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Spectrophotometric Determination of Procaine Hydrochloride in Pharmaceutical Preparations *via* Diazotization-Coupling Reaction with Phenol

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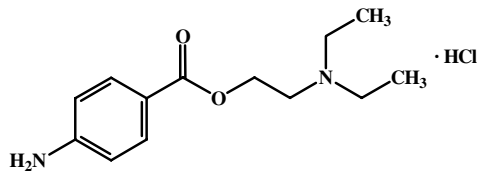
A simple, rapid and sensitive spectrophotometric method has been developed for determination of procaine hydrochloride in pure form and injections. The proposed method is based on coupling reaction between diazotized procaine hydrochloride with phenol in alkaline medium to form an intense yellow, water-soluble dye that is stable and has a maximum absorption at 450 nm. The calibration graph was linear over the concentration range of 2 to 22 μg mL^{-1} with a molar absorbtivity of 2.469×10^4 L mol^{-1} cm^{-1}. Sandell's sensitivity of 11.045×10^{-3} μg mL^{-1}. The optimum conditions for full colour development are described and the proposed method was probably applied satisfactorily to pharmaceutical injections.

Keywords: Procaine HCl, Spectrophotometry, Phenol, Diazotization coupling reaction.

INTRODUCTION

Procaine, 4-aminobenzoic acid 2-(diethyl amino) ethyl ester, is a local anesthetic[1] used alone or with penicillin as an antibacterial drug which is marketed as its hydrochloride salt, with molecular weight of 272.8 and the structure is[2]:



Procaine and the other local anesthetic drugs prevent the generation and the conduction of the nerve impulses. Their main site of action is the cell membrane, since conduction block can be demonstrated in giant axons from which the axoplasm has been removed. It is used in obstetrics and sometimes for relief pain in the lower back and tooth extraction[3].

Currently, several analytical methods for the quantitation of procaine in pharmaceutical formulations have been reported. Examples of these methods are high performance liquid chromatography[4,5], gas chromatography[6], electrophoresis[7,8], differential-plus voltammetry and electrochemical analysis[9-12], chemiluminsense[13], atomic absorption[14], ion association titration[15], flow injection analysis[16,17], fluorimetry[18-20], sequential injection analysis[21,22],liquid chromatography[23], colorimetry and spectrophotometric methods[24-30]. However, some of these methods are time consuming and/or require expensive equipment and conditions. In this work, rapid and sensitive method using spectrophotometric detection at 450 nm was proposed for the determination of procaine in pharmaceutical preparations. The method is based on diazotiazation and coupling reaction between diazotized procaine with phenol reagent in alkaline medium. The yellow product was spectrophotometrically measured at 450 nm. The analytical procedure is simple, fast, accurate and has been applied for the determination of procaine in pure and injections preparations using standard additions method.

EXPERIMENTAL

All spectral and absorbance measurements were carried out on a Shimadzu UV- Visble-260 digital double-beam recording spectrophotometer (Tokyo-Japan), using 1-cm quartz cells.

All chemicals used were of analytical reagent grade. Procaine hydrochloride standard material was provided from the state company for drug industries and medical appliances (SDI) Sammara, Iraq.

Procaine hydrochloride solution (1000 μg mL^{-1}): A 0.1 g of procaine hydrochloride was dissolved in distilled water and the solution was made up to volume of 100 mL in volumetric flask with the same solvent. To obtain procaine hydrochloride working solution (100 μg mL^{-1}) a 10 mL volume of the stock solution was transferred into a 100 mL volumetric flask and made up to the mark with distilled water. More dilute solutions

were prepared daily by appropriate dilution using distilled water.

Sodium nitrite solution (3.65×10^{-4} M): Sodium nitrite solution was prepared freshly by dissolving 0.0126 g of $NaNO_2$ in small amount of distilled water then make up to 500 mL with distilled water.

Phenol (3.66×10^{-4} M): Phenol solution was prepared by dissolving 0.0172 g of phenol in distilled water then completed the volume to 500 mL with distilled water.

HCl (0.1 M): It was prepared by diluting 4.35 mL of 11.49 M of concentrated hydrochloric acid with distilled water then completed the volume to 500 mL with distilled water.

