Mahmoud and Taha, 2016
J. basic appl. Res 2(4): 577-583

JBAAR
Journal of Basic And Applied Research
ISSN 2413-7014

Batch and flow injection spectrophotometric determination of procaine-HCl using diazotization-coupling reaction

Kamal M. Mahmoud* and Suzan S. Taha

Department of Chemistry, College of Science, Salahaddin University-Erbil Iraqi Kurdistan Region

*corresponding author: kamal.mahmoud@su.edu.krd or kamal_k58@yahoo.com

Received: 1-7-2016
Revised: 20-8-2016
Published: 30-9-2016

Keywords:
Spectrophotometry, Procaine-HCl, Diazotization-coupling, Flow Injection Analysis.

Abstract: This paper involves batch and flow injection spectrophotometric determination of procaine-hydrochloride in pharmaceutical formulations. These methods were based on the diazotization reaction of procaine-HCl with sodium nitrite and hydrochloric acid to form diazonium salt, which is coupled with 8-hydroxyquinoline in alkaline medium to form orange-pink water soluble azo dye that was stable and has a maximum absorption at 509 nm. In batch method Beer's law was obeyed in the concentration range of (0.8 - 8.0 µg/mL) and detection limit of 0.12 µg/mL with a correlation coefficient (r) of 0.9954 and a molar absorptivity of 2.0814×10^4 L $mol^{-1}cm^{-1}$. The flow injection analysis (FIA) system was applied for determination of procaine-HCl. The calibration graph is linear in the concentration range of (2.0 – 50 µg/mL) with detection limit (0.75 µg/mL) and correlation coefficient of (0.9927). The precision and accuracy of both methods were checked by calculating relative standard deviation (RSD) and relative error (E %) for two different levels of concentration.

Cite this article as: Mahmoud, K.M. and Taha, S.S. (2016). Batch and flow injection spectrophotometric determination of procaine-HCl using diazotization-coupling reaction. Journal of basic and applied Research 2(4): 577-583
Like us on Facebook - CLICK HERE Join us on academia - CLICK HERE Visit JBAAR on Google Scholar - CLICK HERE

1. INTRODUCTION

Procaine-HCl is a white crystalline powder and the chemical name is 2 diethylaminoethyl-4-aminobenzoate-hydrochloride (BP, 2009). The wide employing of procaine-HCl in both pharmaceutical industries and medicine doses, have prompted extensive literature on selective methods for its determination.

Several methods have been reported for the determination of procaine- HCl in biological sample and pharmaceutical dosage forms; include high performance liquid chromatography (HPLC) (Xiao Jun, *et al.* 2009; Luo, *et al.* 1998; Qing, *et al.* 2009), electrochemical analysis such as differential-pulse voltammetry (Zhang, *et al.* 2010 and Dutu, *et al.* 2014), electrophoresis (Yuan, *et al.* 2007), flow injection analysis (FIA) (Carmona, *et al.* 1992; Al-Abachi, *et al.* 2012; Al-Abachi and Al-Ward, 2012), chemiluminescence (Pasekova and Polasek, 2000), colorimeter and spectrophotometric methods (Irinel and Luminita, 2005; Ashour, *et al.* 2009; Chen, *et al.* 2009; Hamzah, *et al.* 2009; and Al-Abachi, *et al.* 2013).

FIA is always achieve to avoid human errors, decrease analysis time and improve precision. The most common detections in flow injection procedure have been carried out by spectrophotometric measurements.

Flow injection spectrophotometric method was described (Mahmoud, 2007) for the trace determination of chloramphenicol. This method depends upon the reduction of the nitro group of the chloramphenicol to the amino group using Zn-redactor mini-column, then diazotization of amino group with nitrous acid and coupling with 8-hydroxyquinoline (8-HQ) in alkaline medium to give an intense water soluble and stable azo dye with a maximum absorption obtained at wavelength of 485 nm. The calibration curve was applied in the range (0.5 – 50 µg/mL). The RSD was ≤ 1.4 % for (30 µg/mL) solution for 10 successive injections.

