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Colorimetric Determination of Procaine hydrochloride in Pharmaceutical Preparations using Diazotization Coupling Reactions

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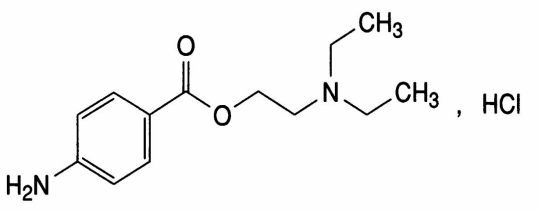
Abstract: A simple and direct spectrophotometric method for quantitative estimation of procaine hydrochloride in pure forms and injections was adopted. The established method is based on coupling reaction between diazotized procaine hydrochloride with 7-iodo-8-hydroxyquinoline 5- sulphonic acid in alkaline medium to form an intense orange, water-soluble dye that is very stable and has a maximum absorption at 500 nm. Regression analysis of Beer's law plot showed a good correlation in the concentration range of 1-14 μg ml^{-1} with a molar absorbtivity of1.429×10^4 L $mol^{-1}cm^{-1}$, Sandell's sensitivity of 19.085 $\times 10^{-3}$ μg ml^{-1}. The optimum conditions for full color development are described and the proposed method was probably applied satisfactorily to pharmaceutical injections.

Keywords: ProcaineHCl, colorimetry, 7-iodo-8-hydroxyquinoline5- sulphonic acid, diazotization coupling reaction.

INTRODUCTION

Procaine is a local anesthetic used alone or with penicillin as an antibacterial drug its chemical name is 2-diethyl aminoethyl-4-aminobenzoate hydrochloride and its formula $C_{13}H_{20}N_2O_2$, with molecular weight of 272.8 the structure of procaine HCl is [1]:



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 [2]. Several methods have been reported for the determination of procaine HCl. Examples of these methods are high performance liquid chromatography [3-5], chemiluminsense [6] gas chromatography [7], ion association titration [8]electrophoresis [9,10]differential-plus voltammetry and electrochemical analysis [11,12], liquid chromatography [13], atomic absorption [14], flow injection analysis [15,16], fluorimetry [17,18], sequential injection analysis [19,20], colorimetry[21] and spectrophotometric

methods [22-25]. However, the direct spectrophotometric methods which reported for the analysis of Procaine are still few. This research describes new spectrophotometric method for determination of Procaine by the diazotization-coupling reaction with a new reagent, 7-iodo-8-hydroxyquinoline 5-sulphonic acid in alkaline medium. This method was found to be useful for assay our target drug, because it produced stable coupling organic product rapidly, with high sensitivity. In addition this method gave a good recovery when applied for the determination of Procaine hydrochloride in pure and injections preparations using standard additions method.

EXPERIMENTAL

Apparatuse

All spectral and absorbance measurements were performed on an Optima Spectrophotometer UV-VIS (Japan) double-beam and using 1 cm quartz cells.

Preparation of solutions

Procaine hydrochloride solution(1000 µg ml^{-1}=3.66× 10^{-3} M)

A 0.1000 gm amount 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 (3.65×10^{-4}M)

Was prepared freshly by dissolving 0.0126g of $NaNO_2$ (Merk) in small amount of distilled water then completed to 500 ml with the same solvent.

7--iodo-8-hydroxyquinoline 5-sulphonic acid (0.1%)

Was prepared by dissolving 0.1g of 7-iodo-8-hydroxyquinoline 5- sulphonic acid (BDH) in distilled water then completed the volume to 100 ml with the same solvent.

HCl (1M)

Was prepared by diluting 43.5ml of 11.49M of concentrated hydrochloric acid(BDH) with distilled water then completed the volume to 500 mL with the same solvent.

