Novel Approach of Oxidative Coupling Reaction with 1-Naphthol of the Simultaneous Metformin Drug Determination in Either Pharmaceutics Formulations or Environmental Samples of Water Using Homemade CFIA-Merging Zones Techniques

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Abstract

A newly developed FIA-merging zones spectrophotometric system, the method is rapid, accurate and sensitive for metformin hydrochloride determination through the oxidation of 1-naphthol by sodium hypochlorite and coupling with metformin.HCl in the presence of sodium hydroxide to form a blue soluble ion pair and this product was determined using homemade CFIA-Merging zones techniques , at 580 nm. Data treatment shows that linear range is (0.5-35) µg/ ml. The optimization conditions for various chemical and physical conditions of [MTF- NaOCl-α-naphthol-NaOH] system were investigated. The LOD was 0.01µg / ml and LOQ 0.1µg/ml from the lowest concentration of the calibration graph with r^2% 99.18 and RSD% did not exceed 3%, sample through put 48 sample/h while percentage recovery (Rec.%) were from 97-100% , indicate no interferences of the tablet excipients. The method was applied successfully, for estimation of metformin in environmental samples of water and pharmaceutical drugs. Statistical analysis of results was compared, shows the method can be regarded as an alternative analytical way for determining of metformin. hydrochloride in bulk and dosage forms samples.

Keywords: CFIA, MTF. HCl, 1-naphthol, Merging Zones, NaOCl, Spectrophotometric determination.

Introduction

Metformin. hydrochloride (MTF.HCl), chemically 1,1-Dimethyl biguanide hydrochloride [1] ($C_4H_{11}N_5$.HCl) is white crystalline powder (fig.1). There are two types of diabetes: (type I) and (type II), the later is the commonest cause one, maintaining blood sugar values as close to the physiological range possible is essential in diabetic patient to prevent mortality and morbidity [2]. Metformin. HCl is an oral anti diabetic drugs that treatment of (type II) diabetes mellitus (non-insulin dependent) that improves the control of sugar level in blood primarily through the inhibiting hepatic glucogenolysis & gluconeogenesis [3-5].

It acts as antimicrobial, antimalerial, analgesic and antimetabolite for organisms that inhibit the metabolism of folic acid [6, 7]. It seems to ameliorate hyperglycemia through improving, peripheral sensitive for insulin, hepatic glucose tolerance production with reducing gastrointestinal glucose absorption. Recently MTF has become affordable for the treatment of polycystic ovary syndrome and has been found to improve vascular function, reverse fatty liver and prevent pancreatic cancer [8]. Various methods have been developed for the determination of MTF.HCl in pharmaceutical formulations and biological samples including; conductometric titrations [9]. Gas chromatography [10,11], Capillary electrophoresis [12], LC [13], adsorptive catalytic square-wave voltammetry [14], potentiometric titration [15] , ion-selective electrode [16] , colorimetric [17-19] , flow injection Chemiluminescence [20-22] , HPLC [23,24] and spectrophotometrically [25-28].

From these, techniques many of disadvantages were observed using complexation extraction methods, which have were time consuming , column – switching technique and ultra filtration have been developed to improve selectivity and specificity. An approach based on the flow injection is a well known technique that offers improvement in most batch methods; providing high sample, through putting rate, sensitive, simple sample preparation [29] and instrumentation.

This work describes a new simple and continuous flow / merging zones technique with spectrophotometric detection for the determination of metformin via the oxidation of 1-naphthol by sodium hypochlorite and coupling with drug in alkaline medium to form colored product measured at 580 nm. The developed method was used in many estimation of environmental water samples and pharmaceutical preparations.

Experimental

Chemicals

All chemicals were of analytical reagent grade and distilled water was used to prepare solutions.

MTF-HCl stock solution (500µg.mL^{-1}) from NDI ($C_4H_{12}N_5$.Cl, 165.63 g.mol^{-1}, NDI, 500 µg.ml^{-1}) was prepared by dissolving 0.05 gm of metformin. hydrochloride pure material in distilled water in 100 ml volumetric flask.

Sodium hypochlorite stock solution: (NaOCl, 74.44g.mol^{-1}, SDI, 2M), was prepared by dissolving 14.888 g of NaOCl in 100 ml of distilled water in a volumetric flask, more dilution for stock solution to have a series of solutions.