Ammonium hydroxide (0.1 M): Ammonium hydroxide solution was prepared by diluting 3.75 mL of 13.36 M of concentrated ammonium hydroxide with distilled water in 500 mL volumetric flask.

More dilute solutions were prepared fresh daily by dilution of the stock solution with distilled water.

Procedure for injections: Two types of injection were analyzed by the developed method, these include:

• Procaine benzyl penicillin injection (300 mg procaine penicillin)-Ajanta House Charkop-India

• Procaine benzyl penicillin injection-(800 mg procaine penicillin)-Troge Medical GMBH -Germany.

For these types of injection, an accurately weighed portion from mixed three vials powder, equivalent to about 0.01 g of procaine HCl, was dissolved in distilled water. The solution was transferred into 100 mL volumetric flask and diluted to the mark with distilled water.

Further appropriate solutions of pharmaceutical preparations were made up by simple dilution with distilled water.

General procedure for calibration: An increasing volumes (0.1-5.5 mL) of 100 μg.mL^{-1} procaine HCl was transferred into a series of 25 mL standard flask. To this solution was added equimolar of sodium nitrite solution (3.65×10^{-4} M) and the acidity was adjusted with 0.8 mL of 0.1 M hydrochloric acid solution with cooling the contents to 10 °C by using an ice bath, then shake well and followed by adding 5 mL of phenol (3.66×10^{-4}) and 4 mL ammonium hydroxide (0.1 M). The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 15 min, the absorbance of the yellow dye formed was measured at 450 nm against a reagent blank. For the optimization of conditions and in all subsequent experiments, a 1 mL of (100 μg mL^{-1}) of procaine in a final volume of 25 mL was used.

RESULTS AND DISCUSSION

Absorption spectra: Factors affecting on the sensitivity and stability of the coloured products resulting from the coupling reaction between diazotized procaine and phenol in an alkaline medium were carefully studied. A typical spectrum for the formed azo dyes were measured *versus* reagent blank which has negligible absorbance at λ_{max} 450 nm (Fig. 1).

Optimization of the experimental conditions: The effects of various parameters on the absorption intensity of the formed products were optimized.

The diazotization reaction of procaine was formed in acidic medium. Therefore the effects of different acids solutions

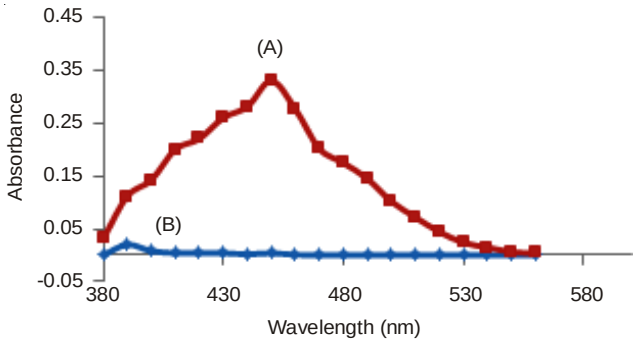


Fig. 1. Absorption spectra of the azo dye (4 μg mL^{-1}) against reagent blank (A) and blank against distilled water (B)

(0.1 M) were studied such as hydrochloric acid, sulfuric acid, phosphoric acid and acetic acid. It was found that hydrochloric acid was the most suitable acidic medium for a maximum absorbance and was used in all subsequent experiments.

The coupling reaction of diazotized procaine with phenol was formed in alkaline medium. Therefore, the effects of different alkaline solutions (0.1 M) were studied such as sodium hydroxide, sodium carbonate, potassium hydroxide and ammonium hydroxide. It was found that ammonium hydroxide was the most suitable alkaline medium for a maximum absorbance and was used in all subsequent experiments.

The effect of different volumes of hydrochloric acid (0.1 M) were studied on the maximum absorbance by varying the volume of HCl between (0.2-1.2 mL) and fixing the other parameters. It was found that 0.8 mL of HCl (0.1 M) gave the highest absorbance and was chosen for further use (Fig. 2).