Ammonium hydroxide (2M)

Was prepared by diluting 74.85 ml of 13.36M of concentrated ammonium hydroxide(BDH) 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:-

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

2-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 0.0100 gm 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 (0.25-3.5 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 1 ml of 1M hydrochloric acid solution with cooling the contents to 15°C . then shake well and followed by adding 3 ml of 7--iodo-8-hydroxyquinoline 5- sulphonic acid (0.1%) and 1 ml ammonium hydroxide (2 M). The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 10 min, the absorbance of the orange dye formed was measured at 500 nm against a reagent blank. For the optimization of conditions and in all subsequent experiments, a 2.5 ml of (100 µgmL^{-1}) of Procaine in a final volume of 25mL was used.

RESULT AND DISCUSSION

ABSORPTION SPECTRA

When a very diluted aqueous solution of diazotized Procaine was mixed with 7-iodo-8-hydroxyquinoline 5-sulphonic acid in alkaline medium, an intense orange dyes produced directly, which became stable after 10min and remain stable for 2 hour at least and have a maximum absorption at 500 nm. Figure 1 shows the spectra of the products formed.

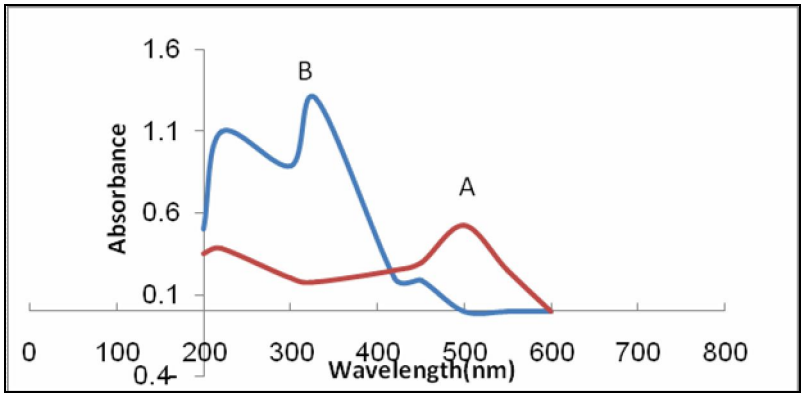


Fig.1: Absorption spectra of the azo dye (10 µg.mL^{-1}) of Procaine hydrochloride against reagent blank(A) and blank against distilled water (B)

STUDY OF THE EXPERIMENTAL CONDITIONS

The effects of various parameters on formation of the products and the absorption intensity were studied and optimized. A 10 µg mL^{-1} of Procaine was used in all optimization experiments.

- **Effect of acid used in diazotization process**

Acidic medium is very essential for accomplished the diazotization reaction. For that reason the effects of different prepared acids solutions (1M) were examined such as hydrochloric acid, nitric acid, sulfuric acid and acetic acid. HCl gave a higher absorbance than other acids, therefore hydrochloric acid was the most suitable acidic medium and was used in all subsequent experiments.