Sodium hydroxide solution: (NaOH, 39.997 g.mol^{-1} , BDH, 2M) was prepared by dissolving 7.999 g of NaOH in 100 mL volumetric flask in distilled water.

1-naphthol stock solution : (M.wt = 144.17 g.mol^{-1}, BDH) (4×10^{-3}M) was prepared by dissolving 0.058 g of 1-naphthol in 100 mL of ethanol and well shacked before use, more dilution was made by ethanol to have a series of working solutions.

Procedure for pharmaceutical preparations (tablets)

Ten tables of pure metformin hydrochlorid (500mg) were accurately weighed and crushed to fine powder . An amount of the powder equivalent to 500 mg of metformin was dissolved in 100 ml distilled water in a volumetric flask , mixed it (30 min), and filtrated off the solution to remove undissolved portion which affect on the response , more dilution by distilled water for this solution was made to have solution with different concentrations , treat these solutions under recommended procedure.

Procedure for tap water

Take water samples from different origins in Iraq then the samples were filtered and analyzed by the proposed method. The result obtained shows negative results then the samples were spiked with the concentration ranging from 1-35 $\mu g.mL^{-1}$ of metformin. HCl then analyzed these samples as recommended procedure and the concentration was calculated by using the calibration curve of the method.

Apparatus

FIA procedure used a quartz flow cell with 100µl internal volume. An injection valve (homemade) (six-three ways which include 3-loops made of Teflon) that loaded by a mixture of metformin with sodium hypochlorite in ($loop_1$) (L_1), 1-naphthol was loaded in ($loop_2$) (L_2) and sodium hydroxide was loaded in ($loop_3$) (L_3) (Figure (2)) based on merging-zones technique were employed using suitable injection volumes of standard solutions and sample. Peristaltic pump (Master flex C/L, USA with power supply (0-250 V)) was used to transport the reagent solutions . All spectral measurements were conducted on an Optima visible SP-300 digital single beam recording spectrophotometer (Japan), for the absorbance measurements as peak height (mV) through (Kompensograph) C1032, Siemens or absorbance with digital multimeter (DT 9205A, China). Flexible vinyl tubes of 0.5 mm internal diameter were used for the peristaltic pump , the reaction coil was made of glass with internal diameter of 2 mm. The distilled water was chosen as carrier which combined with injection samples either pharmaceutical preparation or tap water samples with sodium hypochlorite L_1 . The stream is then merged with the reagent 1-naphthol, L_2, then mixed with sodium hydroxide L_3. All the solutions were mixed in reaction coil with length of 50 cm, injection sample of 100µl. Flow rate of carrier of 3.3 $ml.min^{-1}$, the colored product formed was measured at 580 nm as a peak height in mV. Figure (2) shows the manifold of flow system that is used for determination and detection of metformin.HCl in bulk, pharmaceuticals and tap water samples.

Methodology

A FIA manifold system for determination of MTF-HCl via spectrophotometric reaction with 1-naphthol to form a blue colored product which is measured at λ_{max} 580 nm was constructed of one line as shown in scheme (2).

FIA procedure was applied to the carrier stream (D.W.) at flow rate 3.3ml/min leading to the injection valve which consists of three loops, L_1 for a mixture of metformin with sodium hypochlorite as oxidizing agent, L_2 for 1-naphthol as a reagent and L_3 for sodium hydroxide as a medium. The content of loops mixed together in 50 cm mixing coil and the response of the formed colored product (blue) was detected via Vis. spectrophotometer (homemade with flow cell) and recorded as peak height in mV through the recorder.

Each measurement was repeated three times. Sampling rates were found to be 48 sample / hour . The proposed mechanism of the reaction is shown in scheme (1), according to the results obtained from the plot of mole ratio (Figure (3)) which indicates the existence of the formed product as (1:1) (MTF:α-naph.).

Result and discussion

Spectroscopic study

When a dilute solution of metformin was mixed with 0.5 ml of 1M sodium hypochlorite as an oxidizing agent and 1.5 ml of 1- naphthol $1x10^{-3}$ M followed by the addition of 1 ml of 0.8 M sodium hydroxide. A blue colored product was formed immediately formed [33] , the recorded spectrum of the formed product shows a maximum at 580 nm against reagent blank as shown in figure (4).