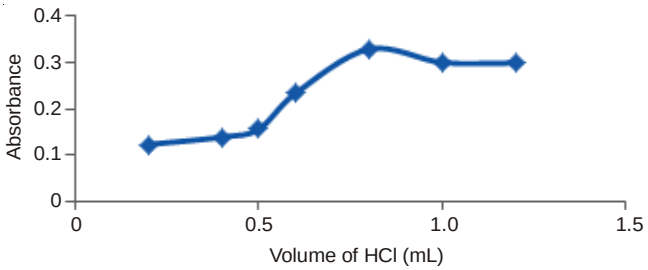


Fig. 2. Effect of the volume of HCl (mL)

Similarly, the effect of different volumes of ammonium hydroxide (0.1 M) was studied on the maximum absorbance by varying the volume of ammonium hydroxide solution between (0.5-6 mL) with fixing the other parameters. Volume of 4 mL of ammonium hydroxide (0.1 M) was enough to obtain the maximum absorbance (Fig. 3).

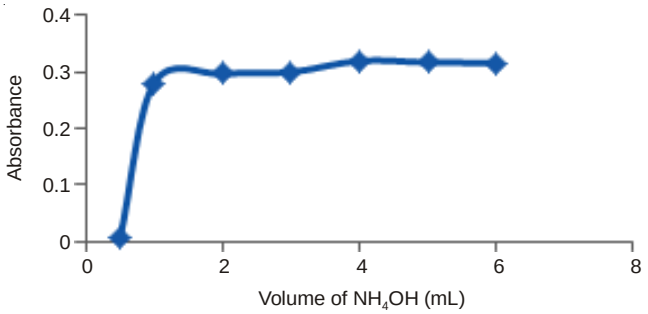


Fig. 3. Effect of the volume of ammonium hydroxide (mL)

Effect of volume of the reagents: Effect of reagent phenol (3.66×10^{-4} M) was studied in the range of (1-7 mL) with fixing the volumes of HCl and NH_4OH. The greatest absorbance intensity was obtained with 5 mL of phenol (Fig. 4).

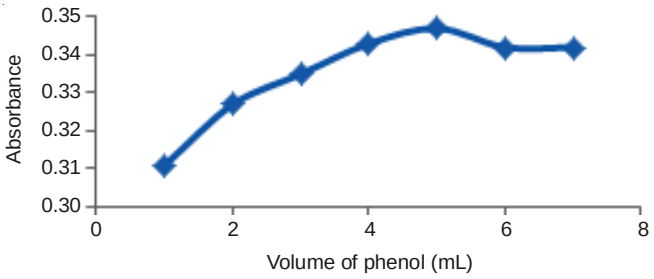


Fig. 4. Effect of the volume of reagents (mL)

Effect of order of addition: Different orders of addition of reagent was examined and it was found that the order of addition of reagent by mixing phenol with cited under general procedure was optimum and was used in all subsequent experiments.

Effect of reaction time: In spite of the rapid colour development (formed immediately) the colour intensity reached a maximum after diazotized procaine solution had been reacted with phenol and ammonium hydroxide for 15 min, therefore a 15 min development time was selected as optimum in the general procedures. The colour obtained was stable for 6 h.

Structures of the products: The stoichiometry of the reaction between diazotized procaine and phenol was investigated using both continuous variation and molar ratio methods respectively, The results (Figs. 5 and 6) show that a (1:1) was formed between diazotized procaine and phenol.

A reaction subsequent based on the above results is shown in **Scheme-I**.

The product formed was soluble in water. The apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of diazotized procaine (3.66×10^{-4} M) (A_S) with that of a solution containing