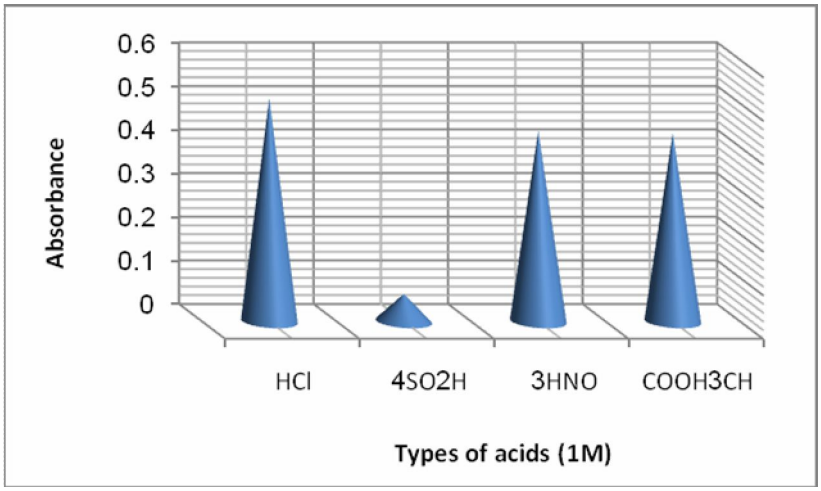


Fig.2: Effect of the type of acid

Consequently the effect of various volumes of hydrochloric acid (1M) was optimized on the maximum absorbance by changeable the volume of HCl between (0.25-4mL) and fixing the other parameters. The highest absorbance was obtained 1 mL of acid and was chosen for further use (Figure 3).

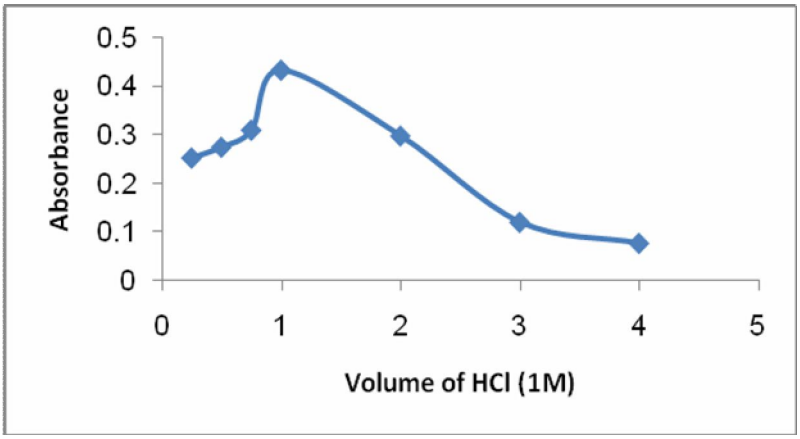


Fig.3: Effect of the volume of HCl (1M)

• Effect of the basic medium(type and volume)

After experimentation of a different medium in order to increase intensity of the reaction, the experiments indicated that the alkaline medium is necessary for developing a more intense color. Accordingly, a different alkaline solutions (2M) were examined such as sodium hydroxide, potassium hydroxide, ammonium hydroxide and sodium carbonate. With regard to Figure 4, it was clear that the ammonium hydroxide was the appropriate alkaline medium for a maximum absorbance was used in all next experiments

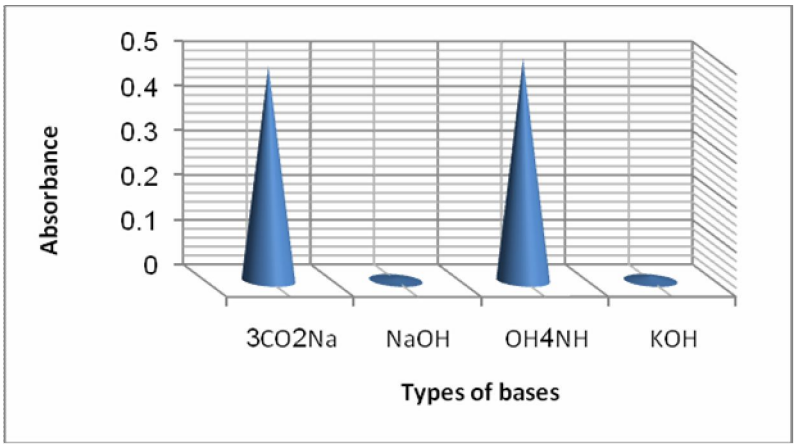


Fig.4: Effect of the type of base

Effect of different volumes of ammonium hydroxide (2M) was studied on the intensity of colored products by varying the volume of the base solution between (0.5-4mL) with fixing the other parameters. A volume of 1mL of ammonium hydroxide (2M) was enough to obtain the maximum absorbance (Figure 5).

