Rafidain Journal of Science

https://rsci.uomosul.edu.iq

Vol. 34, No. 1, pp. 28-42/ March, 2025

Determination of Procaine Hydrochloride by Two Spectrophotometric Methods in its Pharmaceutical Preparation and Human Blood Serum

Gulan S. Qadir **Nabeel S. Othman** **Amer Th. Al-Taee**

Department of Chemistry/ College of Science/ University of Mosul/ Mosul/ Iraq

p-ISSN: 1608-9391
e -ISSN: 2664-2786

Article information

Received: 25/5/2024
Revised: 11/9/2024
Accepted: 21/9/2024

DOI: 10.33899/rjs.2025.186493

Corresponding author:
Gulan S. Qadir
gulan.salah22@gmail.com
Nabeel S. Othman
nsn20002004@uomosul.edu.iq
Amer Th. Al-Taee
amerthanon@uomosul.edu.iq

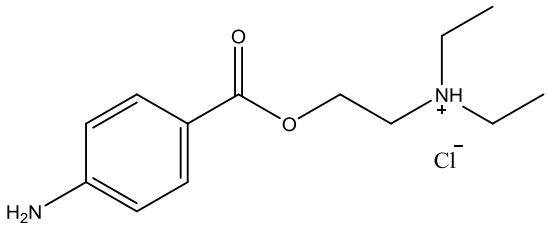
ABSTRACT

Two methods of high sensitivity and simplicity, based on spectrophotometry, have been proposed for the quantitative analysis of local anesthetic drug procaine hydrochloride (PROC-HCl) in its pure state as well as within pharmaceutical formulations and human blood serum. The first methodology is predicated on the diazotization of PROC-HCl to its corresponding diazotized form, employing sodium nitrite under ambient conditions in the presence of acetic acid. Subsequently, coupling with the 2,5-dimethylphenol reagent to produce an intense yellow-orange colored azo dye. Importantly, this dye exhibits a maximum absorption peak at a wavelength of 466 nm. Beer's law was found to be applicable within a concentration range from 1 to 15 µg/ml, yielding a determination coefficient of 0.9997, and molar absorptivity of $2.193x10^4$ l/mol.cm, Sandell's sensitivity 0.0124 µg/cm^2 limit of detection 0.105 µg/ml, and limit of quantification 0.352 µg/ml. The stoichiometric ratio for the reaction involving coupling diazotized procaine with 2,5-dimethylphenol was established at 1:1. Employing the suggested method for the determination of procaine in its dosage form (injection) and serum sample produced analytical results that were deemed acceptable within scholarly standards. The second method depended on the same route of reaction but instead read absorbance measuring the area under the peak, with a fixing range of wavelengths 460-480 nm. Beer's law was applicable in the concentration range of 0.25 to 20 µg/ml, with a determination coefficient of 0.9990. Finally, the method was applied to the injection form of procaine, giving a better recovery. As a comparison between the two methods, in the second method the extent of the linear relationship decreased to concentration lower than the minimum concentration in the first method, and the recovery by the second method was about 100%.

Keywords: Procaine HCl, diazotization, 2,5-dimethylphenol, spectrophotometric determination.

INTRODUCTION

Procaine hydrochloride (PROC-HCl) finds common applications in medical and dental interventions, where its primary function is to induce temporary numbness or the absence of sensation in targeted areas of the body (Mahmoud and Taha, 2016). Initially manufactured in 1905, this medication is often employed as a nerve blocker for minor surgeries like tooth removal because of its local analgesic properties. It's routinely coupled with penicillin to address bacterial infections. This substance has gained popularity over cocaine owing to simpler production and sterilization processes alongside its non-addictive nature. Although it doesn't last as long when compared to cocaine, it presents significantly lower toxicity levels (Qader *et al.*, 2023). Procaine hydrochloride, also known as Procaine-HCl, is categorized as a local anesthetic within the amino ester group. It presents as a white crystalline powder and is identified chemically as "2-(diethylamino) ethyl 4-aminobenzoate hydrochloride" (British Pharmacopoeia, 2009), its chemical structure shown in Fig. (1):



M.wt=272.77 g/ mol.

Fig. 1: The chemical structure of Procaine hydrochloride.

Numerous techniques have been documented in the scientific literature for the quantification of PROC-HCl in both biological samples and pharmaceutical formulations; enlist capillary electrophoresis (Yuan and Wang, 2007), flow injection analysis (FIA) (Mahmoud and Taha, 2016; Carmona *et al*., 1992; Al-Abachi *et al.*, 2012), high-performance liquid chromatography (He *et al.*, 2013), UPLC (Savchenko and Georgiyants, 2018), electrochemical analysis, such as differential-pulse voltammetry (Plotycya *et al*., 2018; Qader *et al*., 2023; Haghighian *et al*., 2023; Zhu *et al*., 2021), differential pulse adsorptive stripping voltammetry (Nianbing *et al.*, 2003), potentiometric titration. (Luminiţa *et al*., 2002), ion-pairing flow injection analysis (Luo *et al.*, 1997) chemiluminescence (Pasekova and Polasek, 2000), spectrophotometric methods (Marin *et al*., 2019; Ashour *et al*., 2009; Chen *et al.*, 2009).

UV-visible spectrophotometry has gained widespread prominence in the field of pharmaceutical analysis owing to its rapidity, ease of use, and applicability across a diverse array of substances (Hussein and Othman, 2023; Zakaria *et al.*, 2022; Li *et al.*, 2003; Al-Uzri and Al-Heeti, 2015; Al-Uzri, 2015). As universally acknowledged, pharmaceuticals are mainly composed of organic compounds exhibiting notable absorption in the ultraviolet range. It is evident that when examining these substances in the ultraviolet range, interference can arise if other compounds absorb light at wavelengths closely aligned with those of the substances.

The present study depends on the diazotization coupling reaction, firstly by reading absorbance, secondly, by studying the area under the peak for the assay of procaine hydrochloride in pharmaceutical formulation and human blood serum. The primary benefit of our methods was the shift of the maximum absorption wavelength of procaine from the ultraviolet range to the visible light range. This alteration enables the determination of procaine within the visible light spectrum.

EXPERIMENTAL

Instruments

Spectral measurements and absorbance readings were meticulously achieved through the utilization of a SHIMADZU UV-VIS spectrophotometer (UV-1900i), featuring two glass cells with

a light path of 1 cm. The experimental setup also involved the use of a BEL-Sensitive balance, ensuring precision and accuracy in the current investigation.

Chemicals and solutions

All chemicals and reagents were of the finest functionality. All reagents from Fluka company.

Sodium nitrite (3.66×10^{-3} M)

This solution was prepared by dissolving 0.0252 g in 10 ml distilled water, then the mixture was diluted to 100 ml with distilled water in a volumetric flask, finally it was transferred to a darken bottle and was used for diazotization of Procaine.