Spectrophotometric method had been developed for the quantitative determination of metoclopramide hydrochloride in both pure form and in its pharmaceutical formulations. The method was based on diazotization of primary amine group of metoclopramide hydrochloride with sodium nitrite and nitric acid followed by coupling with 8-HQ in alkaline medium to form a pinkish –red colored species, which showed a maximum absorption at 528 nm against reagent blank. Beer's law was obeyed over the concentration range of 5.0 - 300 µg/ 25 mL with a molar absorptivity 3.1×10^4 L $mol^{-1}cm^{-1}$ (Sarsam, *et al.* 2011).

Simple and selective spectrophotometric methods had been developed for determination of mosapride citrate (MDC) in pharmaceutical formulations. This method was based on the diazotization of MDC with sodium nitrite and hydrochloric acid, followed by coupling with resorcinol (A), 1-benzoylacetone (B) and 8-HQ (C) in alkaline medium, the coloured azo dyes were quantitated photometrical at 520, 428, and 535 nm for methods A, B and C respectively. Beer's law

was obeyed over the concentration ranges of (1.0 - 24.0, 1.0 - 28.0 and 1.0 - 20.0) µg/mL for methods A, B, and C, respectively (Satyanary and Nagesara, 2012).

The main goal in the present work involves batch and flow injection spectrophotometric determination of procaine-hydrochloride in pharmaceutical formulations. These methods were based on the diazotization reaction of procaine-HCl with sodium nitrite and hydrochloric acid to form diazonium salt, which is coupled with 8-HQ in alkaline medium to form orange-pink water soluble azo dye that was stable and has a maximum absorption at 509 nm.

2. Experimental

2.1. Apparatus

The spectral measurements were carried out on a (CECIL CE3021-ENGLAND) UV/Vis spectrophotometer. Absorbance measurements of batch method were carried out on JENWAY 6300 spectrophotometer using 1.0 cm quartz cell.

The flow injection analysis (FIA) spectrophotometric system used in this work consists of peristaltic pump (Carter12/6 cassette pump with five channels variable speed) to deliver flow streams. The tygon pump tubes with (1.05 mm i.d.) were used to transport the carrier solutions. The rotary valve (Rheodyne U.S.A.) with variable sample volumes was used to inject the sample into the flowing carrier stream. The valve was mode of a poly tetra flouroethylene (PTFE) with good resistance against different solvents and the corrosion by the chemical acids or bases. A Y-shaped Perspex piece was used for mixing the two streams of reagents. The absorbance measurements of the FIA were carried out using spectrophotometer (JENWAY 3600 UV/Vis spectrophotometer) with flow cell Sterna-micro (100 µL) with 1.0 cm optical path length. The absorbance was recorded by means of x-t recorder (type 0.5 A kip and zone, one line recorder) with varies amplification factors and different chart speeds.

2.2. Reagents and solutions

All chemicals and reagents were of analytical grade. Distilled-deionized water used for preparation of all solutions.

Stock solution of procaine- HCl

Procaine- HCl stock solution [(1000 µg /mL) = 3.66 x 10^{-3} M] 0.10 g amount of pure Procaine -HCl (SDI) was dissolved in amount of water then complete to 100 mL in a volumetric flask with the same solvent(Al-Abachi, *et al*. 2012). Procaine-HCl working solution (200 µg/mL), was prepared by dilution of 20.0 mL of the stock solution to 100 mL volumetric flask with distilled-deionized water.

Sodium nitrite solution (0.50 M)

A fresh stock solution of 0.50 M was prepared daily by dissolving 3.45 g of sodium nitrite (FLUKA) in little volume of distilled-deionized water and then the volume completed to 100 mL.

8-hydroxyquinoline solution (3.4x 10^{-4}M)

The solution of 3.4 x10^{-4} M 8-HQ was prepared by dissolving 0.005 g of 8-HQ (Annular) in (0.5 mL of 0.1 M HCl) and complete the volume to 100 mL volumetric flask with distilled-deionized water.

Sodium and potassium hydroxide and sodium carbonate solutions:

1.0 M NaOH solution prepared by dissolving 20.0 g of sodium hydroxide (Annular), 1.0 M KOH solution prepared by dissolving of 28.05 g of potassium hydroxide (RIEDDEL-DEHAEN) and 1.0 M sodium carbonate solution prepared by dissolving 10.6 g of Na_2CO_3 (FLUKA) in a little volume of water separately, the volume complete to 500 mL in volumetric flask with distilled-deionized water.