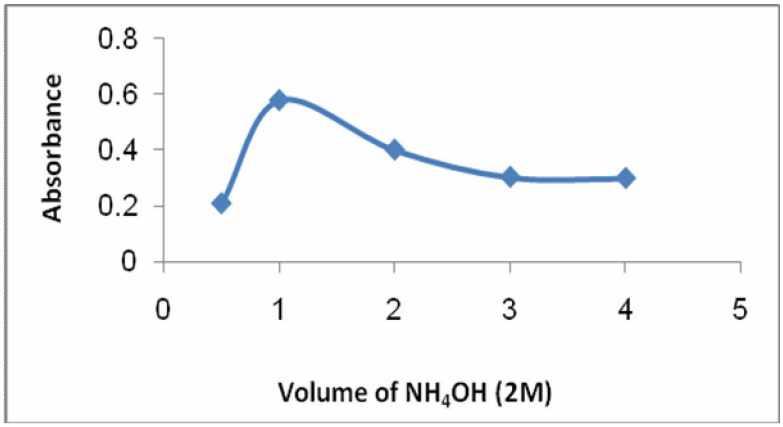


Fig.5: Effect of the volume of NH_4OH (2M)

- **Effect of coupling reagent concentration**

Different volumes of reagents (7-iodo-8-hydroxyquinoline 5-sulphonic acid (0.1%) was studied in the range of (0.5-4mL) with fixing the volumes of HCl and NH_4OH. The greatest absorbance intensity was obtained with 3ml of 7-iodo-8-hydroxyquinoline 5-sulphonic acid (Figure 6).

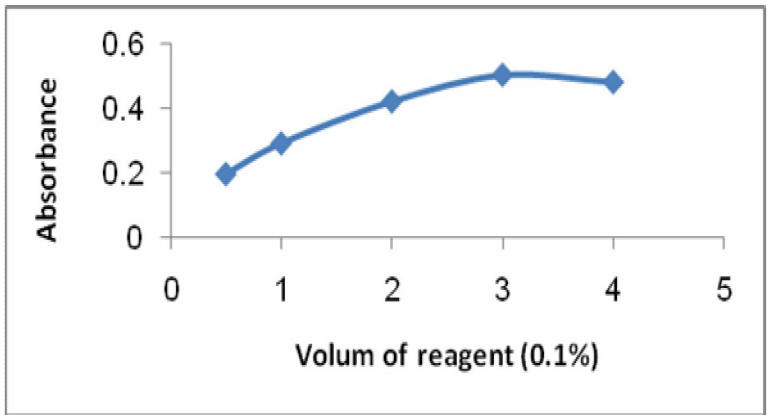


Fig.6: Effect of the volume of reagent (0.1%)

- **Order of addition of reagents**

The order of reagent addition is very important, so different orders of addition of reagent were examined and it was found that the order of addition of reagent by mixing Procaine with sodium nitrite then HCl, 7-iodo-8-hydroxyquinoline 5-sulphonic acid and ammonium hydroxide gave the highest absorbance and was used in all later experiments.

- **Effect of reaction time and color stability**

The resultant colored product of the proposed method was found to be formed rapidly and immediately, but the color intensity reached a maximum absorbance for 10min, therefore a 10 min development time was selected as optimum in the general procedure. The color obtained was stable for 2hr.

COMPOSITION OF THE PRODUCT

Continuous variation and mole ratio methods[26], had been established under the recommended optimum conditions in order to study the composition of the formed complexes between diazotized Procaine and 7-iodo-8-hydroxyquinoline 5-sulphonic acid .The results obtained in (Figure 7) and (Figure 8) show that a 1:1 azo dye was formed between diazotized Procaine and reagent at 500 nm.