Acetic acid solution (1M)

A few ml of distilled water was added to a 100 ml volumetric flask followed by adding 5.7 ml of concentrated acetic acid (17.4 M), added distilled water to the markup. This solution was stored in a dark bottle.

Diazotized PROC-HCl solution (100 µg/ml, 3.66×10^{-4} M)

The PROC-HCl solution was prepared by dissolving 0.0100 g of PROC-HCl in 10 ml distilled water and 5 ml of 1 M acetic acid, then the mixture was transferred to a 100 ml volumetric flask, and 10 ml of sodium nitrite solution (3.66×10^{-3} M) was added to the mixture, finally the volume was completed to 100 ml in a volumetric flask with distilled water.

2,5-Dimethylphenol solution, 4×10^{-2} M

This solution was prepared by dissolving 0.1250 g of 2,5-dimethylphenol in 2 ml ethanol, then the mixture was transferred to a 25 ml volumetric flask and marked up with distilled water. Finally, the reagent transferred to a darken bottle. This solution was prepared every day.

Sodium hydroxide (1M)

To prepare this solution, 100 ml of sodium hydroxide in a plastic vial with a concentration of 10 M was diluted to 1000 ml of distilled water in a volumetric flask. typically stored within a plastic container.

Pharmaceutical preparation (injection form, 100 µg/ml), diazotized procaine

The contents of 2 powder injections of procaine penicillin (300,000 I.U/injection, equivalent to 0.295 g) (Jaber Ebne Hayyan company, Iran) and (600,000 I.U/ injection, equivalent to 0.590 g) (Devapen, Türkiye), were weighed individually as a total weight.

For Iranian injection, an aliquot quantity, equivalent to 0.0100 g of pure PROC-HCl from the powder was taken, and dissolved in 10 ml of distilled water then 5 ml of acetic acid (1M) was added to it, in addition, 10 ml of sodium nitrite (3.66×10^{-3} M) was added to the procaine injection powder, finally the all mixture was transferred to a 100 ml volumetric flask and made up to the mark with distilled water.

For Turkish injection, an aliquot quantity, that equivalent to 0.0100 g of pure PROC.HCl from the powder was taken, and dissolved in 10 ml of distilled water then 5 ml of acetic acid (1M) was added to it, in addition, 10 ml of sodium nitrite (3.66×10^{-3} M) was added to the procaine injection powder, finally the mixture was transferred to a 100 ml volumetric flask, and made up to the mark with distilled water. Those mixtures were unstable, so they were prepared at the same day of measurement.

Human blood serum sample

Specimens of blood were procured from healthy individuals not undergoing procaine-containing treatments. Following coagulation, the blood samples underwent centrifugation at 4000 rpm for 15 minutes, facilitating the separation of serum (Taha and Ali, 2023). The samples underwent no pre-treatment procedures prior to the analysis.

Method I :

Spectrophotometric method on the basis of absorbance

Methodology and calibration curve

Added 0.1-1.5 ml of 100 µg/ml diazotized PROC-HCl solution to a series of 10 ml volumetric flasks, then 1.5 ml of the 2,5-dimethylphenol reagent solution and 2.5 ml of 1M NaOH are added. The volume was completed to the mark with distilled water and the absorbance of the colored azo dye solutions was measured at 466 nm against the blank. A linear calibration graph was obtained over a concentration of 1-15 µg/ml PROC-HCl as shown in Fig. (2), and there is a negative deviation above 15 µg/ml with molar absorptivity 2.193 x 10^4 L/mol.cm.

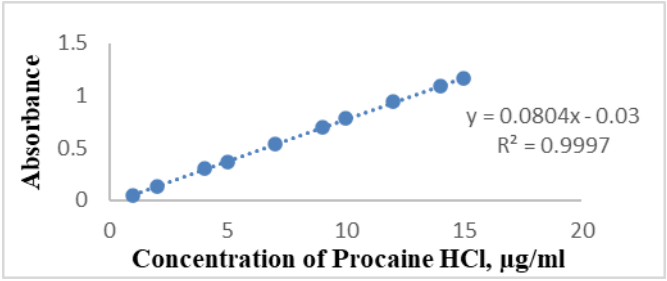


Fig. 2: Calibration curve for PROC-HCl determination via the suggested method.

(Table 1) contain the values of Sandell′s sensitivity index, LOD, LOQ and other parameters extracted from calibration curve.

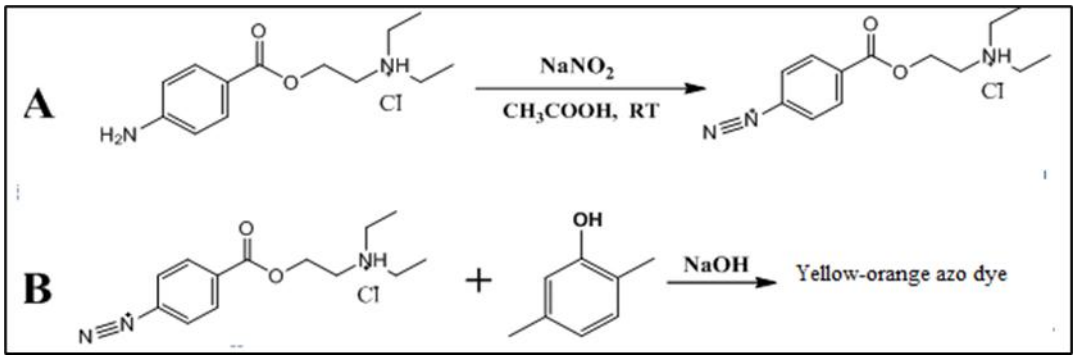
Table 1: Optical characteristics and statistical data from calibration curve.

Variable	Optimal
λ_{max} (nm)	466
Linearity range (µg/ ml)	1-15
Molar absorptivity (ε, L/mol.cm)	2.193×104
Sandell′s sensitivity (µg /cm2)	0.0124
Intercept	0.03
Slope	0.0804
Determination coefficient (R^2)	0.9997
LOD (µg/ml)	0.105
LOQ (µg/ml)	0.352

RESULTS AND DISCUSSION

Principle of the method

The suggested approach revolves around the diazotization of Procaine hydrochloride in the presence of sodium nitrite and acetic acid, leading to the formation of a diazonium salt. Subsequently, this salt undergoes a reaction with the coupling agent, 2,5-dimethylphenol, resulting in the generation of an orange azo dye characterized by a maximum absorption peak at 466 nm. The mechanistic details of this process are elucidated as shown in (Scheme 1):



Scheme 1: Steps of the generation of the produced azo dye. (A) Diazotization reaction step, (B) Coupling reaction step.

