2,4-di-nitrophenylhydrazine with potassium periodate and reaction with PPH in an alkaline medium to form a colored product.

Experimental

Apparatus

Shimadzu UV-1800 pc, UV-vis spectrophotometer model T80 (U.K) with 1cm quartz cells.

MATERIALS AND METHODS

The chemical used had been of analytical reagent grad BDH and Pancreas.

Pharmaceutical grad propranolol hydrochloride powder which has been received in the pure from (i.e. 99.99%) has been obtained from state company for medical applications and drug industry Samara-Iraq (SDI).

Stock Solution of Propranolol Hydrochloride (1000 µg.mL^{-1})

The Stock solution of (PPH) has been prepared by dissolving the precisely weighed 0.1000g of the pure drug in 10 mL of ethanol and the volume has been to the mark in a volumetric flask 100 mL with the ethanol. stock solution was protected from light and stored at 5°C in refrigeration.

Propranolol Hydrochloride Working Solution (200 µg.mL^{-1})

They were prepared from the dissolve of 20 mL of the stock solution to 100 mL in a volumetric flask with the ethanol.

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2,4-dinitrophenyl hydrazine solution (2, 4-DNPHz) (1×10^{-3}M)

It was prepared from the dissolve of 0.0198 g of 2,4-DNPHz in 2 mL of the concentrated sulfuric acid, and this volume has been completed to a mark in 100 mL volumetric flask with distilled water.

Potassium Periodate Solution (3×10^{-3}M)

Prepared from the dissolve of 0.069 g of KIO_4 in a proper amount of the distilled water and the volume has been completed to the mark in a 100 mL volumetric flask.

Sodium Hydroxide Solution (~1M)

4.000 g of the NaOH dissolved in a convenient volume of distilled water and relocated to a 100 mL volumetric flask and completing the mark with distilled water.

Propranolol Hydrochloride Tablets Solution (800 µg.ml^{-1})

The content of 10 tablets has been precisely weighed, then grinded to fine powder and mixed well afterward, and the mean value of the weight has been computed. A powder amount that has been equal to 0.1930 g and 0.1905 g (containing 40 mg of the drug propranolol hydrochloride) of Indicardin–40 mg and propranolol–40 mg respectively was accurately and separately weighed, dissolved in 10 mL of ethanol, and stirred for 5 minutes to 50 mL volumetric flask and diluted to a mark with the ethanol for the purpose of getting 800 µg/mL (PPH). The solution has been filtered through the use of the Whatman filter paper N041 for avoiding any un-dissolved or suspended material prior to the usage.

Working solution (200 µg.mL^{-1}) was freshly prepared then analyzed according to the presented procedure.

General Recommended Procedure for Calibration

In a series of 10 mL volumetric flask, 1 mL of 1.5 ×10^{-3} M 2,4-DNPHz and 3 ×10^{-3} M potassium periodate were added to each flask. The resulting oxidized product was coupled with PPH by adding 1 mL aliquots of the standard solution containing 100–300 µg followed by 1-mL of 3 M sodium hydroxide to each flask with shaking. After 4 minutes, the solutions were making up to the mark with distilled water, mixed well, and left to stand for 4 min. The absorbance greenish brown colored chromogen was measured at 481 nm against the reagent blank.

RESULTS AND DISCUSSION

Absorption Spectra for Primary Test

This method's primary test has involved the oxidation of 2,4-dinitrophenyl hydrazine with potassium periodate and coupling with Propranolol hydrochloride in an alkaline medium. The test was done by adding 1-mL of 1×10^{-3} M 2,4-DNPHz and 1 mL of oxidizing agent 3×10^{-3}M (KIO_4) in 10 mL volumetric flask. The resultant oxidized product has been coupled with the (PPH) through the addition of 1 mL of the standard solution's aliquots (200µg/mL), which has been succeeded by 1-mL of ~1 M sodium hydroxide with shaking. The contents were diluted to a mark with the distilled water. Absorbance and λ max of the brown color product has been measured against reagent blank. Figure 1 illustrates that the maximal absorption has been produced at a wavelength of 479.5 nm

Optimization of Reaction Variables

The various parameters related to the colored product formation have been studied through the variation of parameters one at a time controlling all others fixed and optimum conditions have been selected.

