Bull. Chem. Soc. Ethiop. **2025**, 39(1), 1-13.   ISSN 1011-3924
© 2025 Chemical Society of Ethiopia and The Authors   Printed in Ethiopia
DOI: https://dx.doi.org/10.4314/bcse.v39i1.1   Online ISSN 1726-801X

SPECTROPHOTOMETRIC DETERMINATION OF PROCAINE HYDROCHLORIDE USING OXIDATIVE COUPLING AND CLOUD POINT EXTRACTION

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(Received June 05, 2024; Revised August 19, 2024; Accepted September 3, 2024)

ABSTRACT. A method with rapid, sensitive, accurate, and selective for estimation of local anesthetic procaine hydrochloride (PRO.HCl) is crucial, due to its bad damage to health if administrated excessively. In this work two spectrophotometric methods oxidative coupling and cloud point extraction (CPE) were utilized for determination of PRO.HCl in its standard state and pharmaceutical preparations. Method A, based on the oxidative coupling reaction using sodium hypochlorite as an oxidant and 2,5-dimethylphenol as coupling agent. The blue colored product has high absorption at 600 nm. Method B based on the CPE relying on the previous reaction, with addition of Triton X-114, after extracting the colored product, it was measured at 630 nm. Beer's law of both methods are linear in the concentration ranges 2.50-50 µg/mL (method A) and 1.25-3.75 µg/mL (method B). The molar absorptivity coefficients of methods (A) and (B) are 4.01 x 10^3 and 1.34 x 10^4 L/mol.cm, respectively. For (method A) limit of detection and limit of quantification were calculated and found that 0.26 µg/mL and 0.87 µg/mL, respectively, while for (method B) were found to be 0.16 and 0.54 µg/mL, respectively. Both methods (A and B) are applied to evaluate PRO.HCl in pharmaceutical preparations (injection).

KEY WORDS: Cloud point extraction, 2,5-Dimethylphenol reagent, Oxidative-coupling, Procaine-hydrochloride, Spectrophotometry, Triton X-114.

INTRODUCTION

Procaine hydrochloride (PRO.HCl) in which, it is known by its trade name Novocain, is one of the oldest local anesthetic drugs administrated by human. It was introduced for the first time by the German chemist Alfred Einhorn, in 1905, it is routinely performed as nerve block during medical procedures, this is made possible by diminishing the permeability of neuronal membranes to sodium ions, which then leads to a reduced rate of depolarization and, as the final outcome, the inhibition of action potential generation and propagation. Also, acting as a site-specific pain reliever during minor surgical operations such as tooth extraction, when administered in suitable amounts [1].

Chemically, PRO.HCl is identified chemically as "2-diethylaminoethyl-4-aminobenzoate hydrochloride" with a chemical formula $C_{13}H_{20}N_2O_2$.HCl presents as a white crystalline powder very soluble in water, and soluble in alcohol [2]. If PRO.HCl is administered excessively, it causes negative consequences such as cardiological and neurological toxicities, and reactions such as nausea, respiratory distress, and in severe cases, even anaphylaxis, and death. Therefore, it is critical to establish a simple, efficient and sensitive analytical method for the evaluation of PRO.HCl [3, 4]. This drug is also administered in combination with penicillin as antibiotics for the treatment of bacterial infections. It is developed as an alternative to cocaine because of its ease of preparation, ease of sterilization, and the fact that it is non-addictive. Its active duration is shorter than cocaine and it is considerably less toxic. In contrast to cocaine, PRO.HCl is a vasodilator and is usually administered with a vasoconstrictor like epinephrine, so that low drug concentrations could be used with a longer duration of action. Additionally, it has advantages compared to other local anesthetic drugs, as it is simple to prepare and has a shorter recovery time [5-7].

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Many analytical methods have been used for determination of PRO.HCl including the following methods: spectrophotometric and colorimetric methods [8-11], high-performance liquid chromatography [12, 13], gas chromatography [14], liquid chromatography and liquid chromatography-mass spectrometry [15, 16], flow injection analysis [17, 18], fluorometric methods [19], electrochemical methods [2, 5, 20-22], and chemiluminescence [23]. Despite these methods having high accuracy, they are usually not cost-effective, sophisticated, time-consuming, require pre-treatments, and most often require skilled operators trained in handling certain procedures of these techniques. Among the different analytical techniques available, spectrophotometry has gained significant attention because of its simplicity, sensitivity, and cost-effectiveness. The sensitivity and selectivity of spectrophotometric methods can be enhanced by using one of the extraction techniques that in this study liquid-liquid extraction was depended on. Spectrophotometric methods involve the measurement of light absorption or transmission by a sample at specific wavelengths, allowing for the quantification of target compounds based on their absorption characteristics [24].