Hydrochloric acid (1.0 M)

A 1.0 M hydrochloric acid solution was prepared by diluting 85.9 mL of HCl (BDH) (36%, sp.gr.1.18) in 1.0 L volumetric flask with distilled-deionized water. Other solutions were prepared by serial dilutions.

Sample preparation

An accurately weighed amount of mixed content of 10 vials. Powder equivalent to 60 mg of the pure drug was dissolved in which a little mount of water in a small beaker and then transferred to a 100 mL volumetric flask and completed to the mark with distilled-deionized water to obtain 600 µg/mL. Working solutions were prepared by diluting appropriate amounts of the stock solution with distilled-deionized water.

2.3. Preliminary work

To 25 mL volumetric flask containing 1.0 mL (0.05 M) of hydrochloric acid solution and 1.0 mL (0.01M) of sodium nitrite solution, and 1.0 mL of 200 µg/mL procaine- HCl were added then shake well following by the addition of 1.0 mL (3.4x10^{-4} M) of 8-HQ and 1.0 mL of sodium hydroxide (1.0 M). The contents of the flasks diluted to the mark with distilled -deionized water, which mixed well and left for 2.0 min at room temperature, the absorbance of the orange-pink dye formed was measured at 509 nm against a reagent blank containing all materials except Procaine -HCl.

3. RESULTS AND DISCUSSION

3.1. Batch spectrophotometric determination of procaine-HCl:

The absorption spectrum for the azo dye was measured at λ_{max} = 509 nm, versus reagent blank which has negligible absorbance at this λ_{max}.

The factors that affecting on the sensitivity and stability of the colored product were carefully studied, the following parameters have been investigated, which includes chemical and physical optimizations. Table (1) shows optimum conditions for spectrophotometric determination of procaine-HCl.

Recommended procedure

A 0.8 mL of 0.05 M HCl and 0.8 mL of 0.01 M $NaNO_2$ were added to a 25 mL volumetric flask, then aliquot of standard procaine-HCl (containing 0.8 to 8.0 µg/mL) and 1.5 mL of $3.4x10^{-4}$ M of coupling reagent 8-HQ and 0.2 mL of NaOH were added, the mixture was diluted to the mark with distilled-deionized water. The blank solution was prepared in the same way in the absence of procaine-HCl. After 2.0 min the absorbance of orange-pink azo dye was measured at 509 nm at room temperature.

Table (1): Optimum conditions for spectrophotometric determination of procaine-HCl:

Parameter	Optimum condition
HCl	0.8 mL of 0.05 M
$NaNO_2$	0.8 mL of 0.01 M
8-HQ	1.5 mL of 3.4 x 10^{-4} M
NaOH	0.2 mL of 1.0 M
λ_{max}	509 nm
Temperature	Room temperature (25ºC)
Time for developing the color	2.0 min

Calibration graph and statistical data

The calibration graph was constructed between the absorbance and the concentration of procaine-HCl (µg/mL) using optimal experimental conditions described in Table (1), the coloured system obeyed Beer's law in the concentration ranges of (0.8 - 8.0 µg/mL) of procaine–HCl with a detection limit 0.12 µg/mL. It was found a good correlation coefficient (0.9954) and high molar absorptivity ($2.0814x10^4$ L mol^{-1} cm^{-1}) with the value of Sandell's index equal to 13.106 µg/cm^2, which indicate that the method is very sensitive.

Precision and accuracy

To determine the precision and accuracy of the proposed method, depending on the two different concentration of pure procaine-HCl , the relative standard deviation (RSD) and relative error (E%) for five replicate were determined. The results were shown in Table (2).

Effect of interferences

The selectivity of the proposed method was investigated, the effect of some foreign substances (e.g. glucose, urea, sucrose, starch, Fe^{3+} and Al^{3+}), that commonly company procaine-HCl preparation forms were studied by adding different amounts of foreign substances to 3.2 µg/mL of procaine- HCl, the results summarized in Table (3) . The tolerance content, define as the amount of foreign substance that produce an error not exceeding ± 5% in the determination of 3.2 µg/mL of procaine-HCl.