Effect of Concentration of 2, 4-DNPHz

The impact of concentration of the 2,4-DNPHz on the absorbance of colored products has been investigated in the range between (7 x 10^{-4} - 5 x10^{-3})M as can be seen from Figure 2. It has been found that the maximum of greenish-brown color absorbance was achieved with 1.5 x 10^{-3} M of the reagent. Above this value a decrease in absorbance was observed. Therefore, 1mL of 1.5 x 10^{-3} M was used during the subsequent work.

Impact of Concentration Potassium Periodate

The study of potassium periodate concentration has shown that the reaction has been dependent upon the KIO_4 as on agent of

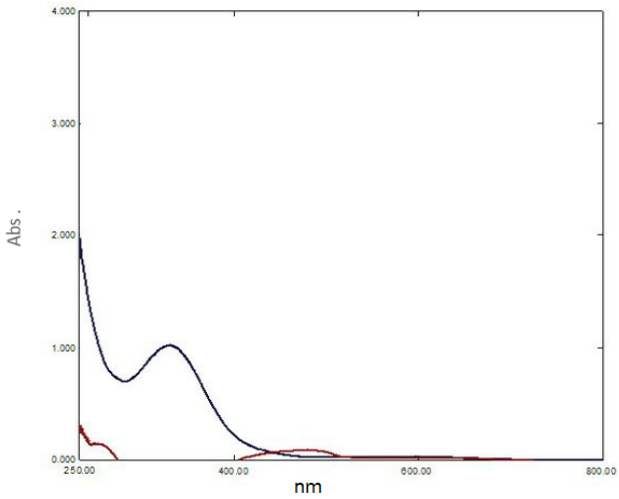


Figure 1: Absorption spectra of: (a) 20 µg/mL (PPH) against the reagent blank, (b) blank solution against solvent under primary conditions of the test

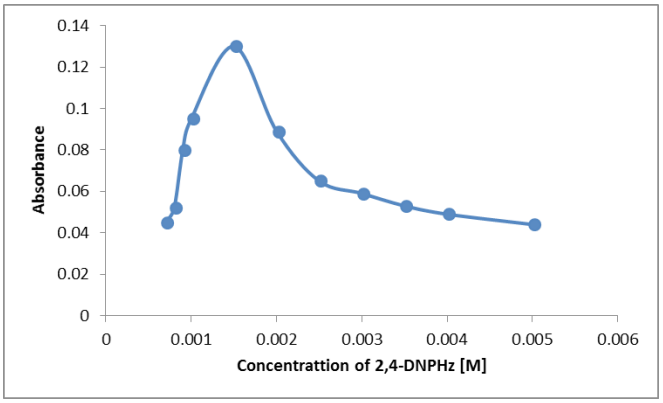


Figure 2: The effect of the concentration of 2, 4-DNPHz on the development of the colour in determining 20 µg/mL (PPH)

oxidization. The maximum absorbance has resulted in the case where the KIO_4 concentration has been 3 x 10^{-3} M, as shown in Figure 3. Therefore 1-mL of 3 x 10^{-3} M was used during the subsequent work.

Effect of Different Bases

The impact of different alkaline with a concentration of 1 mL on absorption intensity of colored dye fromed was investigated. Four types of bases namely; sodium hydroxide, sodium carbonate, potassium hydroxide, and ammonium hydroxide were tested and the results have been listed in Table 1.

Impact of the Concentration of NaOH

The impact of the concentration of sodium hydroxide on measured absorbance of formed colored product has been investigated through the use of 1mL of various concentration levels of the NaOH solution has been in the ranged between 0.5-5 M.