One of the best and known alternative methods that utilizes surfactants for extraction of organic/inorganic parts is the cloud point extraction (CPE) It is a fast, easy and budget-friendly extraction technique involves assembly of monomers of nonionic surfactant to form a hydrophobic core (micelle), in which, the micelles trap the hydrophobic bioactive mixes inside the micellar extraction. This field of extraction greatly promotes innovative and active analytical approaches that are applicable to many systems. Spectrophotometric technique combined with CPE is still provided attractive features in routine analyses of metals and organic compounds as an alternative to the earlier cited costly instrumentation. This technique bases on the preconcentration of metal ions in aqueous samples, and it is common that surfactant in the aforementioned technique have the ability to concentrate materials. The CPE technique receives great attention, because of the simplicity, and fastness of the method, and the extraction process is useful by improving the experimental conditions by adjusting the temperature, extraction time, and surfactant concentration. The surface tension factor results in the separation of the phase called the surfactant-rich phase and the surfactant aliquot phase. Comparing the maximum temperature with the critical temperature is called the cloud point temperature, in which, it will be the substance to be analyzed is concentrated, and the preconcentration factor is high [25-28].

Oxidative coupling reactions are one of the most important organic reactions that have wide applications in the field of analytical chemistry, as they are simple, rapid, and highly sensitive methods. These reactions often involve the coupling of two or more organic substances in the presence of an oxidizing agent, as the oxidation of these substances leads to the formation of intermediate compounds that interact with each other. The other is to form a colored product that can be measured spectrophotometrically [29].

The aims of the present work to identify the most accurate, precise, and reliable spectrophotometric method for PRO.HCl determination, on the basis of oxidative coupling reaction and for the first-time using cloud point extraction in estimation PRO.HCl.

EXPERIMENTAL

Instruments

A double beam Shimadzu UV-VIS spectrophotometer (UV-1900i) was used, featured with two glass cells and two plastic cells with a light path of 1 cm. The experimental setup also involved the use of the ABS-120-4 by Kern and Sohn-sensitive balance, water bath of type Karl Kolb, MSE centrifuge, and the BP3001 pH-meter.

Chemical reagents and standard solutions

All chemicals and reagents used in this study were achieved a high purity level. All materials were used without further purification.

Standard solution of (PRO.HCl) solution (250 µg/mL) (≥97% Sigma-Aldrich). It was prepared by dissolving 0.0250 g in 100 mL volumetric flask and completed to the mark with distilled water.

2,5-Dimethylphenol solution (8.18 x 10^{-3} M) (BDH). It was prepared by dissolving 0.1000 g of 2,5-dimethylphenol in 3 ml ethanol, then the solution was transferred to a 100 ml volumetric flask and marked up with distilled water.

Sodium hypochlorite solution (1.6 x 10^{-2} M) (BDH). 2 mL of the liquid sodium hypochlorite with the concentration 6% was added to a volumetric flask of 100 mL and marked up with distilled water.

Sodium hydroxide solution (1 M) (BDH). It was prepared by diluting a plastic vial of sodium hydroxide with a concentration of (10 M) and a volume of 100 mL in a volumetric flask of 1000 ml with distilled water.

Triton X-114 (T-X-114) (10% v/v) (Merck). It was prepared by adding 10 mL of T-X-114 to 100 mL volumetric flask and completed to the mark with distilled water.

Hydrochloric acid solution (1 M) (BDH). 8.624 mL of 12.1 M HCl was diluted with 100 mL distilled water.

Analysis of procaine hydrochloride

Method A (oxidative coupling). Under optimum conditions, 0.1-2 mL of 250 µg/mL PRO.HCl solution covering the concentration range of 2.5-50 µg/mL are added to a series of 10 mL volumetric flasks, then 0.1 mL of 1 M HCl solution was added followed by 1 mL of 1.6 x 10^{-2} sodium hypochlorite solution. Next, 1.25 mL of the 2,5-dimethylphenol reagent solution is added after elapsing 10 min and 1 mL of 1 M NaOH is added. The volume was completed to the mark with distilled water and the absorbance of the colored dye solutions was measured at 600 nm against the blank solution.