Optimization of the variables:

All factors that affected the intensity of the colored azo dye were investigated by adjusting the factor under investigation while holding the other factors constant, and the most favorable conditions were approved.

The first step in the preparation of diazo compounds is the production of the diazonium salt, which requires an acidic medium; thus, the effect of four acids on the reaction process of 10 µg/ ml has been investigated and shown in Fig. (3, A), and also the amount of acetic acid was fixed and the results are in Fig. (3, B).

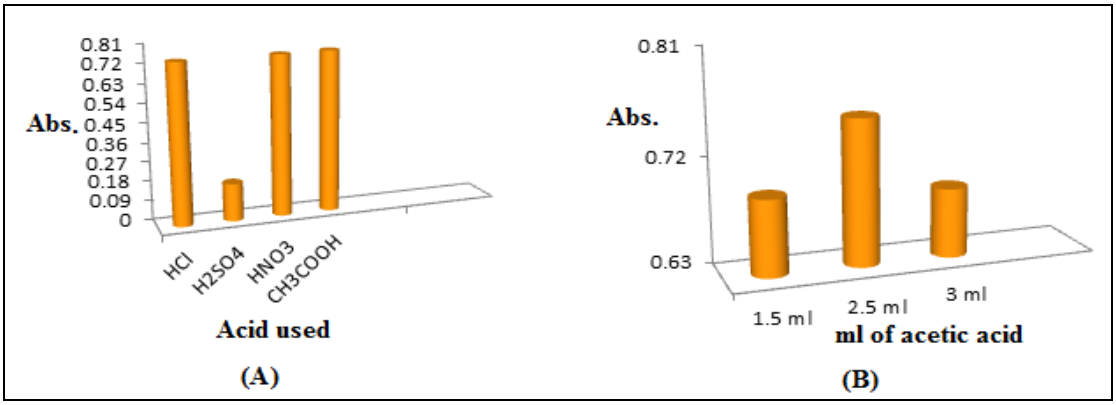


Fig. 3: Effect of (A) type and, (B) amount of acetic acid.

As it is obvious from the above Figure that the acetic acid gave the highest absorbance, and the ideal value of the acetic acid is 2.5 mL, so they were kept for the next experiments.

Effect of 2,5-dimethylphenol amount:

Various quantities ranging from 0.5-2.0 ml of a 2,5-dimethylphenol solution with a concentration of 4 x 10^{-2} M were introduced into a set of 10 ml volumetric flasks. These flasks already contained varying volumes of diazotized procaine, ranging from 0.25-1.5 ml, along with a 100 µg/ml diazotized PROC-HCl standard solution, corresponding to concentrations of 2.5-15 µg/ml. The outcomes of these combinations are detailed in (Table 2).

Table 2: Effect of reagent amount on the colored azo dye.

Volume (ml) of 2,5-dimethyl phenol (4x10^{-2} M)	Absorbance/ µg/ ml of diazotized PROC-HCl				R^2
	2.5	5	10	15	
0.5	0.172	0.375	0.575	1.152	0.9535
1	0.187	0.401	0.785	1.242	0.9986
1.5	0.255	0.434	0.811	1.368	0.9886
2	0.192	0.383	0.774	1.201	0.9994

Based on the findings presented in (Table 2), the highest absorbance for the produced azo dye was observed with 1.5 ml. Consequently, 1.5 ml was chosen for use in the subsequent experiments.

Effect the type and amount of base:

Various bases have been employed as a reaction medium, as shown in Fig. (4), sodium hydroxide gave high sensitivity, and was used in subsequent experiments.

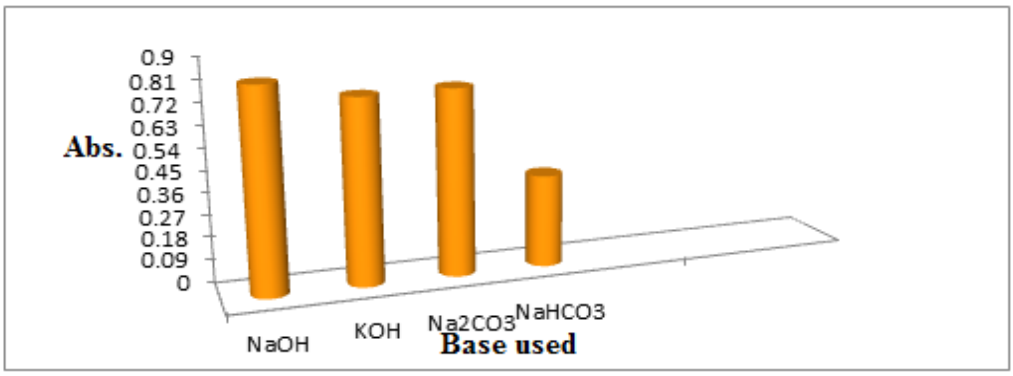


Fig. 4: Effect of base type.

Furthermore, the ideal quantity of NaOH was investigated by introducing varying volumes, ranging from 0.5-3 ml, of a 1M sodium hydroxide solution to a mixture containing 1 ml of diazotized PROC-HCl and 1.5 ml of 2,5-dimethyl phenol ($4x10^{-2}$ M). The findings from this investigation are documented in (Table 3).

Table 3: Effect of sodium hydroxide amount

Sodium hydroxide (1M)	Absorbance / 10 µg / ml of diazotized PROC-HCl
0.5	0.776
1	0.786
1.5	0.788
2	0.797
2.5	0.846
3	0.764

The data presented in (Table 3) elucidates that the maximum intensity of the colored azo dye was achieved with a sodium hydroxide (1M) volume of 2.5 ml. Consequently, this specific volume is recommended for utilization in subsequent experiments.

After optimizing all of the factors the final spectrum was obtained with an acceptable absorbance as shown in Fig. (5) below:

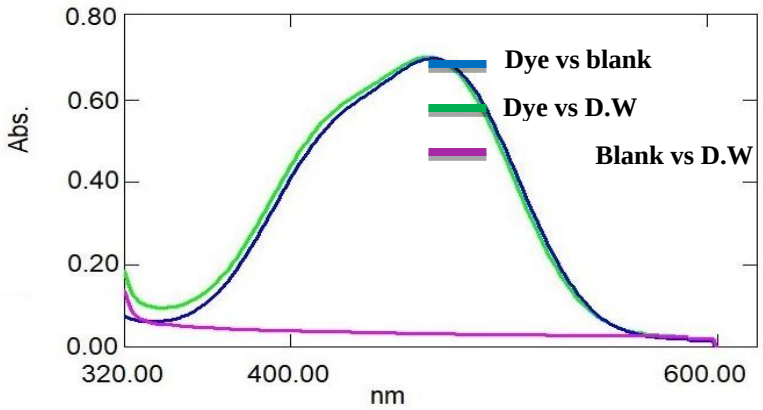


Fig. 5: Final spectrum of the produced azo dye using 10 µg/ml diazotized PROC-HCl.