Structure of the dye

The stoichiometry of reaction between procaine-HCl and 8-HQ in azo dye was checked by Job's continues variation method (Harris, 2007). For this method, a series of solutions was prepared in which the total volume of procaine-HCl and 8-HQ were kept at 1.0 mL, the result obtained in Fig. (1) showed the existence of 1:1 procaine-HCl: 8-HQ reagent at 509 nm. Therefore, the structure of the dye may be written as indicated in Fig. (2).

The suggested mechanism

The possible mechanism for the reaction of diazotized procaine-HCl with 8-HQ illustrated in Fig. (2), which can situated two steps, step -1 describe the reaction between procaine-HCl with nitrous acid to form diazonium ion, while step-2 was described the coupling of diazonium ion with 8-HQ in alkaline medium (NaOH) to form orange-pink stable azo dye.

Applications

In order the applicability of the proposed method to determination of procaine-HCl, the method was applied for analysis of procaine-HCl in pharmaceutical formulations, for the aim of comparison the samples were also analyzed by reference method in British pharmacopoeia (2009) and the result was summarized in Table (4).

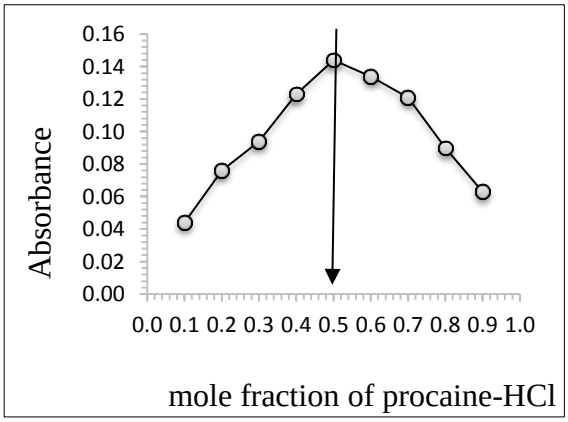

Fig. (1): Jobs method of procaine-HCl: 8-HQ in azo dye.

Table (2): precision and accuracy of the proposed method

Analyte	Analyte concentration (µg/mL)		RSD %	E%
	Standard solution	Calculation from proposed method		
Procaine- HCl	2.40	2.34	2.13	2.50
	6.40	6.26	0.95	2.18

Table (3): Effect of interferences on the absorbance of 3.2µg/mL procaine HCl:

Interferences	MAIC[a] (µg/mL)	Absorbance		E%	TCR[b]
		With interference	Without interference		
Glucose	3.2	0.299	0.304	1.64	1.0
Sucrose	3.2	0.307	0.304	-0.98	1.0
Starch	16.0	0.310	0.304	-1.97	5.0
Urea	3.2	0.315	0.304	-3.61	1.0
Fe^{3+}	6.4	0.294	0.304	3.28	2.0
Al^{3+}	19.2	0.300	0.304	1.31	6.0

(a) MAIC: Maximum Allowable Interference Concentration.
(b) TCR: Tolerable concentration Ratio [conc. of Interference (µg/mL) / conc. of sample (µg/mL)].