Method B (CPE). This method based on the (method A) reaction (oxidative coupling), adding Triton X-114 (T-X-114) to the produced blue colored product. The same optimal amount of the chemicals from the method A that calibration curve treated with were examined with addition of 0.05-1.5 mL of 250 µg/mL standard PRO.HCl over the concentration range 1.25-37.5 µg/mL, after addition of 1 mL of T-X-114 a turbid solution formed. The mixture was placed in the water bath for 10 min at 40 ^{o}C for the enhancement of the phase separation. To accelerate the phase separation more a centrifuge instrument was used at 4000 rpm for 5 min. After cooling the mixture surfactant rich-phase has changed to viscous, while aqueous phase was removed by decantation. Finally, 1 mL of absolute ethanol was added to the surfactant rich phase and transferred to 1 cm plastic cell. The absorbance was measured against the absolute ethanol as blank solution by UV-Visible spectrophotometer at 630 nm.

Calibration curve

Under the optimum conditions, a linear calibration curves were obtained over the concentration ranges of 2.5-50 µg/mL and 1.25-37.5 µg/mL of PRO.HCl with a molar absorption coefficients (ε) of 4.01 x 10^{3} and 1.34 x 10^{4} L/mol.cm for methods A and B, respectively. The values of

Sandell's sensitivity index were 0.068 μg/cm^2 for method A and 0.020 μg/cm^2 for method B. Figure 1 indicates the calibration curves for both proposed methods A and B.

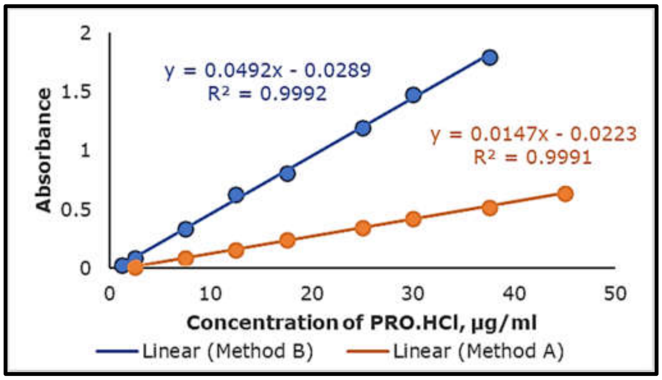


Figure 1. Calibration curves of PRO.HCl evaluation in both methods A and B.

Essential methodology for assaying PRO.HCl in pharmaceutical formulations

For pharmaceutical sample A (300,000 IU) injection powder

One ampoule of sample A is weighed totally (which contains 300,000 IU PRO.HCl). An aliquot quantity, that equivalent to 0.0250 g of PRO.HCl, was taken, and is dissolved in 10 mL of distilled water. The solution was transferred to a 100 mL volumetric flask and marked up with distilled water. An aliquot of the diluted solution from the injection is analyzed employing the procedures outlined in methods (A) and (B).

For pharmaceutical sample B (600,000 IU) injection powder

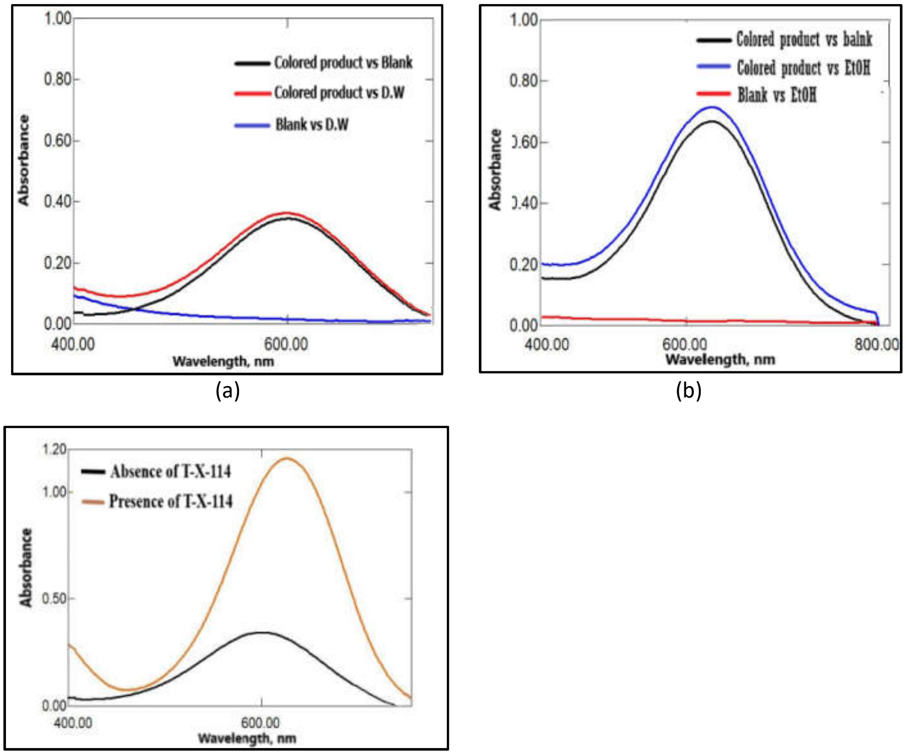
One ampoule of sample B is weighed totally (which contains 600,000 IU PRO.HCl). An aliquot quantity, that equivalent to 0.0250 g of PRO.HCl, is transferred to a 100 mL volumetric flask and marked up with distilled water. An aliquot of the diluted solution of the injection is analyzed employing the procedures outlined in methods (A) and (B).