As shown in Figure 4, exposes that adding 1 mL of 3 M NaOH exhibited a maximum level of absorbance. Above this concentration the absorbance value decreased. Therefor, 1-mL of 3 M NaOH was used in all subsequent experience

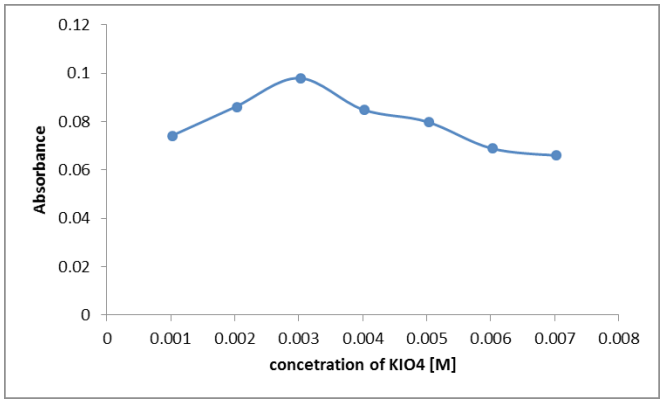


Figure 3: The impact of potassium periodate concentration on the development of the colour in determining the 20 µg/mL (PPH)

Table 1: The effect of different bases on coupling reaction

Base (1M)	*Absorbance*
NaOH	0.100
KOH	0.032
Na_2CO_3	0.014
NH_4OH	0.070

Table 2: The Impact of coupling reaction time

Time (minutes)	*Absorbance*
0	0.390
1	0.425
2	0.446
4	0.455
5	0.436
7	0.419
10	0.410
15	0.402
20	0.389
30	0.383

Effect of Coupling Reaction Time

The optimum time for the reaction between (PPH) and 2,4-DNPHz was studied at a fixed concentration of (PPH). 20 µg.mL^{-1} reacted with 2,4-DNPHz and potassium periodate in an alkaline medium. The values of the absorbance have been they are recorded at different intervals ranging from immediate measurement to a waiting period of 30 minutes. The oxidative coupling reaction completed in 4 min as shown in Table 2.

Effects of the Mixing Order of the Reagents

Effects of different orders of components on chromogen formation has been investigated by changing the order of addition of reactants four times as shown in Table 3. From results shown, it is obvious that mixing order number one was recommend as it resulted in obtaining a maximum absorbance and hence was followed in the subsequent experiments.

Stability

The stability of the colored product which has been produced upon the reaction of the (PPH) with 2,4-DNPHz has been performed through the measurement of its absorbance at various intervals of time. A 25 minutes has been selected as optimal time under general recommended procedure. The color of the solution has been stable for the least 60 minutes as can be seen in Figure 5.

Final Spectra of Absorption

The greenish-brown product's absorption spectrum, which has been produced from the treatment of PPH with 2,4-DNPHz in the presence of the potassium periodate in alkaline mediums under optimum conditions was recorded and has shown a

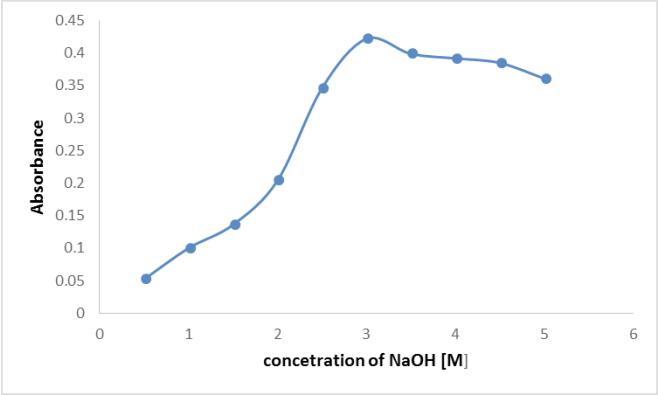


Figure 4: The impact of the concentration of the sodium hydroxide on the development of the colour in determining 20µg/mL (PPH)

Table 3: Variations of the absorbance with changing the order of the addition of the reactants in determining 20µg/mL (PPH.)

No	*Sequences*	*Absorbance*
1	R+O+D+B	0.485
2	R+D+O+B	0.466
3	D+R+O+B	0.415
4	D+B+R+O	0.005

R: reagent O: oxidizing agent D: drug B: base

maximum absorption at 481 nm against the reagent blank as can be seen from Figure 6.