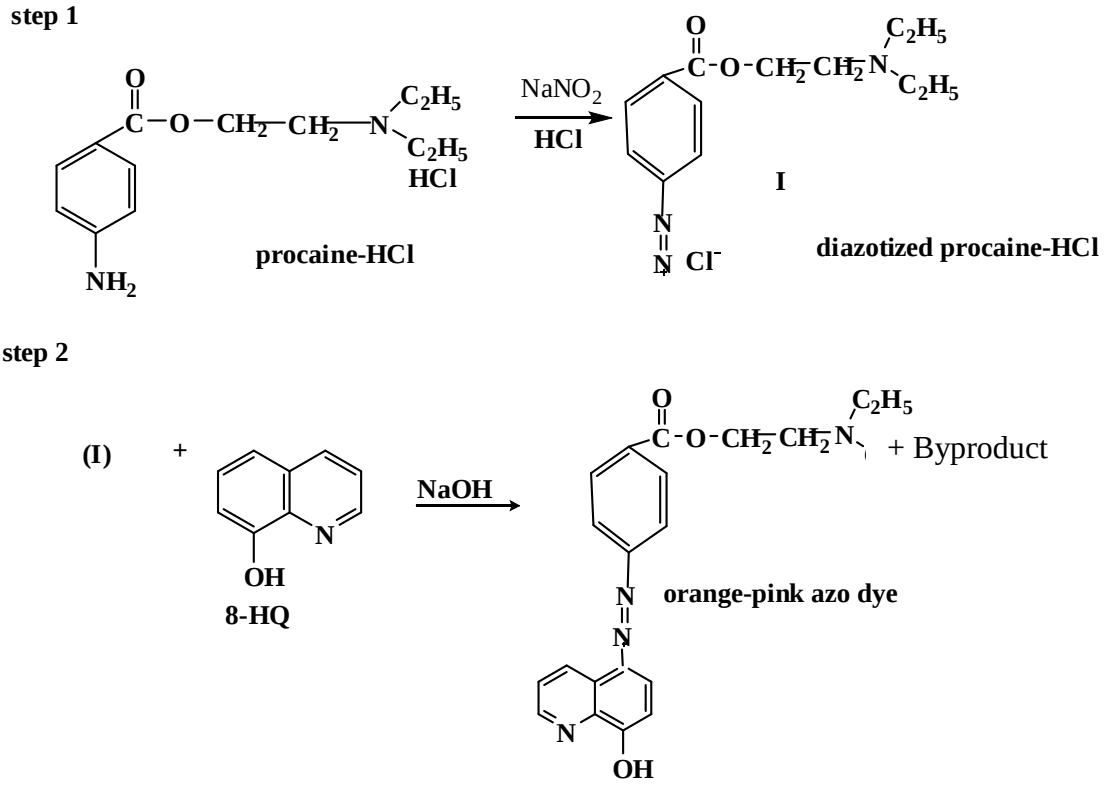

Fig. (2): The possible mechanism reaction

Table (4): Comparison of present method with standard method (BP 2009) for determination of 6.4 µg/mL procaine-HCl:

Injection Samples	Amount found (µg/mL)		Difference Between two method	E%
	Present Method	Standard Method		
Procaine injections (600 mg of Procaine, Iran)	6.867	6.765	-0.102	- 1.507
Procaine injections (600 mg of Procaine Turkey)	6.762	6.634	- 0.158	- 2.390
Procaine injections (300 mg of Procaine Iran)	6.474	6.547	0.073	1.115
Procaine injections (300 mg of Procaine Turkey)	6.723	6.590	- 0.133	- 2.018

The results of two methods were compared using the t-tests and F-test with a confidence limit of 95 % indicates that there were no significant differences between accuracy and precision of two methods (BP 2009). This indicates that there was no any systematic error in our method of analysis.

3.2. FIA-Spectrophotometric determination of procaine-HCl:

General procedure

The FIA system, Fig. (3-I), was operated. A sample volume of 100 µL was used to inject 100 µg/mL of procaine-HCl into the solution stream. Two reaction coils was used in the system with length equal to 30 cm for first coil (a) which represent to reaction of sodium nitrite solution with the hydrochloric acid (to produce nitrous acid) to form diazonium ion; while the second coil (b) with length 10 cm was used for the coupling of diazonium ion with 8-HQ in the presence of

sodium hydroxide solution to formation of azo dye. The absorbance was measured as peak height in (mm) and monitored at 509 nm. At least three injections were mode for each sample solution.

Optimization of manifold designs

Two manifolds designs shown in Fig. (3-I and II) had been tested for the determination of procaine-HCl. Among these two manifolds it was found that the manifold (I) was the best in the stability and peak height (mm) obtained, compared with the manifold (II), which gives lower measuring, may be due to the presence of water which decreases the intensity of the azo dye due to dilution. Therefore, design (I) had been selected in further studies.