RESULTS AND DISCUSSION

Principle of the methods (A and B)

In method (A) an oxidative coupling reaction occurred, in which two organic compounds PRO.HCl and 2,5-dimethylphenol are coupled in the presence of oxidizing agent sodium hypochlorite, under acceptable conditions to produce a colored product that can be measured spectrophotometrically, and exhibit a peak at 600 nm. Figure 2(a) shows the spectrum of the produced blue colored product after optimization.

In method (B) non-ionic surfactant T-X-114 which was added to the blue colored product that formed in the method (A). When the temperature falls below the cloud point, a cloudy dispersion forms. Upon heating, the surfactant-rich phase separates, and after centrifugation, it is analyzed spectrophotometrically for the presence of the analyte (PRO.HCl). It exhibits maximum absorption at 630 nm. Figure 2(b) shows the absorption spectrum of the colored product. If we take a look at the two absorption spectra, we notice the shift of the wavelength (bathochromic shift) and absorbance (hyperchromic shift). Figure 2(c) explains these two shifts of absorption. This shift takes apart because of the presence of T-X-114. It indicates that method (B) is more sensitive than method (A).



(a) (b)

(c)

Figure 2. Absorption spectra of the blue colored product a) in method (A), b) in method (B), c) in the presence and absence of T-X-114.

Optimum conditions in method (A)

Various volumes ranging from 0.5-1.5 mL of a 2,5-dimethyl phenol solution with a concentration of 8.18 x 10^{-3} M were introduced into a set of 10 mL volumetric flasks. These flasks already contained varying volumes of PRO.HCl, ranging from 1.0-3.0 mL, along with a 250 µg/mL PRO.HCl standard solution, corresponding to concentrations of 25-75 µg/mL. The outcomes of these combinations detailed in Figure 3. Based on the results the highest absorbance for the produced colored product was observed in 1.25 mL yielding a good determination coefficient R^2 = 0.9991. Consequently, 1.25 mL was chosen for use in the next experiments.

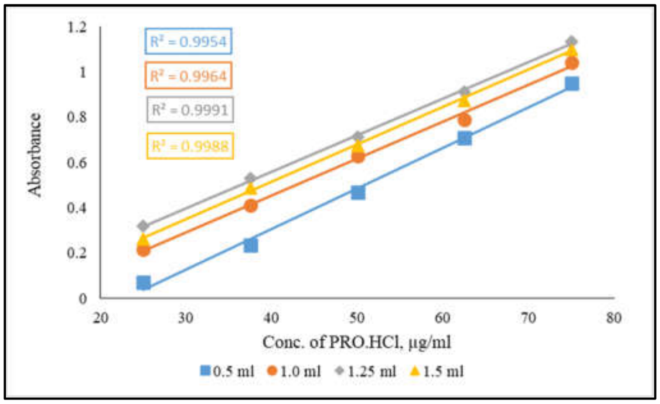


Figure 3. Effect of amount of 2,5-dimethylphenol reagent.

Different oxidizing agents including N-bromosuccinimide (NBS), sodium and potassium periodate, ferric sulfate, and sodium hypochlorite were used to know which one is the best one for the reaction among them only sodium hypochlorite produced the blue colored dye. Therefore, optimal value for sodium hypochlorite (1.6 x 10^{-2} M) were studied with varying volumes of procaine from 0.5-2 mL as shown in Figure 4. 1 mL of sodium hypochlorite gave the highest absorbance with (R^2 = 0.9993) and the rest of experiments depended on.