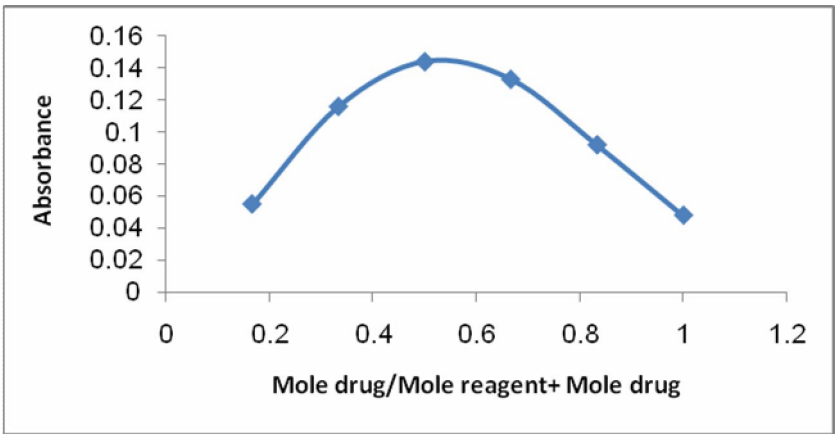


Fig.7: Continuous variation plot

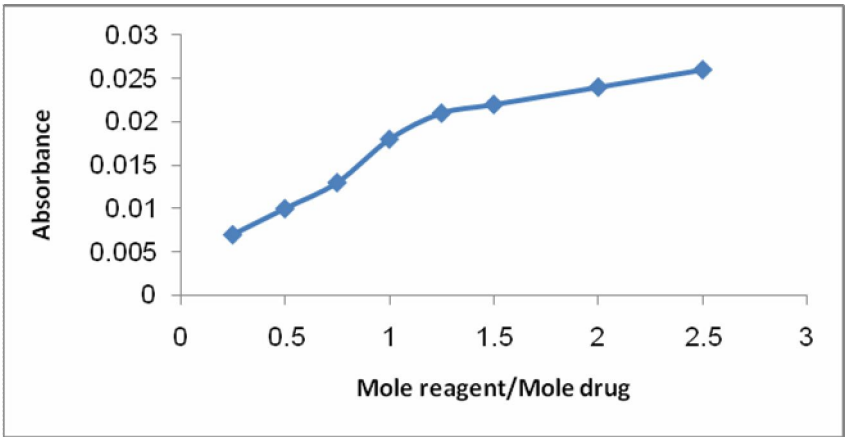
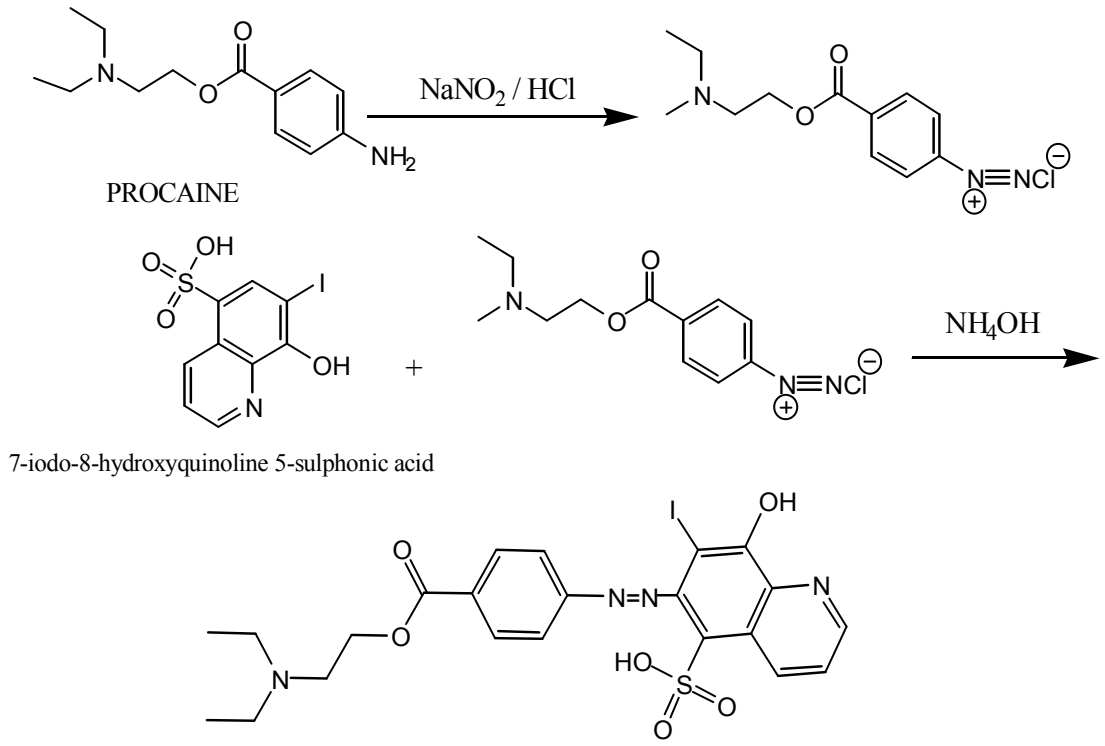