Calibration Curve and Analytical Data

Utilizing the optimum experimental condition, the measured values of the absorbance at 481 nm vs. various standard concentrations values of PPH have been plotted to cinstruct a calibration curve. The linearity of the obtained plot of the PPH has been in the concentration range 1-30 µg/mL concentration range as can be seen from Figure 7. The statistical treatments of analytical data are summarized in Table 4.

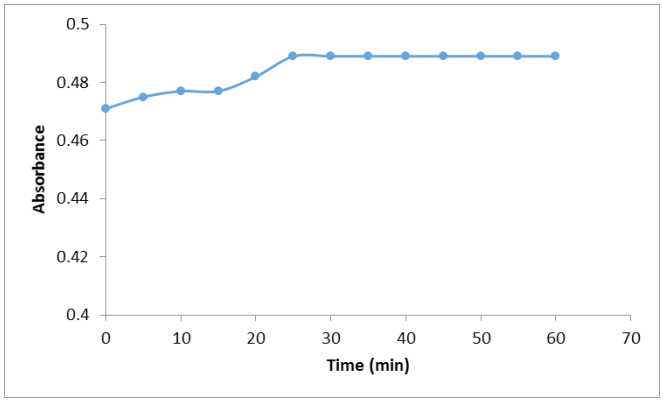


Figure 5: The stability of the colored product with time

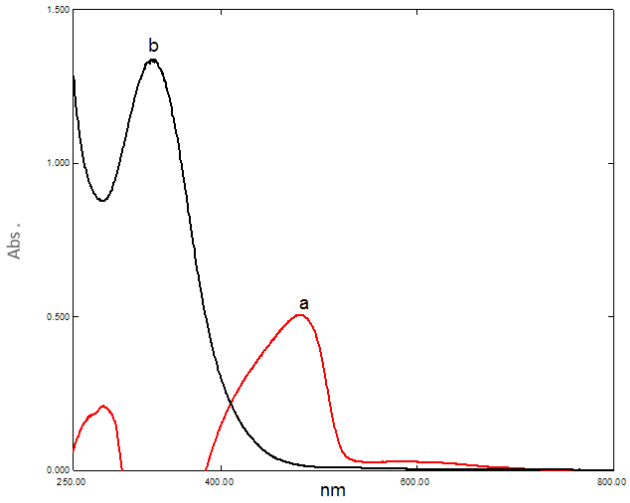


Figure 6: Absorption spectra of (a) 20 µg/mL(PPH) against the reagent blank, (b) blank solution against solvent under optimal conditions

Table 4: Optical characteristics and statistical data for determining of PPH

Parameter	*Value*
Optical characteristics	
1- λ max (nm)	481
2- Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	6224.16
3- Sandell's sensitivity ($\mu g.cm^{-2}$)	0.0416
Regression	
1- Slope ($\mu g.mL^{-1}$)	0.024
2- Intercept	0.0045
3- Correlation coefficient (r)	0.9997
Validation Parameters	
1-Beere's Law Limit ($\mu g.mL^{-1}$)	1-35
2- L.O.D. ($\mu g.mL^{-1}$)	0.1375
3- L.O.Q. ($\mu g.mL^{-1}$)	0.4166

Structure of the Product

Job's method[18] and mole ratio method[19] have been used to determine the stoichiometry of the reaction between PPH and 2,4-DNPHz. The results as shown in Figures 8 and 9 showed that 1:1 PPH to 2,4-DNPHz ratio is obtained. The proposed mechanism of reaction between PPH and 2,4-DNPHz can be represented in Scheme 2.