Stoichiometric ratio:

Job's (continuous variation), mole, and slope ratio methods were used via using equimolar solutions of diazotized drug solution and reagent (2,5-dimethylphenol) solution (3.66 x 10^{-4} M).

Using Job's method, various drug quantities ranging from 0.5 to 4.5 ml were added to a series of 10 ml volumetric flasks, and various reagent volumes were added to ensure that the final volume of the two components in each volumetric flask was the same then 2.5 ml of NaOH (1M) was added, the volume was completed with distilled water, and the absorbance was read at 466 nm. The result is in Fig. (6).

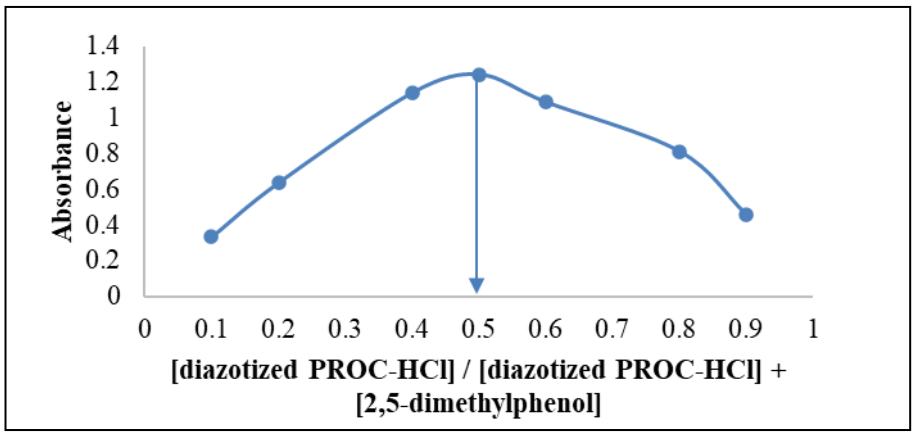


Fig. 6: Plot of Job's method for diazotized PROC-HCl and 2,5-dimethylphenol.

As depicted in Fig. (6), the ratio is equal to 1:1 diazotized PROC-HCl : 2,5-dimethylphenol.

The mole ratio method was additionally executed by introducing different volumes ranging from 0.25 to 4 ml of the 2,5-dimethylphenol reagent to 1 ml of the diazotized PROC-HCl solution, maintaining consistent concentrations (3.66x10^{-4} M). The outcomes presented in Fig. (6) validate the findings derived from Fig. (7).

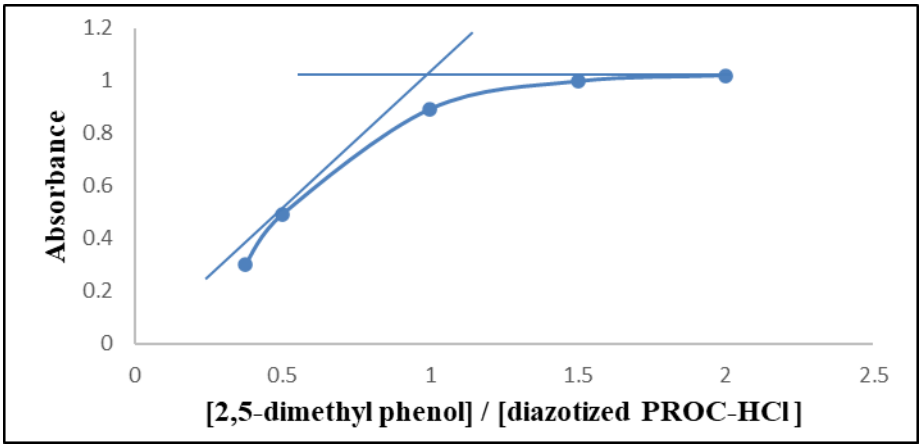


Fig. 7: Plot of mole ratio method.

Slope ratio method also has been worked to expect the final structure of the produced azo dye. First, it was done by fixing the concentration of diazotized PROC-HCl, and changing the concentration of the 2,5-dimethylphenol, then the concentration of the 2,5-dimethylphenol was fixed and diazotized PROC-HCl concentration is changed obtained two linear graphs as shown below in Fig. (8: A, B):

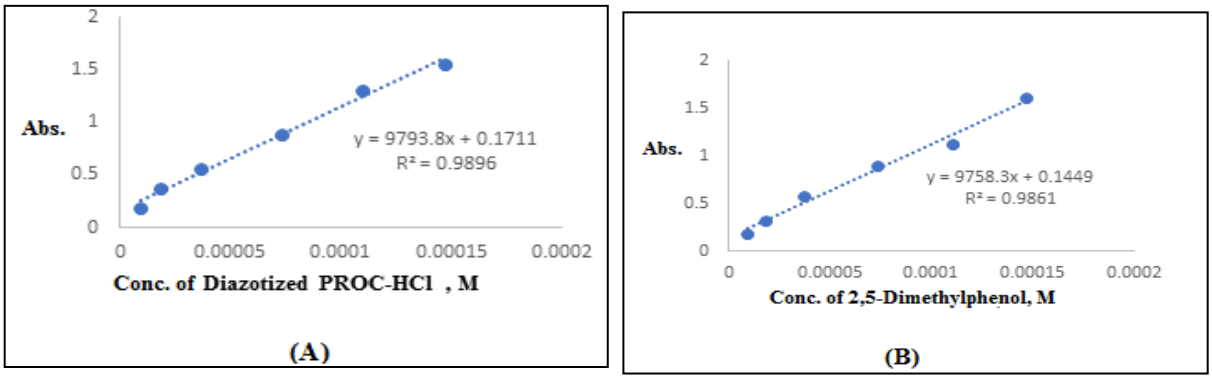


Fig. 8: Plot of slope ratio method. (A) Concentration of diazotized PROC-HCl gains absorbance. (B) Concentration 2,5-dimethylphenol against absorbance.

If we divide slope of Fig. (8, A) by slope of Fig. (8, B) we get the result 1.022, which means our product is 1:1.