Fig.8: Mole ratio plot

MECHANISM OF REACTION

Usually two steps are required to accomplish the diazotization coupling reaction. The first step is converted the amino compound (Procaine) to diazo compound by reaction with nitrous acid ($NaNO_2$/HCl), while the second step is involved a coupling between diazotized drug and the coupling reagent [27]. The orange dye product was only formed in alkaline medium (ammonium hydroxide). According to mentioned mole ratio and continuous variation results, and the obtained ratios, the reaction pathway were postulated to proceed as shown in scheme(1) [28].



Scheme-1: **reaction sequence**

DETERMINATION OF STABILITY CONSTANT OF COMPLEX

The apparent stability constant [29] was calculated by comparing the absorbance of a solution containing stoichiometric amount (3.66×10^{-4}M) of diazotized Procaine and reagent 7-iodo-8-hydroxyquinoline 5-sulphonic acid (A_S) with that of a solution containing a five- fold excess of reagent (A_m) and according to analytical procedure. The average stability constant (K) = 0.575×10^{4} L mol^{-1} where [$K = (1-\alpha) / \alpha^2 C$; $\alpha = (A_m - A_s) / A_m$].

CALIBRATION GRAPH

Calibration graph (Figure 9) for the proposed method, was obtained by the procedure described before in which a sequence of standard solutions were analyzed in triplicates to test the linearity. All the analytical characteristics, like the slope (a), the intercept (b), and the correlation coefficient (r) were validated by a least-squares regression analysis and are incorporated in Table (1). The linearity was also obtained and the value of molar absorbtivity insure that method was sensitive for determination of Procaine.

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)

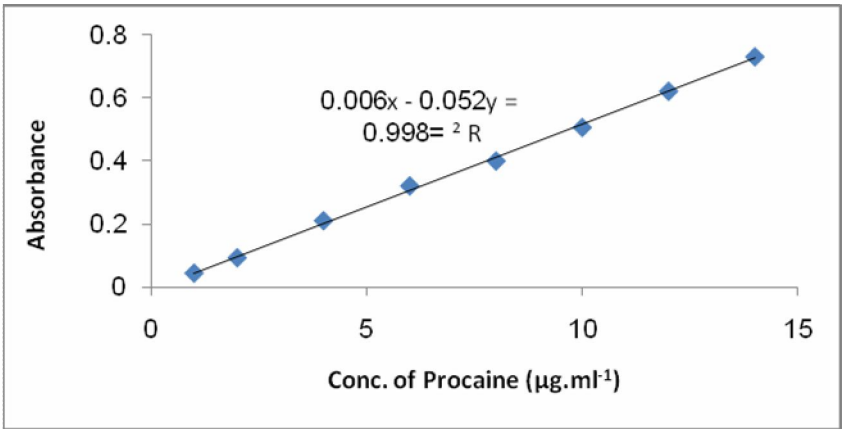


Fig.9: Calibration graphs of Procaine

Table 1: Data for calibration graph for Procaine using the proposed method.