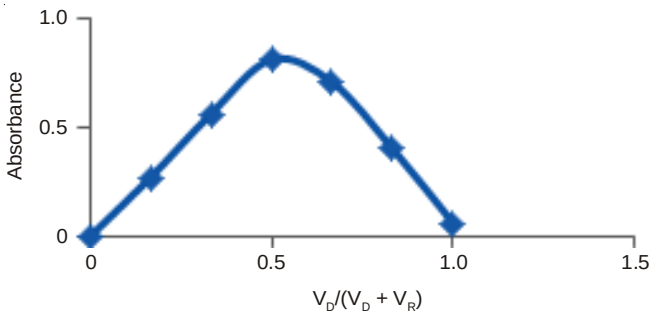


Fig. 5. Continuous variation plot

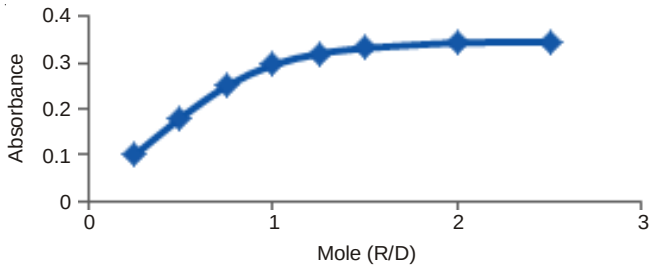
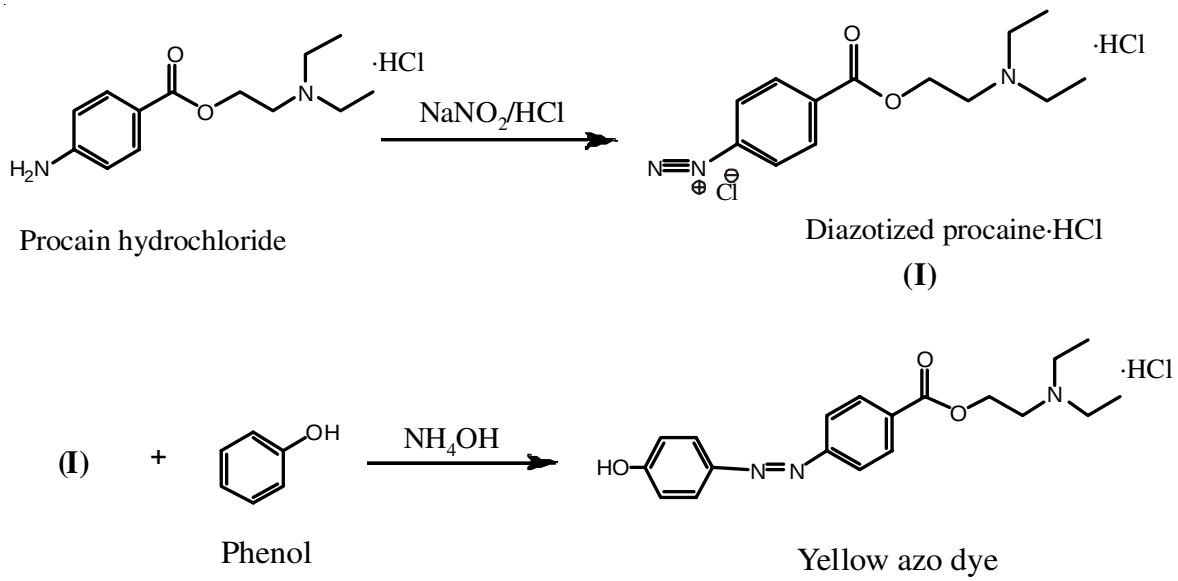


Fig. 6. Mole ratio plot

a five-fold excess of phenol reagent (A_m) and according to analytical procedure. The average stability constant (K) = 2.433 $\times 10^6$ L mol^{-1}, where is [$K = (1-\alpha)/\alpha^2 C$] and $\alpha = A_m - A_s/A_m$[31].

Analytical characteristics of spectrophotometric method: For the proposed method, a calibration graph was obtained and a series of standard solutions was analyzed in triplicate to test the linearity. The molar absorptivity (ε), the Sandell's sensitivity (S), the slope (a), the intercept (b), the correction coefficient (r) and correlation of determination (r^2) were determined and are included in Table-1.

Statistical evaluation[32] of the regression line gave the values of standard deviations for residuals ($S_{y/x}$), slope (S_a) and intercept (S_b) at 95 % confidence are shown in the same table. The limit of detection (LOD) and limit of quantitative (LOQ) were determined by using the formula: LOD or LOQ



Scheme-I: Reaction sequence

TABLE-1
DATA FOR CALIBRATION GRAPH FOR PROCAINE USING THE PROPOSED METHOD

Parameters	Value
Linearity range (μg mL^{-1})	2 - 22
Molar absorbtivity (L mol^{-1} cm^{-1})	2.469×10^4
Sandell's sensitivity (μg cm^{-2})	11.045×10^{-3}
Correction coefficient	0.9983
Correlation of determination	0.9966
Slope (a)	0.0798
Intercept (b)	0.051
$S_{y/x}$	3.4396×10^{-2}
Standard deviation of slope (S_a)	1.5442×10^{-3}
Standard deviation of intercept ($S_{b)}$	1.8939×10^{-2}
LOD (μg mL^{-1})	1.2931
LOQ (μg mL^{-1})	4.3103
Molar ratio (D:R)	1:1

= k $S_{y/x}$ /b, where k = 3 for LOD and 10 for LOQ. The LOD and LOQ values are shown in Table-1.