Therefore, according to the obtained results fixed in Figs. (6, 7, and 8), the suggested structure of the yellow-orange azo dye (Hasan *et al.*, 2020; Hiba and Othman, 2022) as in Fig. (9):

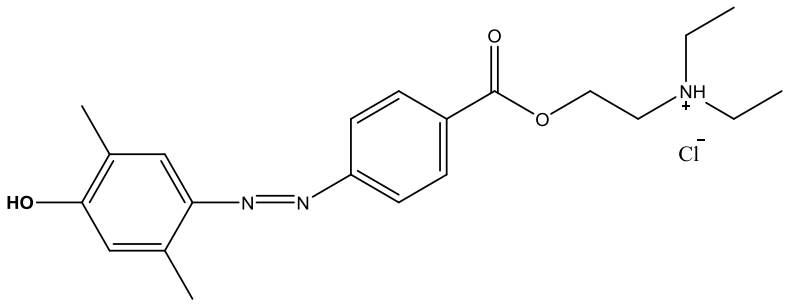


Fig. 9: Structure of yellow-orange azo dye formed.

Stability constant of the produced azo dye

The stability constant (K) is calculated for the product formed in a 1:1 ratio (diazotized PROC-HCl:2,5-dimethylphenol) by preparing solutions containing equivalent amounts of diazotized PROC-HCl and the reagent 2,5-dimethylphenol at a concentration of 3.666 x 10^{-4} M of each. The absorbance of each solution was measured against its blank solution and its absorption value was expressed in as solutions were also prepared containing the same amount of diazotized PROC-HCl and an excess amount of 4 ml of 2,5-dimethylphenol and the absorption value here is in Am, and by applying the following relationship, the degree of disintegration was calculated (Hargis, 1988):

$$\alpha = \frac{A_m - A_s}{A_m} \qquad K_s = \frac{1-\alpha}{a\alpha^2 C}$$

a: degree of dissociation

C: concentration of the product

The results are shown in (Table 4).

Table 4: The stability constant of the produced azo dye

Amount of 3.66×10^{-4} M PROC-HCl (ml)	Conc. of PROC-HCl (M)	Absorbance		α	K ($L.mol^{-1}$)
		As	Am		
1.5	5.49×10^{-5}	0.754	1.050	0.281	1.658×10^{5}
2.0	7.32×10^{-5}	0.913	1.201	0.239	1.820×10^{5}

The average value of the stability constant is 1.7390×10^{5} $L.mol^{-1}$, and this indicates that the produced azo dye has very good stability.

Analytical application

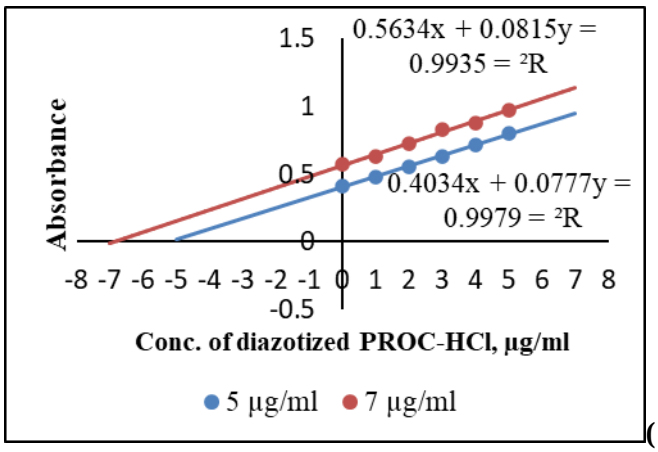
The suggested methodology has been employed for the quantification of PROC-HCl in injection form. The application of this proposed procedure has yielded favorable outcomes in terms of recovery, accuracy, and precision (expressed as Relative Standard Deviation (RSD%)), as illustrated in (Table 5).

Table 5: Analytical application for pharmaceutical in dosage form (injection).

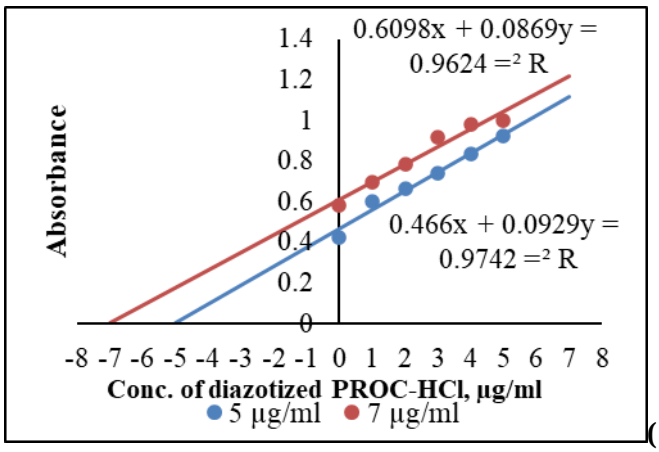
Pharmaceutical preparation	Amount taken µg/ml	Amount measured µg/ml	Recovery %	Relative error %	RSD %	Drug contains (g)
Procaine/ injection (300 000IU, 0.295 g) (Jaber Ebne Hayyan, Iran)	3	2.90	96.6%	-3.4%	0.89	0.284
	5	5.06	101.2%	+1.2%	0.86	0.298
	10	9.91	99.1%	-0.9%	0.32	0.292
Procaine/ injection (600 000IU, 0.590 g) (Devapen, Türkiye)	3	2.96	98.6%	-1.4%	0.94	0.581
	5	5.01	100.2%	+0.2%	1.21	0.591
	10	9.94	99%	-0.6%	0.49	0.584

Standard addition method

The standard addition method was also used to estimate the drug content in the injection ampoule to prove that there was no interference with additives used in pharmaceutical industries. Two concentrations of 5 and 7 µg/ml were taken from the solution of the pharmaceutical drug procaine penicillin for the ampoule, followed by adding different concentrations of the drug solution of procaine under study in its pure form, provided that it does not exceed the maximum estimate range in the calibration curve, noting that one of the volumetric bottles should contain only a known concentration of the pharmaceutical solution without the pure compound. These additions were made to two series of 10 ml volumetric bottles. The additions were made according to the optimal conditions, and the absorbance of the solutions was read against the blank solution. At the wavelength of 466 nm, Fig. (10) and (Table 6) show the results we obtained.



A)



B)

Fig. 10: Standard addition method curve for the determination of PROC-HCl (injection). (A) Iranian company, (B) Turkish company.

Table 6: Standard addition method for the determination of PROC-HCl.

Pharmaceutical preparation	Amount taken (µg/ml)	Recovery%	Drug contains (g)
Procaine / injection (300000IU, 0.295 g) (Jaber Ebne Hayyan company, Iran)	5	103.8%	0.3062
	7	98.7%	0.2911
Procaine / injection (600000IU, 0.590 g) (Devapen, Türkiye)	5	100.2%	0.5911
	7	100.1%	0.5905

Application for blood serum

Procaine hydrochloride was determined in the blood serum sample by adding 0.2 ml of the blood serum, then 0.5 ml and 1 ml of 100 µg/ml diazotized PROC-HCl with the optimal values of 2,5-dimethylphenol and 1M NaOH solutions (Table 7).