Value	Parameters
500	$\ddot{e}_{max}$ (nm)
1 - 14	Linearity range, µg ml^{-1}
1.429×10^{4}	Molar absorbtivity (L mol^{-1} cm^{-1})
19.085×10^{-3}	Sandell's sensitivity(µg cm^{-2})
y = 0.0524x - 0.0067	Regression equation
0.9993	Correlation coefficient, r
0.9987	correlation of determination
99.87	Linearity percentage, r^{2}%
0.0524	Slop(a)
0.0067	Intercept (b)
0.062	LOD, µg ml^{-1}
1:1	Molar ratio (D:R)
2	Stability (hr)

Table 2: Accuracy and precision for the proposed method.

Amount of procaine(µg.ml^{-1})		RSD%	E_{rel}%	Recovery%
present	found			
4.00	3.950	5.150	-1.25	98.75
8.00	8.024	0.787	0.30	100.30
12.00	12.294	2.836	2.45	102.45

PHARMACEUTICAL APLICATION:

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 122 % ,this might be due to the interaction of the benzyl penicillin that present with procaine injections. Therefore, a standard additions method is applied (figure 10) which involves adding increment volumes

(0-1 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[1]. Table 3.

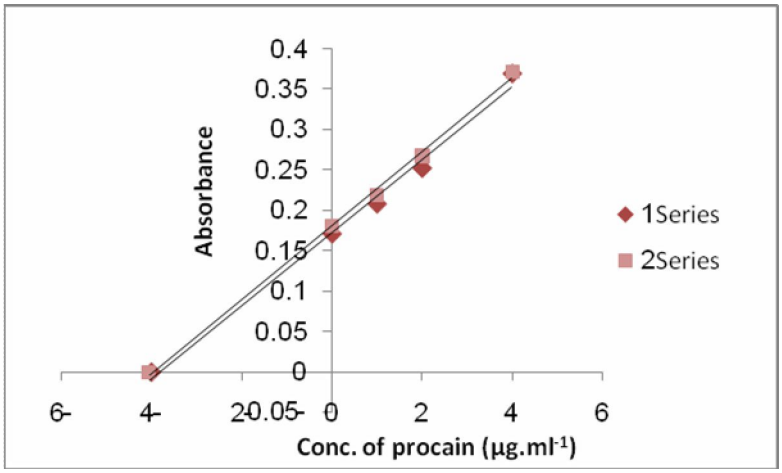


Fig.10: Standard additions method for determination of procaine in pharmaceutical injections (Series1:procain India , Series2:procain Germany

Table 3: Application of the standard additions method and official methods for the determination of Procaine in injections

Injection samples	[Procaine] depend on st.* additions	Standard additions method		Official method[1]
		Recovery, %	RSD%	Recovery, %
Procaine benzyl penicillin injection(300 mg Procaine penicillin)-Indian.	4.01	100.25	2.223	99.82
Procaine benzyl penicillin injection-(800 mg Procaine Penicillin)-Germany.	4.05	101.25	2.612	101.50

***Standard additions**

Statistical analysis [30] and by applying F-test and t- test, at 95% confidence level. The calculated values for F(3.141) and t (0.5163) 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.

CONCLUSIONS

This research offers new spectrophotmetric method using new reagent (7-iodo-8-hydroxyquinoline 5-sulphonic acid) as chromogenic agent for determination of Procaine drug. The developed method is very effortless and effective for the determination of Procaine in its injections without requiring any complicated steps. In addition the present method, as compared with other expensive techniques such as HPLC-MS, GC, fluorimetry, electro sensors and capillary electrophoresis, are economical and cheap and have an excellent accuracy and precision.

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