Optimization of experimental parameters

All chemical and physical conditions that effected on the peak height of the azo dye were studied and the reaction conditions were optimized. The conditions include concentration of the reactants, length of the mixing coils, flow rate and sample volume. The optimization starts with 0.01M hydrochloric acid, 0.002 M of sodium nitrite, $6x10^{-5}$ M of 8-HQ, 0.2 M of NaOH, reaction coil with length (a = 30 and b =10) cm, 1.0 mL/min flow rate

100 µL sample volume at the maximum wavelength of 509 nm for the determination of procaine-HCl as preliminary conditions.

Chemical and physical optimizations were illustrated as shown in Table (5).

Calibration graph

Under the recommended conditions described in Table (5), the calibration curve was produced by plotting the peak height (mm) against the concentration of procaine-HCl (µg/mL). Beer's law was obeyed over the concentration range of 2.0 - 50 µg/mL of procaine-HCl, with a correlation coefficient of 0.9927 and detection limit of 0.75 µg/mL.

Table (5): Optimum chemical and physical conditions for the determination of 100 µg/mL procaine-HCl:

Parameters	Optimum values
HCl	0.05 M
$NaNO_2$	0.01 M
8-HQ	$1.7x10^{-4}$ M
NaOH	0.1 M
Flow rate	2.0 mL/min
Mixing coils	a = 30 cm
Sample volume	150 µL

Accuracy and precision

The accuracy and precision had been established by five replicate determination made on two different concentration of pure procaine-HCl solution. The (E %) and (RSD %) were checked for the same solution. The RSD% values for both levels below 2%. These results confirm that the method was precise and accurate. Table (6) shows the accuracy and precision for the proposed method.

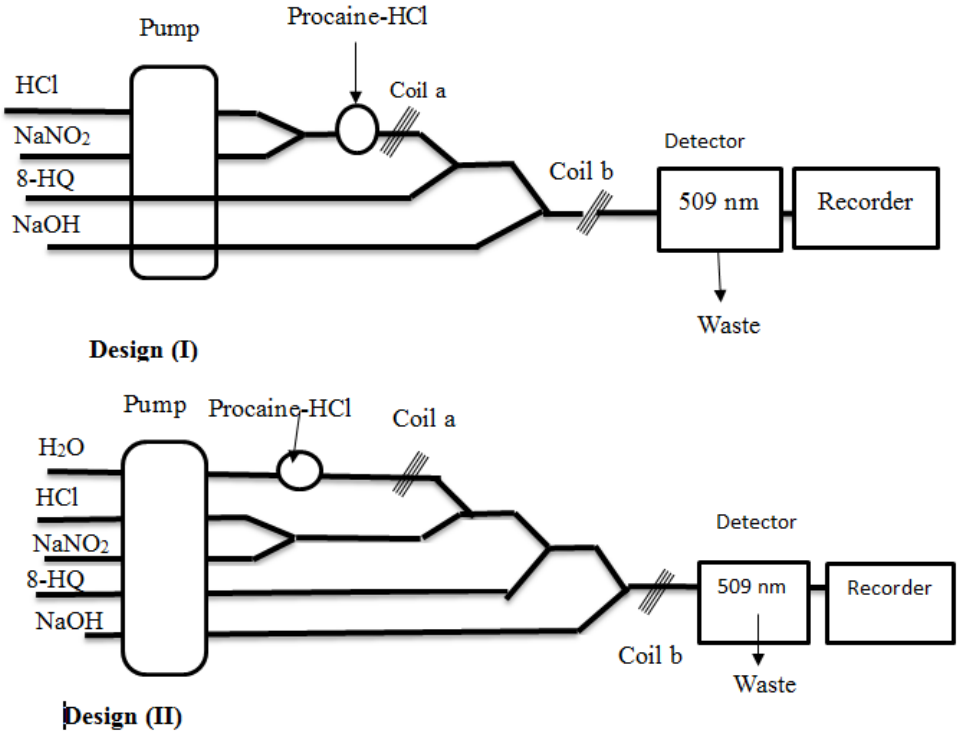

Fig. (3): Schematic diagram of FIA-Spectrophotometric designs (I and II) for determination of procaine-HCl.