Table 7: Analytical application for procaine in human blood serum.

Amount added µg/ml	Amount measured µg/ml	Recovery %	Relative error %	RSD %
5	4.98	99.6%	-0.4%	2.26
10	9.98	99.8%	-0.2%	2.37

The outcomes obtained from the suggested method validate its precision and accuracy in retrieving data from a serum blood sample. The recovery range, falling within 99.6-99.8%, attests that the proposed method is proficient in recovering the majority of data from a serum blood sample. This substantiates its efficacy and establishes it as a dependable solution for data recovery.

Method II:

Studying area under the peak

By using the same optimum conditions that indicated previously peak areas were read instead of absorbance, by selecting and fixing a range of wavelengths from 460-480 nm in which the maximum absorption is included. The Fig. (11) below shows the spectrum of the azo dye and the selected area under the peak used in our investigation.

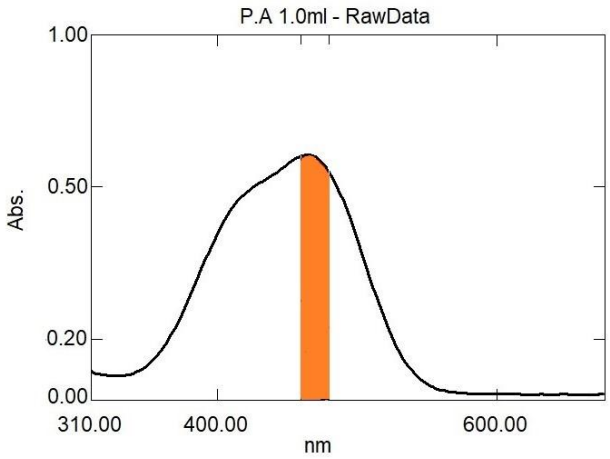


Fig. 11: Spectrum of the colored azo dye, showing area under the peak.

Methodology and calibration curve

0.025-2 ml of 100 µg/ml of diazotized PROC-HCl was added to a series of 10 ml volumetric flasks, then 1.5 ml of $4x10^{-2}$ M of 2,5-dimethylphenol was added to them, finally 2.5 ml of 1M NaOH solution was added. The volume was completed to the mark with distilled water and the peak

area of the colored azo dye solutions was measured at 460-480 nm against the blank solution. A linear calibration graph was obtained over a concentration of 0.25-20 µg /ml diazotized (PROC-HCl), as shown in Fig. (12) with molar absorptivity 8.919x103 L/mol.cm, and Sandell's sensitivity index equal to 0.030 µg/cm^2.

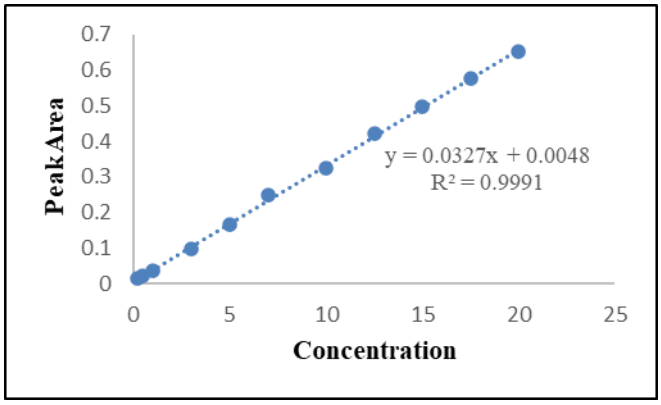


Fig. 12: Calibration curve between concentration and peak area.

Analytical application

The suggested methodology has been employed for the quantification of Procaine in injection form. The application of this proposed procedure has yielded favorable outcomes in terms of recoveries, accuracy (expressed as Relative error %), and precision (expressed as Relative Standard Deviation (RSD%)), as illustrated in (Table 8).

Table 8: Analytical application for pharmaceutical in dosage form (injection), using peak area.

Pharmaceutical preparation	Amount taken (µg/ml)	Amount measured (µg/ml)	Recovery %	Relative error %	RSD %	Drug contains (g)
Procaine/ injection (300000IU, 0.295g) (Jaber Ebne Hayyan company,Iran)	5.0	4.96	99.20%	-0.8%	2.06	0.2926
	10.0	9.93	99.30%	-0.7%	1.02	0.2929
	12.5	12.38	99.04%	-0.96%	0.415	0.2921
Procaine/ injection (600000 IU) 0.590 g (Devapen, Türkiye)	5.0	5.0	100.00%	0%	1.67	0.5900
	10.0	10.0	100.00%	0%	0.81	0.5900
	12.5	12.47	99.70%	-0.3%	0.66	0.5882

Standard addition method

The standard addition technique also has been employed to calculate procaine drug quantity, ensuring there was zero interferences with pharmaceutical industry additives. Two different concentrations of procaine penicillin (5 and 7 µg/ml) from the pharmaceutical solution for injection ampoule, augmented by various proportions of pure-form studied diazotized PROC-HCl under one condition: It should not surpass the highest estimation range on our calibration graph. It's worth noting that at least one volumetric flask had a known amount of pharmaceutical formulation without its standard compound as a reference in this experiment. These insertions took place into dual series within 10 ml volumetric flasks, with optimal conditions defined priory. Absorbance readings were performed against blank solutions, in which visible at a wavelength of 466 nm. Figures reflecting results have been shown in Fig. (13), and (Table 9).

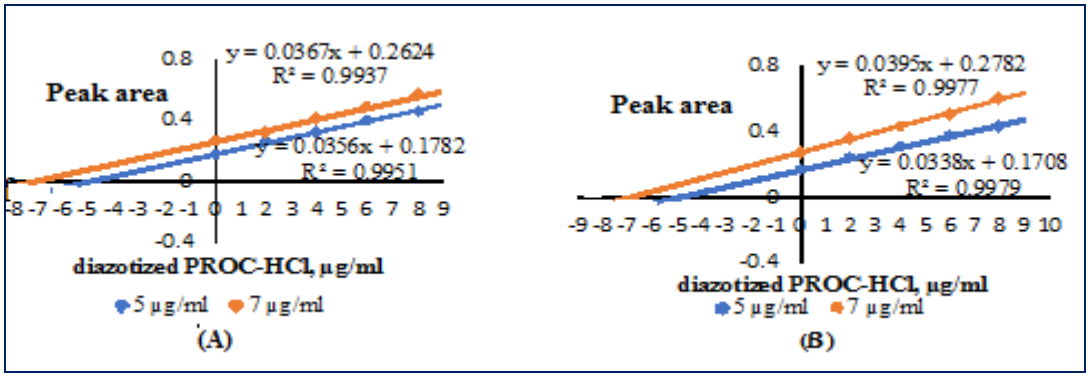


Fig.13: Standard addition method curve for the determination of procaine (injection). (A) Iranian company, (B) Turkish company.