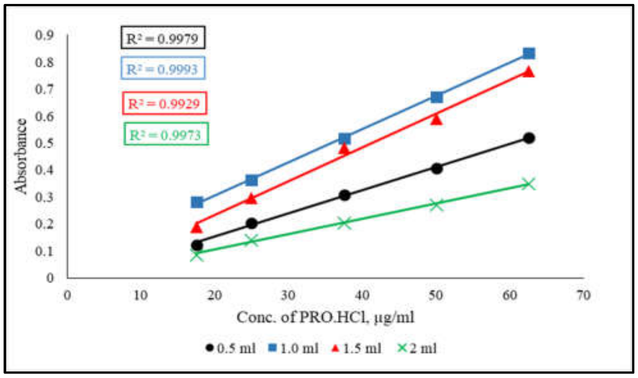


Figure 4. Effect of amount of sodium hypochlorite (1.6 x 10^{-2} M).

The oxidation step was conducted in the presence of an acid, five different acids were examined with 1 M concentration were evaluated hydrochloric acid, sulfuric acid, acetic acid, phosphoric acid, and nitric acid. None of them were able to oxidize the compound except for hydrochloric acid, which indicated that strong acid required for oxidation step, while in case of sulfuric acid a turbid solution has formed as a result of precipitation of some ions in the solution. As a consequence, optimal volumes of hydrochloric acid studied, from 0.05 mL to 0.75 mL of 1 M. Because 0.1 mL HCl obtained as an ideal value, it was used in the next experiments.

Oxidation step can occur immediately after the addition of the oxidant or maybe by elapsing some time. Thus, its influence was studied in a range 0-15 min. 10 min for oxidation gave the highest absorbance, and the whole study was relied on it.

The coupling step with 2,5-dimethylphenol happened in the presence of a base. Four bases (NaOH, KOH, Na_2CO_3, and $NaHCO_3$) added one by one to a series of four volumetric flasks of 10 mL the same optimal amount of the variables that were studied before. It was determined that NaOH and KOH did not have significant difference in absorbance but NaOH had greater absorbance, while in using $NaHCO_3$ and Na_2CO_3 a turbid solution has been observed. It indicates that the reaction required strong base to produce colored product, and NaOH was kept in using in the whole experiments.

Sodium hydroxide effectiveness was studied at two different concentrations of procaine-HCl 25 and 50 µg/mL, in a range of volumes (0.5-1.5 mL). 1.0 mL of 1 M sodium hydroxide was found to be optimum value so, this amount kept in using.

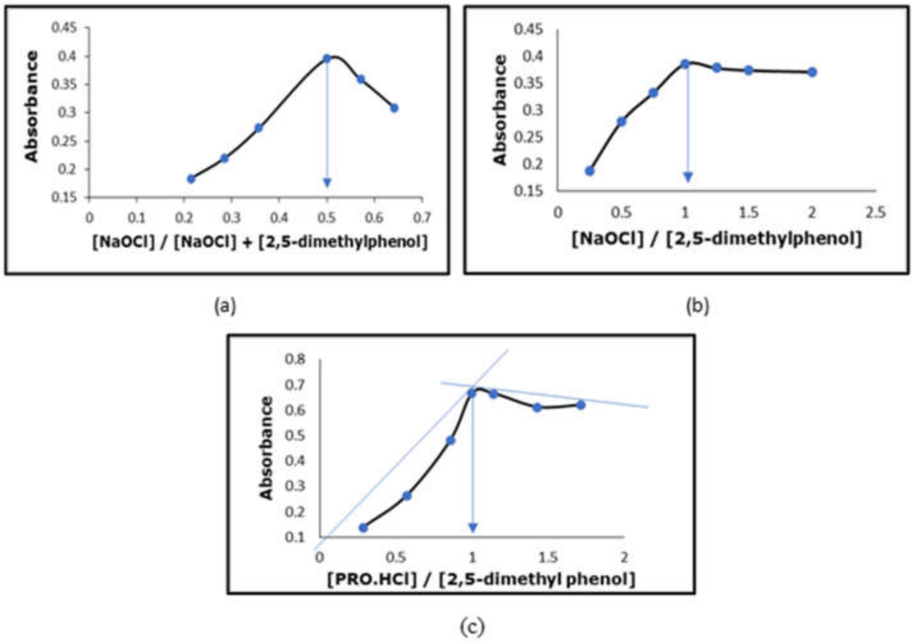
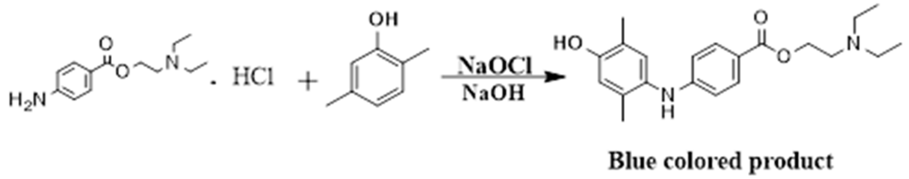