The accuracy and precision of the proposed method was tested by analyzing four replicate of diazotized procaine using the proposed spectrophotometric method for three different concentrations of diazotized procaine. The values of relative standard deviation RSD % and relative error E_{rel} % are summarized in Table-2.

TABLE-2
ACCURACY AND PRECISION OF THE PROPOSED METHOD

Amount of procaine (μg mL^{-1})		RSD (%)	E_{rel} (%)	Recovery (%)
Present	Found			
6.00	6.00	0.360	0.00	100.00
8.00	7.857	1.319	-1.788	98.213
12.00	12.112	0.612	0.933	100.933

Pharmaceutical application: Two types of injections containing procaine have been analyzed. It was found that when the proposed method was applied to the determination of procaine in injections, the recovery % was around 115 %. This might be due to the interaction of the benzyl penicillin that present with procaine injections. Therefore, a standard additions method is applied (Fig. 7) which involves adding increment volumes (0-2 mL) of a standard solution of 100 μg mL^{-1} of procaine to a fixed volume sample (1 mL of 100 μg mL^{-1} of pharmaceutical preparations) and employing the conditions described under procedure. They gave a good accuracy and precision (Table-3).The proposed method was compared successfully with the British pharmacopeia's standard method[2] (Table-3).

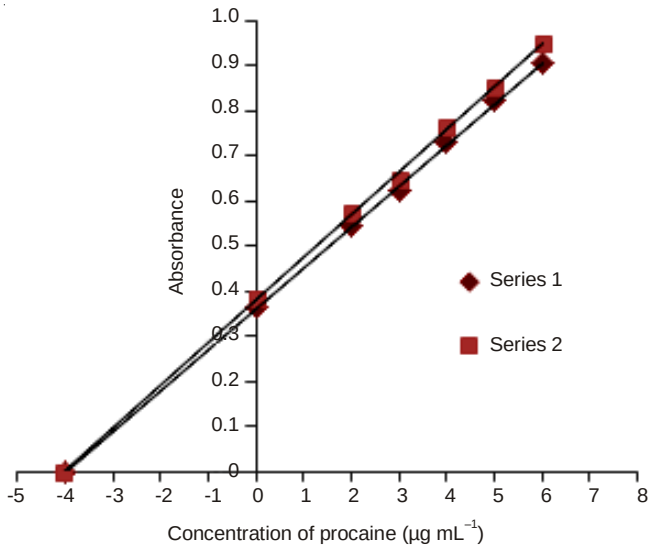


Fig. 7. Standard additions method for determination of procaine in pharmaceutical injections (Series 1: Procain India, Series 2: Procain Germany

Statistical analysis[32] and by applying F-test and t- test, at 95 % confidence level. The calculated values for F(4.326) and t (0.765) did not exceed the critical values of F = 19.00 and t = 2.776 (n_1+n_2-2=4).These confirming that there are no significant differences between the proposed method with the official method with respect to precision and accuracy in the determination of procaine in pharmaceutical preparations.

Conclusion

The proposed method was found to be simple, rapid, low cost and fairly selective than some of the reported methods. They had an advantage of being accurate, did not require the removal of excipients, any chemical sample pretreatment, temperature control, pH control, solvent extraction step and high cost reagents and solvents. The proposed method was applied to the analysis of procaine in injections and can be used for the routine analysis.

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TABLE-3
APPLICATION OF THE STANDARD ADDITIONS METHOD AND OFFICIAL METHODS FOR THE DETERMINATION OF PROCAINE IN INJECTIONS

Injection samples	[Procaine] depend on standard additions	Standard additions method		Official method [Ref. 2]
		Recovery (%)	RSD (%)	Recovery (%)
Procaine benzyl penicillin injection (300 mg procaine penicillin), Indian	3.99	99.75	1.101	99.82
Procaine benzyl penicillin injection (800 mg procaine Penicillin), Germany	4.02	100.50	2.201	101.50

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