Table (6): Accuracy and precision of the FI-spectrophotometric determination of procaine-HCl using diazotization method:

Analyte	Analyte concentration (µg/mL)		E%	RSD%
	Standard solution	Calculation from proposed method		
Procaine-HCl	8.0	7.77	2.87	1.81
	35	34.69	0.88	0.72

Effect of interferences

Interference studies were carried out in order to investigate the effect of excipients that might be present in procaine-HCl dosage forms. The study was carried out by synthetic solutions containing 20 µg/mL pure procaine-HCl and varying amounts of the interfering compound up to equal or greater amount than that of the pure procaine-HCl. The results obtained were tabulated in Table (7) which shows the maximum concentration of the various compounds that leading to obtain the error ±5%.

Applications

The present FIA-spectrophotometric method was applied for determination of procaine-HCl in pharmaceutical samples, Potentiometric titration method was used as standard method in (BP, 2009) for analyzed of the samples.

The results of two methods were compared for the determination of procaine-HCl injection samples using the F-test and t-tests (Harris, 2007). Table (8) observed that there was no significant difference between accuracy and precision of the two methods at 95% level of confidence when (t-calculated = 0.48 less than t-table = 2.254, F-calculated =3.891 less than F-table =9.28).

CONCLUSIONS

A batch and flow injection spectrophotometric method was described for the determination of procaine-HCl in pharmaceutical formulations.

The batch method was found to be very simple, low cost and rapid for determination of procaine-HCl in pharmaceutical dosages. They had an advantage in which did not require the removal of excipients, any chemical sample pretreatment, temperature control, solvent extraction step, and expensive reagents and solvents. The present method was based on the diazotization reaction of procaine-HCl with coupling reagent (8-HQ) in alkaline medium to for orange-pink azo dye which was water soluble, stable and shows maximum absorption at 509 nm. The method was applicable for low concentration of procaine-HCl with precision (RSD%; 2.13, 0.95), high accuracy (E%; 2.50, 218) and reasonable sensitivity in which the molar absorptivity was found to be $2.0814x10^4$ L mol^{-1} cm^{-1} and sandal's index 13.106 µg/ cm^2. Beer's law was obeyed in the range (0.8 - 8.0) µg/mL of procaine- HCl concentration.

FI-Spectrophotometric method utilizes for determination of procaine-HCl in injection samples and the proposed method was also simple, rapid, and precise. Sensitivity of this method was not less than obtained for determination of procaine-HCl using FI method (Al-Abachi, *et al.* 2012), that gave linear range between (5.0 – 400.0 µg/mL).

As comparison in sensitivity between batch and FIA techniques observed that batch techniques more sensitivity than FIA technique in which linear ranges for both techniques (0.8 – 8.0 µg/mL) and (2.0 – 50 µg/mL) respectively and detection limit for batch method 0.20 µg/mL while for FI method gave 0.75 µg/mL. However, FIA had some advantages over the batch method due to simplicity and rapidity.

ACKNOWLEDGEMENT: We would like to express our deep gratitude to the Salahaddin University-Hawler; College of Science - Department of Chemistry for their help during the work.

Table (7): Effect of interferences on the peak height of 20 (mg/mL) procaine-HCl:

Interferences	**MAIC (mg/mL)**	**Peak height (mm)**		**E%**	**TCR**
		With interference	**Without interference**		
Glucose	20.0	72	73	1.36	1.0
Sucrose	20.0	74	73	-1.36	1.0
Starch	40.0	71	73	2.73	2.0
Urea	40.0	74	73	-1.36	2.0
Fe^{3+}	20.0	75	73	-2.73	1.0
Al^{3+}	40.0	76	73	-4.10	2.0

Table (8): Comparison of present method with standard method (BP, 2009) for determination of 20 µg/mL procaine-HCl:

Injection Samples	**Amount fount (µg/mL)**		**Difference Between two methods**	**E%**
	Present Method	Standard Method		
Procaine injections (600mg of Procaine, Iran)	21.535	21.142	-0.393	-1.858
Procaine injections (600 mg of Procaine Turkey)	20.125	20.732	0.607	2.927
Procaine injections (300 mg of Procaine Iran)	20.944	20.460	-0.484	- 2.365
Procaine injections (300 mg of Procaine Turkey)	20.806	20.596	0.210	-1.019

REFERENCES:

Al-Abachi M. and Al-Ward H.; (**2012**), "Batch and flow-injection spectrophotometric determination of thymol using procaine hydrochloride as a new chromogenic reagent", *Journal of Baghdad for Science*, 9(2), 302-310.