Figure 5. Plot of a) Job's method and b) mole ratio for 2,5-dimethylphenol and sodium hypochlorite. c) Mole ratio for standard PRO.HCl and 2,5-dimethylphenol.

Stoichiometric ratio and stability constant

Job's method, or continuous variation, and mole ratio were studied to determine the ratio of the starting materials in the reaction. Firstly, the ratio between 2,5-dimethylphenol and sodium hypochlorite was determined using equimolar concentrations of (8.1×10^{-4} M) each. It was performed by fixing the amount of 1 mL of 8.1×10^{-4} M PRO.HCl and varying volume of 2,5-dimethylphenol (0.5-1.1 mL) and sodium hypochlorite (0.9-0.3 mL) that final volume was 1.4 mL. The results in Figure 5(a) reveal that the ratio is 1:1 of 2,5-dimethylphenol: NaOCl. To ensure the ratio that obtained from continuous variation method the mole ratio was also studied using equimolar (8.1×10^{-4} M) of 2,5-dimethylphenol and sodium hypochlorite. It was performed by fixing both PRO.HCl and 2,5-dimethylphenol (8.1×10^{-4} M) amounts with 1 mL and varying the volume of sodium hypochlorite in range (0.25-2 mL). Figure 5(b) shows that the ratio between 2,5-dimethylphenol and sodium hypochlorite is 1:1. Secondly, in order to know the ratio between standard PRO.HCl and 2,5-dimethylphenol the mole ratio method was studied using equimolar between all PRO.HCl, NaOCl, and 2,5-dimethylphenol. It was also performed by fixing PRO.HCl amount with 1 mL of 8.1 x 10^{-4} M and varying volumes of both 2,5-dimethylphenol and sodium hypochlorite (8.1 x 10^{-4} M) amounts in range (0.25-2 mL). Figure 5(c) reveals a 1:1 ratio between standard PRO.HCl and 2,5-dimethylphenol.

So, all the material's ratio is 1:1:1 of Pro.HCl: NaOCl: 2,5-dimethylphenol consequently. Therefore, the chemical reaction progress of PRO.HCl with the oxidizing agent NaOCl and the coupling agent 2,5-dimethylphenol in the basic medium can be written as it is shown in Scheme 1.



Scheme 1. Proposed oxidative coupling reaction progress of PRO.HCl

The conditional stability constant the complex was calculated depending 1:1 ratio using the following equations [30]:

$$K_{1:1} = \frac{1-\alpha}{\alpha^2 C} \text{ and } \alpha = \frac{A_m - A_s}{A_m} \qquad (1)$$

where $K_{1:1}$ is the stability constant (L/mol), α is the dissociation degree, C is the molar concentration of the colored product, A_m is the excessive amount of the reagent and As is the stoichiometric amount of reagent. The result is 3.69×10^5 L/mol indicated the colored product has good stability.

Optimum conditions of method (B)

Cloud point extraction method offers a variety of benefits over other extraction processes, including: environmental friendliness due to the little amount of non-flammable, non-toxic, and non-volatile surfactants required [27].

Crucial parameters affecting the efficiency of the cloud point extraction, the sensitivity and stability of the produced colored product were studied by varying one parameter and fixing the others. The amount of T-X-114 proved to be the most influential factor that affects the efficiency of extraction in the cloud point extraction method through lessen the phase volume ratio and sensitivity of the colored product that formed. This effect was studied by using different volumes in a range (0.3-2.5 mL) of 10% of T-X-114. As shown in Figure 6, the absorbance of the colored product was increased by increasing the T-X-114 amount up to 1.0 mL. A considerable decrease in the absorbance is observed with increasing the surfactant amount higher than 1.0 mL. This can be attributed to an increase in volume and viscosity of the micellar phase. At concentrations below this value, the extraction efficiency of colored product was low because there are few molecules of the surfactant to entrap the oxidative coupling colored product quantitatively. Thus, T-X-114 amount of 1.0 mL was selected for subsequent experiments.