Comparison of the Approaches

Table 5 lists a comparison between the proposed approach

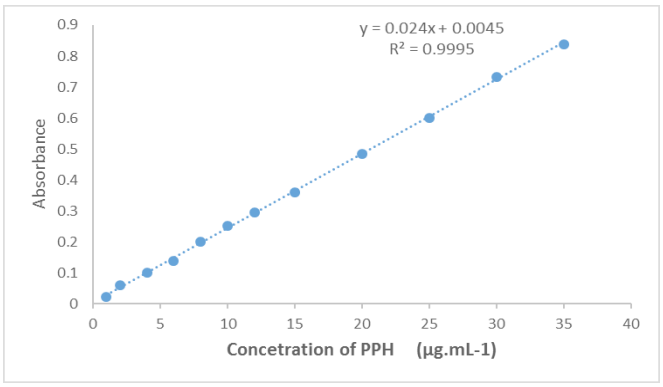


Figure 7: Calibration curve for determining the PPH under the optimal conditions

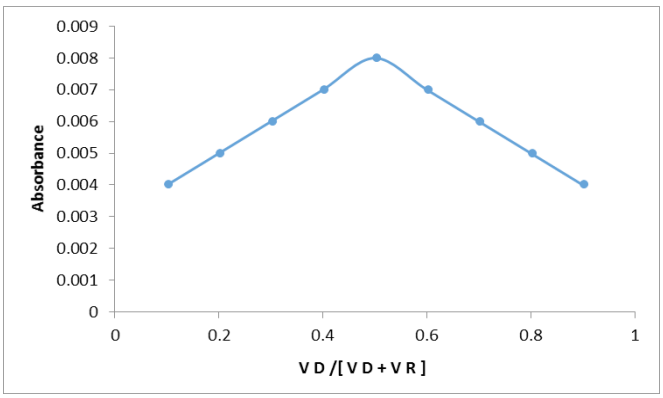


Figure 8: Continuous variation method for the reaction of PPH with 2,4-DNPHz

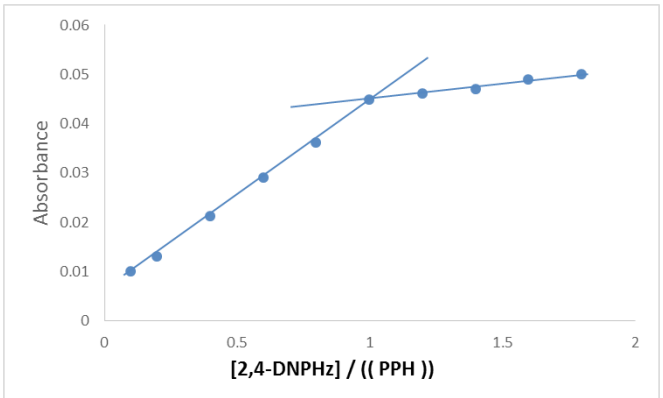


Figure 9: Mole ratio method for the reaction of PPH with 2,4-DNPHz

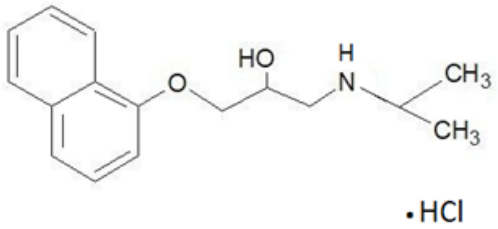
and that of another Literature spectrophotometric methods throughout some measure analytical parameters.

Accuracy and Precision

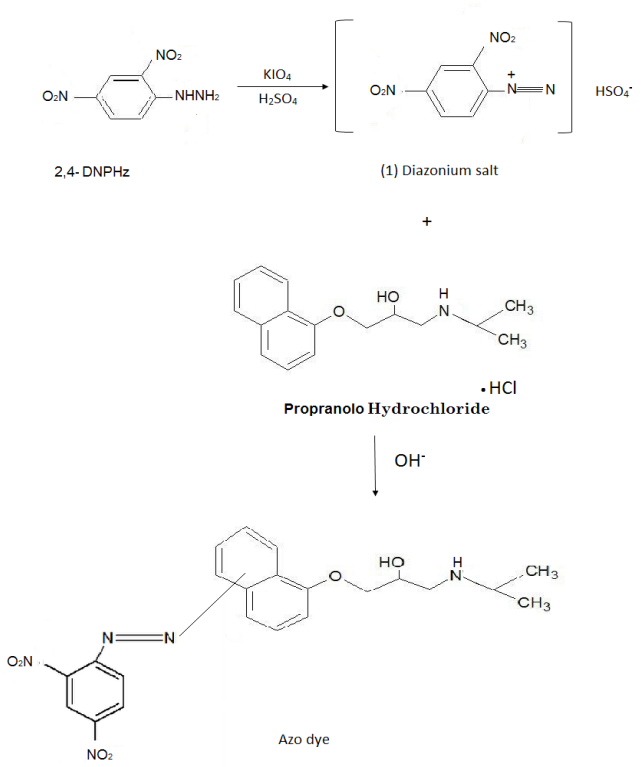
The precision and accuracy of the proposed method has been tested by analyzing of 3 replicate (PPH) samples in 3 different concentration (within Beer's law range). The results listed in Table 6 indicate an acceptable precision and accuracy of this approach.