Al-Abachi M., Al-Uzri W. and Al-Ward H.; (**2012**), "Batch and flow-injection spectrophotometric determination of procaine-HCl in pharmaceutical preparations via using diazotization and coupling reaction", *J. Baghdad for Sci*, 9(3), 521-531.

Al-Abachi M., Al-Ward H. and Al-Samarrai E.; (**2013**), "Spectrophotometric determination of procaine hydrochloride in pharmaceutical preparations via oxidative coupling reaction", *Tikrit Journal of Pharmaceutical Sciences*, 9(2), 213-222.

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

British Pharmacopoeia, 4th Ed., Pharma. Press London; (**2009)**, 4985.

Carmona M., Silva M. and Bendito P.; (**1992**), "A selective and sensitive kinetic method for the determination of procaine and benzocaine in pharmaceuticals", *Journal of Pharmaceutical Biomedical Analysis*, 10(2-3), 145-152.

Chen Y., Tian F. and Song M.; (**2009**), "Spectrophotometric determination of procaine hydrochloride with hemoglobin as catalyst", *Journal of analytical chemistry*, 64(4), 366-370.

Dutu G., Tertis M., Sondulescu R. and Ckistea C.; (**2014**), "Differential pulse and square wave voltammetry methods for procaine hydrochloride determination using graphite based spes modified with p-tertbutyl-diester-calix [4] arene", *Rev.Chim.*, 65(2), 142-147.

Hamzah M., Al – Abachi M., Al-Daamy M. and Farid Y.; (**2009**), "A new colorimetric method for determination of procaine in pharmaceutical preparations via oxidative coupling organic reaction", *Journal of Kerbala University*, 7(1), 236-242.

Harris D.C.; (**2007**), "Quantitative chemical analysis", 7th Ed., W.H. Freeman and Company, New York, 62- 63.

Irinel A. and Luminita V.; (**2005**), "Imultaneous determination of procaine and benzoic acid by derivative spectrometry", *Chemie Anul*, (1-2), 401-406.

Luo, X., McNamara B., Fennell M., Uboh D. and Soma L.; **(1998)**, "Quantification of penicillin-G and procaine in equine urine using HPLC", *Journal of chromatography B: Biomedical Science and Application"*, 714 (2), 269-276.

Mahmoud K. M.; (**2007**), "Determination of chloramphenicol in acidic medium using a chromogenic compound by FIA spectrophotometric method", *National Journal of Chemistry*, (28), 634-641.

Pasekova H. and Polasek M.; (**2000**), "Determination of procaine, benzocaine and tetracaine by sequential injection analysis with permanganate-induced chemiluminescence detection", *Talanta*, 52(1), 67-75.

Qing X., Hong-jie S. and Li-fu S.; (**2009**), "Determination of contents of procaine hydrochloride and its degradation product p-amino benzoic acid in myocardial", *Pharmaceutical Care and Research*, 1.

Sarsam L., Mohammed S. and Al-Abbasi K.; (**2011**), "Spectrophotometric determination of metoclopramide hydrochloride in pharmaceutical preparations using diazotization reaction", *Rafidain Journal of Science*, 22(3), 76-88.

Satyanary K. and Nagesara P.; (**2012**), "Simple and selective s spectrophotometric methods for the determination of mosapride citrate by diazo coupling reaction in pharmaceutical formulations", *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(2), 363-368.

Xiaojun H., Hongli L. and Yanming, F.; (**2009**), "Determination of procaine hydrochloride and procaine hydrochloride injection by HPLC", *Chirapharmaceticals*, 24.

Yuan J., Yin J., and 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. Chromatogram A.*, 1154(1-2), 368-372.

Zhang X., Zhao D., Feng L., Jiao L. and Wang S.; (**2010**), "Electrochemical sensor for procaine based on a glassy carbon electrode modified with poly-amidosulfonic acid and multi-walled carbon nanotubes", *Microchimica Acta*, 169 (1-2), 153-159.