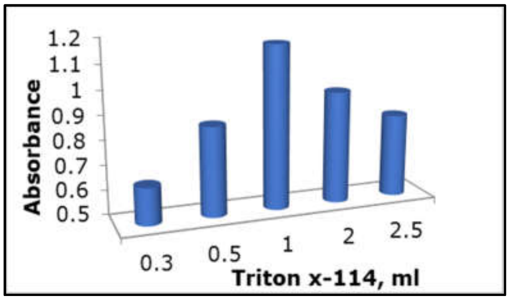


Figure 6. Effect of T-X-114 amount.

It is essential to study the optimal extraction temperature, to obtain an effective phase separation and easy preconcentration. Different temperatures were studied ranging from room temperature (23 ºC) to 70 ºC. The optimal extraction temperature was found to be 40 ºC.

In cloud point extraction proper time is required to reach the equilibrium between surfactant rich phase and aqueous phase. The mixture was put in a water bath and studied the intensity of the colored product at different time in it in the range of 5 to 15 min. 10 min is the optimal time for extraction.

Centrifugation was carried out for isolating aqueous phase and surfactant rich-phase, at speed of 3000 rpm and 4000 rpm, over the duration ranging from 5 to 20 min. As the result, 5 min had maximum intensity with 4000 rpm.

Application

Both methods (A) and (B) are applied to assess PRO.HCl in its pharmaceutical preparations (injection) for three different concentrations 12.5, 17.5, and 25 µg/mL (method A) and 12.5, and 17.5 µg/mL of PRO.HCl (methods B). The results listed in Table 1 reveal that the proposed methods (A) and (B) are consistent with the stated content. To evaluate the results of the both proposed procedures (A) and (B), a t-test has been performed four degrees of freedom (N = 4), indicates that the difference is not statistically significant, which validate the effectiveness of the two proposed methods for assaying PRO.HCl in its drugs (injection).

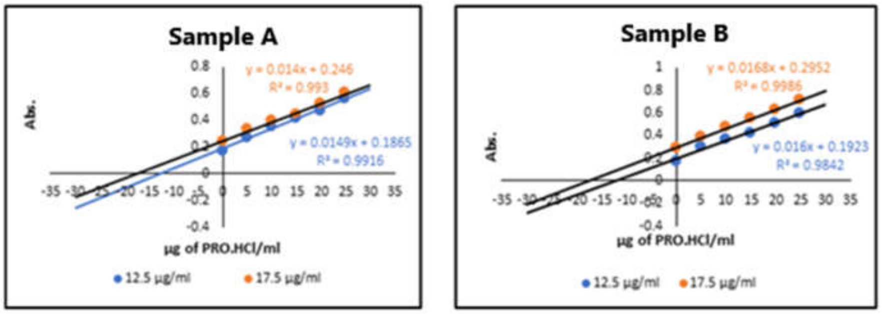
Table 1. Analysis of PRO.HCl in pharmaceuticals preparations for methods (A) and (B).

Drug	Certified value (mg)	Method (A)			Method (B)		
		Found (µg/mL)	Rec.(%) ± RSD% (N=5)	Measured value (mg)	Found (µg/mL)	Rec.(%) ± RSD% (N=5)	Measured value (mg)
Sample A injection	295 mg	12.79	102.32 ± 1.77 t_{exp} = 3.04	301.84	12.47	99.70 ± 0.55 t_{exp} = 0.31	294.11
	295 mg	25.20	100.80 ± 0.73 t_{exp} = 2.78	297.36	17.52	100.10 ± 0.54 t_{exp} = 0.23	295.29
Sample B injection	590 mg	12.67	101.36 ± 3.40 t_{exp} = 0.93	598.02	12.47	99.70 ± 0.28 t_{exp} = 0.68	588.23
	590 mg	24.92	99.68 ± 1.93 t_{exp} = 0.18	588.11	17.45	99.70 ± 0.39 t_{exp} = 1.05	588.23

Evolution of the proposed methods

The standard addition method was applied to estimate PRO.HCl in pharmaceutical dosage forms. The good recovery implied the high selectivity, efficiency of the method and free of interferences by additives. Results listed in Table 2 and shown in Figure 7 indicate that there is a high agreement between the standard additions method and the proposed methods (A and B) for the determination of PRO.HCl in its pharmaceutical formulations.