Interference Study

The level of the interference by some of the excipients that are often accompanied pharmaceutical preparations has been studies through the measurement of absorbance of the solution which contains 20µg/mL of the PPH and 1000 µg/mL of the excipient. The results in Table 7 show that the studied excipients aren't interfering in determining the PPH in its dose forms.



Scheme 1: The structural formula of propranolol hydrochloride



Scheme 2: The suggested reaction mechanism between PPH and 2, 4-DNPHz

Table 5: Analytical parameters for the analysis of PPH. by the proposed method comparing to other methods

Methods	*Linear range of compliant concentrations of Lambert Bear Law µg.mL^{-1}*	*Correlation coefficient (R)*	*C.V% range*	*Ref.*
Proposed method	1-35	0.9995	0.3190-2.7997	
RP-HPLC–UV	0.050-0.030	0.9998	1.0 – 4.50	20
spectrophotometric	2-42	0.999	0.257-0.435	21
HPLC Methods	0.12 - 0.60	0.9989	0.1	22
spectrofluorimetric method	0.050 – 2.250	0.9999	-	23
RP-HPLC	64-96	0.9954	-	24
Spectrophotometric	20 - 80	0.9994	1.869-0.209	25
RP-UPLC	10 - 50	0.9994	0.72	26
SPECTROPHOTOMETRIC	1.0-40.0	0.9994	0.46 – 1.01	27

Table 6: Evaluation of the accuracy and precision of the proposed method for the determination of PPH

Conc. of (PPh) µg/mL			
Taken	*Found**	*Er%*	*C.V%*
4	3.9375	-1.5625	2.7997
10	10.31	1.0416	0.4123
20	19.85	-0.2430	0.3190

Table 7: Recovery values for 20µg/mL of the PPH with the presence of 1000 µg/mL of various excipients

Excipients		*Carbamazepine Conc.*		*Recovery Name*
Name	*Conc. (µg/mL)*	*Taken (µg/mL)*	*Found (µg.m^{-1})*	*(%)*
Lactose	1000	20.000	19.8541	99.2708
Glucose			19.9791	99.8958
Sucrose			19.8125	99.0625
Starch			20.0625	100.3125

Table 8: Determination of PPH in the pharmaceutical formulations (tablets) by the proposed method

		Concentration ($\mu g.mL^{-1}$)			
Sample	*Weight found* (mg)*	*Taken*	*Found**	*Recovery %*	*C.V %*
Propranolol 40 mg	39.75	10	9.9375	99.375	0.65
	39.958	20	19.9791	99.8958	0.715
INDICARDIN 40 mg	39.9164	10	9.9791	99.7916	0.409
	39.7916	20	19.8958	99.4791	0.328

*Average of three measurements

Application in the Pharmaceutical Preparations

For the purpose of demonstrating the proposed method applicability for determining the PPH, this method has been applied to three types of pharmaceutical formulations (tablets) from different manufacturing sources containing PPH the results of the application were statisfac as shown in Table 8.

CONCLUSIONS

The proposed method is rapid, simple, sensitive, and accurate in determination of propranolol hydrochlorid. The method is free from the interference by the excipients. The wide application of the new procedure for routine quality control was well established by the assay of propranolol hydrochloride in the pure forms and in the pharmaceutical preparations.

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