Table 9: Standard addition method for the determination of procaine.

Pharmaceutical preparation	Amount taken (µg/ml)	Recovery %	Drug contains (g)
Procaine / injection (300000 IU, 0.295 g) (Jaber Ebne Hayyan company,Iran)	5	100.0%	0.290
	7	102.0%	0.300
Procaine / injection (600000 IU, 0.590 g) (Devapen, Türkiye)	5	101.0 %	0.595
	7	100.5%	0.592

Comparison of the methods

Some analytical variables of the two proposed spectrophotometric methods for the determination of PROC-HCl were compared with each other using different reactions, reagents, and conditions. The results are in (Table 10).

Table 10: Comparison of some of the analytical variables of the two proposed methods with literature method for estimation of PROC-HCl.

Parameter	Method I	Method II	Literature method
Method/ Measurement	Diazotization and coupling/ Abs.*	Diazotization and coupling/ AUC**	Diazotization and coupling
Reagent used	2,5-Dimethylphenol	2,5-Dimethylphenol	Phenol
Wavelength (nm)	466	460-480	450
Product colour	Yellowish-orange	Yellowish-orange	Yellow
Beer's law range (µg/ml)	1-15	0.25-20	2-22
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	2.193 x 104	8.919×10^3	2.469×10^4
Sandell's index ($\mu g/cm^2$)	0.0124	0.030	11.045×10^{-3}
Determination coefficient (R^2)	0.9997	0.9990	0.9966
LOD (µg/ml)	0.105	----	1.2931
RSD%	0.324-1.215	0.415-2.060	

*Absorbance, **Area under the curve

According to the results of the comparison table, the sensitivity of the method used for comparison is higher than the two proposed methods, but the two methods are no less important than the comparison method and have an application scope with analytically satisfactory results.

CONCLUSIONS

Two sensitive, simple, and cost-effective methods are proposed for the quantification of procaine, involving the coupling of diazotized PROC-HCl with the 2,5-dimethylphenol reagent under alkaline conditions. The first method has been adeptly applied for the assay of procaine in its formulation (injection), and also in human blood serum. In addition, area under the peak was studied and applied to pharmaceutical formulation of procaine. The second method showed more enhancement in the recovery and expanded the linearity range of the calibration curve. The results obtained affirm the absence of interference in the commercial dosage form, further attesting to the method's reliability and specificity. Consequently, it can be deduced that the developed method is well-suited for the routine analysis of procaine.

REFERENCES

Al-Abachi, M.Q.; Al-Uzri, W.A.; Al-Ward, H.S. (2012). Batch and flow-injection spectrophotometric determination of procaine-HCl in pharmaceutical preparations via using diazotization and coupling reaction. *J. Baghdad Sci*, **9**(3), 521-531. DOI: 10.21123/bsj.2012.9.3.521-531

Al-Uzri, W.A. (2015). Spectrophotometric determination of procaine hydrochloride inpharmaceutical preparations via diazotization-coupling reaction with phenol. *Asian J. Chem.*, **27**(12), 4449-4453. DOI: 10.14233/ajchem.2015.19169

Al-Uzri, W.A.; Al-Heeti, M. (2015). Colorimetric determination of procaine hydrochloride in pharmaceutical preparations using diazotization coupling reaction. *Inter. J. Pharm. Tech. Res.*, **8**(5), 1042-1052.

Ashour, S.; Boudakji, A.; Al-farers, B. (2009). Simultaneous determination of procaine hydrochloride and phenazone in pharmaceutical preparations by derivative spectrophotometric method. *Res. J. Aleppo. Uni*., **64**(5), 52-63.

British Pharmacopoeia (2009), 4th ed., Pharma, Press London, 4985.

Carmona, M.; Silva, M.; Bendito, P. (1992). A selective and sensitive kinetic method for the determination of procaine and benzocaine in pharmaceuticals. *J. Pharm. Biomed. Ana.*, **10**(2-3), 145-152. DOI: 10.1016/0731-7085(92)80022-f

Chen, Y.; Tian, F.; Song, M. (2009). Spectrophotometric determination of procaine hydrochloride with hemoglobin as catalyst. *J. Ana. Chem.*, **64**(4), 366-370. DOI: 10.1134/s106193480904008x

Haghighian, F.; Ghoreishi, S.M.; Attaran, A.; Kashani, F.Z.; Khoobi, A. (2023). Electrochemical study for simultaneous detection of procaine hydrochloride and its metabolite in biological samples using a nanostructured strong sensor. *Korean J. Chem. Eng.*, **40**(3), 650-656. DOI: 10.1007/s11814-022-1290-1

Hargis, L.G. (1988). "Analytical Chemistry: Principles and Techniques". Prentice Hall International, London, pp: 424-427.

Hasan, S.H.; Othman, N.S.; Surchi, K.M. (2020). Using of diazotized 2,4-dinitroaniline in spectrophotometric estimation of phenylephrine hydrochloride. *Raf. J. Sci.*, **29**(3), 37-46. DOI: 10.33899/rjs.2020.166310

He, Y.T.; Peng, J.D.; Tang, J.X.; Zhang, C. (2013). Incorporation of high-performance liquid chromatography with resonance Rayleigh scattering detection for determination of procaine and lidocaine in human plasma. *Ana. Meth*., **5**(24), 7110. DOI: 10.1039/c3ay40725j

Hiba, A.A.; Othman, N.S. (2022). Estimation of p-aminophenol via diazotization and coupling reaction with 4-chlororesorcinol-Application on paracetamol. *Raf. J. Sci.*, **31**(1), 35-46. DOI: 10.33899/rjs.2022.172930

Hussein, A.M.; Othman, N.S. (2023). Development of a new analytical method for estimation of trifluoperazine-hcl in pharmaceutical formulation. *Israa Uni. J. App. Sci.*, **6**(2), 56–70. DOI: 10.52865/rwic5362

Li, X.X.; Yun, X.S.; Huai, Y.W.; Ji, G.J.; Yan, X. (2003). Spectrophotometric determination of procaine hydrochloride in pharmaceutical products using 1,2-naphthoquinone-4-sulfonic acid as the chromogenic reagent. *Spec. Acta Mol. Biom. Spec.*, **59**(13), 3103-3110. DOI: DOI: 10.1016/S1386-1425(03)00125-2

Luminiţa, V.; Dana, M.; Irinel, B.; Carmencita, C. (2002). Determination of procaine hydrochloride by potentiometric titration. *R. Roumaine Chim.*, **47**(8–9), 911–915.