Method (A)



Method (B)

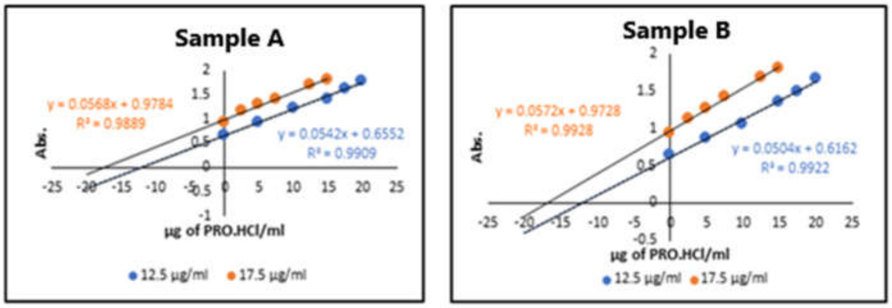


Figure 7. Plot of standard addition method for determination of PRO.HCl in pharmaceutical dosage forms using both methods (A) and (B).

Table 2. The results of standard addition methods for analysis of PRO.HCl in its drugs.

Method (A)					
Drug	Certified value (mg)	PRO.HCl taken (µg/ml)	PRO.HCl measured (µg/ml)	Average of recovery (%)	Average of measured value (mg)
Sample A injection	295	12.5	12.51	100.24	295.70
		17.5	17.57		
Sample B injection	590	12.5	12.01	98.24	578.63
		17.5	17.57		
Method (B)					
Sample A injection	295	12.5	12.08	97.5	287.62
		17.5	17.22		
Sample B injection	590	12.5	12.22	97.4	574.66
		17.5	17.00		

Comparison of the proposed methods with another method

The limit range of Beer's law, molar absorptivities, Sandell's sensitivities, accuracy (recovery %), and precisions (RSD%) of the two methods (A) and (B) were assayed. The linearity of both methods is described by the regression equation ($y = mx+b$), as well as the determination coefficient (R^2) for PRO.HCl is determined for two proposed methods demonstrating excellent linearity. Limits of detection (LOD), and quantitation (LOQ) were also determined. The results summarized in Table 3, in which indicate that the proposed both methods are precise, accurate, and sensitive. In addition the two proposed methods has been compared to another literature method.

Table 3. Analytical data and optical characteristics of the proposed methods (A) and (B), and literature method.

Parameters	Value		
	Method (A)	Method (B)	Literature method [31]
Beer's law range (µg/mL)	2.50-50	1.25-3.75	2-80
Molar absorptivity (L/mol.cm)	4.01x10^{3}	1.34x10^{4}	5.51x10^{3}
LOD (µg/mL)	0.26	0.16	--
LOQ(µg/ml)	0.87	0.54	--
Recovery (%) range*	99.68 to 102.32	99.7 to 100.1	99.98 to 101.98
RSD*	0.73 to 3.40	0.28 to 0.54	1.72 to 0.28
Determination coefficient (R^2)	0.9991	0.9992	0.9993
Slope (a)#	0.014	0.049	0.024
Intercept (b)#	0.022	0.028	0.012
Sandell's sensitivity	0.06	0.02	4.28x10^{-2}

*Average of five estimations, #Regression equation ($X = b + ac$), where c is (PRO.HCl) in µg/mL. Based on the data in Table 3 method (B) sensitivity is higher than other methods, and has the best recovery range (99.7-100.1%) compared to others.

CONCLUSION

The spectrophotometric estimation of PRO-HCl by oxidative coupling and cloud point extraction has been carried out. Via detailed optimization of experimental variables and through validation, the methods offer high sensitivity and accuracy for the quantification of PRO-HCl in pharmaceutical samples (injection). As a comparison between both methods, method B is more sensitive than method A based on its higher molar absorptivity (1.34 x 10^{4} L/mol.cm) with a linear range of concentration 1.25-37.5 µg/mL PRO.HCl and 0.9992 determination coefficient. In addition, method B has lower limit of detection of 0.16 µg/mL. İn case of recovery and RSD also method B was found to has better recovery than method A (with range 99.7% to 100.1%) and lower RSD (with range range 0.28% to 0.54%).

ACKNOWLEDGMENTS

The authors intend to thank the University of Mosul for offering up the necessary resources and facilities.

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