Luo, M.J.; Xiaohui, L.; Ziling, X. (1997). Determination of procaine hydrochloride by ion-pairing flow injection analysis with piezoelectric detection. *Fresenius J. Ana. Chem.*, **358**, 556–558. DOI: 10.1007/s002160050469

Mahmoud, K.M.; Taha, S.S. (2016), Batch and flow injection spectrophotometric determination of procaine-HCl using diazotization-coupling reaction. *J. Basic App. Res.*, **2**(4), 577-583.

Marin, N.M.; Batrinescu, G.; Nita-Lazar, M.; Pascu, L.F.; Lehr, C.B. (2019). Simultaneous determination of procaine hydrochloride, procainamide hydrochloride and lidocaine by molecular absorption spectrometry. *Inter. Sym. Envir. Industry*, Simi 2019, Proceedings Book, 318-324. DOI: 10.21698/simi. 2019.fp40

Nianbing, L.; Jianping, D.; Guonan, C. (2003). Determination of trace procaine hydrochloride by differential pulse adsorptive stripping voltammetry with a nafion modified glassy carbon electrode. *Ana. Sci.*, **19**, 1587-1592. DOI: 10.2116/analsci.19.1587

Pasekova, H.; Polasek, M. (2000). Determination of procaine, benzocaine and tetracaine by sequential injection analysis with permanganate-induced chemiluminescence detection. *Talanta*, **52**(1), 67-75. DOI: 10.1016/S0039-9140(99)00341-0

Plotycya, S.; Strontsitska, O.; Pysarevska, S.; Blazheyevskiy, M.; Dubenska, L. (2018). A new approach for the determination of benzocaine and procaine in pharmaceuticals by single-sweep polarography. *Inter. J. Electr.* **2018**, 1376231. DOI: 10.1155/2018/1376231

Qader, I.B.; Ismail, H.K.; Alesary, H.F.; Kareem, J.H.; Maaroof, Y.T.; Barton S. (2023). Electrochemical sensor based on polypyrrole/ triiron tetraoxide (PPY/Fe_3O_4) nanocomposite deposited from a deep eutectic solvent for voltammetric determination of procaine hydrochloride in pharmaceutical formulations. *J. Electr. Chem.*, **951**, 117943. DOI: 10.1016/j.jelechem.2023.117943

Savchenko, L.; Georgiyants, V. (2018). Development and validation of the UPLC method for the simultaneous assay of the compounding ointment components. *J. App. Pharm. Sci.*, **8**(5), 61–67. DOI: 10.7324/japs.2018.8508

Taha, S.S.; Ali, D.S. (2023). Oxidative coupling assay for mesalazine determination in pharmaceuticals and spiked human plasma utilizing syringic acid. *J. Spectr.*, **2023**, Article ID 4583013. DOI: 10.1155/2023/4583013

Yuan, J.; Yin, J.; Wang, E. (2007). Characterization of procaine metabolism as probe for the butyrylcholinesterase enzyme investigation by simultaneous determination of procaine and its metabolite using capillary electrophoresis with electrochemiluminescence detection. *J. Chrom. A.*, **1154**(1-2), 368-372. DOI: 10.1016/j.chroma.2007.02.024

Zakaria, S.A.; Talal, Z.; Othman, N.S. (2022). Using 2,4-dinitrophenylhydrazine in spectrophotometric determination. *Samarra J. Pure App. Sci*., **4**(2), 107-117. DOI: 10.54153/sjpas. 2022.v4i2.368

Zhu, Y.; Xu, Y.; Liu, G. (2021). Electrochemical detection of the anesthetic drug procaine hydrochloride based on molecularly imprinted polymer/diamond-graphite composite electrode. *Inter. J. Electro. Sci.*, **16**(12), 211237. DOI: 10.20964/2021.12.35

تقدير هيدروكلوريد البروكايين بطريقتين طيفيتين في مستحضراته الصيدلانية ومصل الدم البشري

كولان صلاح قادر نبيل صبيح عثمان عامر ذنون عبد الرحمن

قسم الكيمياء/ كلية العلوم/ جامعة الموصل/ موصل/ العراق

الملخص

تم اقتراح طريقتين عاليتي الحساسية والبساطة، استنادًا إلى القياس الطيفي، للتقدير الكمي لعقار التخدير الموضعي بروكايين هيدروكلوريد (PROC-HCl) في حالته النقية وكذلك في المستحضرات الصيدلانية ومصل الدم البشري. تعتمد الطريقة الأولى على ازوتة PROC-HCl إلى ملح الديازونيوم المقابل، باستخدام نتريت الصوديوم في الظروف المحيطة وفي وجود حامض الخليك. بعد ذلك، يتم الاقتران مع كاشف 2،5- ثنائي ميثيل فينول لتكوين صبغة آزوية ذات لون أصفر- برتقالي. الصبغة تظهر ذروة امتصاص قصوى عند الطول الموجي 466 نانومتر. وجد أن قانون بير قابل للتطبيق ضمن نطاق تركيز من 1 إلى 15 ميكروغرام/مل، ومعامل تقدير 0.9997، ومعامل امتصاص مولاري 2.1×10^4 لتر/مول سم، وحساسية ساندل 0.012 ميكروغرام/سم^2 وكان حد الكشف 0.105 ميكروغرام/ مل، وحد التقدير الكمي 0.352 ميكروغرام/ مل. تم تحديد النسبة المتكافئة للتفاعل الذي يتضمن اقتران البروكائين المؤزوت مع 2،5- ثنائي ميثيل فينول عند 1:1. إن استخدام الطريقة المقترحة لتقدير البروكايين بمستحضره (الحقن) وعينة المصل أدى إلى نتائج تحليلية تعتبر مقبولة ضمن المعايير العلمية.

تعتمد الطريقة الثانية على نفس مسار التفاعل ولكن بدلاً من قياس الامتصاص يتم قراءة المساحة تحت الذروة، مع تحديد نطاق الطول الموجي بـ 460-480 نانومتر. كان قانون بيرة قابلاً للتطبيق في نطاق التركيز من 0.25 إلى 20 ميكروغرام/مل، مع معامل تقدير 0.9990. وأخيراً، تم تطبيق الطريقة على مستحضره حقن البروكايين مما أدى إلى نتائج أفضل. وعلى سبيل المقارنة بين الطريقتين، فإن الطريقة الثانية وسعت نطاق التركيز الخطي في منحنى المعايرة مما يؤدي إلى طريقة أكثر حساسية، وعززت الاستعادة اذ وصلت الى 100%.

الكلمات الدالة: بروكائين هيدروكلوريد، الأزوتة، المنطقة تحت الذروة، 2،5-ثنائي مثيل فينول، التقدير الطيفي.