Optimization of experimental parameters

The chemical (concentrations of reagent solution used in the reaction) and physical parameters (sample volume, reaction coil length, purge time and flow rate) were examined**.**

Optimization of Chemical parameters

The influence of sodium hydroxide in the range of (0.1-1.5 M) was studied, 1M seems to be the optimum concentration as indicated in figure (5) and table (1).

The influence of sodium hypochlorite was investigated by using solutions of various concentrations (0.01-1.7M) . A concentration of 1.2M gave the highest peak height and chosen to be the optimum concentration as shown in figure (6).
The results obtained were summarized in table (2)

The influence of 1- naphthol which was used as a reagent was studied by using different concentrations ($1x10^{-4}$ – $2.5x10^{-3}$ M) .It was found that $2.5x10^{-3}$M was the ideal concentration of 1-naphthol as shown in figure (7) and table (3) , and chosen for further experiments .

Optimization of physical parameters

The effect of flow rate was studied by using different flow rates (1-7ml/min) , The results show that in the slow flow rates the response was suffers sharp decline which may be due to the high dispersion . In high flow rates mix time will not be enough to diffusion of metformin in reagent 1-naphthol this is due to lose in the peak height therefore, 3.3 ml/min of flow rate was chosen to be ideal flow rate because it gave a good response as indicated in figure (8).

The volume of injection sample was examined in the range of (20-130 µL) and it was found that 100µl was the optimum sample injection volume and chosen for subsequent experiment as shown in figure (9) .

The length of reaction coil was studied, from the results obtained it was observed that 50 cm was optimum reaction coil and if the reaction coil is taller than 50 cm the response will be distribution as shown in figure (10), while if the reaction coil is lower than 50 the peak will suffer a decline due to not enough time for completing the reaction. Therefore 50 cm was chosen to be the optimum reaction coil.

Purge time of the injected sample under optimal parameters that were achieved in the previous studies . The purge time of the distilled water was studied. Different purge times from (5-25) seconds and open valve (> 25) injected mode was adopted throughout the research work, Figure (11), shows that the purge time more than 25 sec. giving a highest response. Open valve was selected to complete transportation of sample from sample loop to flow cell.

Calibration Graph

A series of different concentrations of metformin.HCl ranging from (0.1-35 µg.mL^{-1}) were prepared by appropriate dilution a standard solution of metformin 500 µg.mL^{-1}. All chemical and physical parameters were set at their ideal values.

The results obtained indicate that the response is changed linearly with the variation of metformin.HCl concentration as shown in table (4) which indicated that $F_{tab} = F_{V_2}^{V_1} = F_7^1 = 8.073 << F_{Stat.} = 845.5631$ Therefore , there is a significant relation between the response obtained and metformin.HCl concentration , as shown in figure (12).

The limit of detection was evaluated using three different methods and the result obtained are tabulated in table (5).

Repeatability study

The repeatability of the method was acheaved by carrying out ten injection sample measurements for two concentrations 8 and 12µg.mL^{-1} , the results obtained were summarized in table (6) which indicated that the method has a high sensitive and good repeatability as shown in figure (13).

Analytical applications

The proposed method was used for estimation of metformin.HCl in tap water and in pharmaceutical formulations (tablets) available in the markets.

The results obtained were compared with classical spectrophotometric method. The standard addition method was used and the result were treated mathematically and tabulated in table (7). From table (8) observed that's no significant differences between the two method, then the proposed method adopted for determination of MTF in different environmental water samples and in dosage forms that treatment of diabetes mellitus (type II) (non-insullin dependent).

Conclusion

The proposed flow injection/merging zones method was a novel method; it is characterized by inexpensive, rapid, sensitive and simple for determination of metformin.HCl. The method based on reaction between metformin and 1-naphthol after oxidized 1-naphthol by sodium hypochlorite in the presence of sodium hydroxide as a medium to yield a blue colored product.

Good reproducible for different ranges of concentrations were achieved in all the experiment carried out through this project and that was indicated that the proposed method have a good accuracy and can be used as an alternative simple method for the determination of metformin drug in pure, pharmaceutics formulations and environmental samples, the method (standard addition) was used to avoid matrix effects.

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Table (1):Effect of sodium hydroxide concentration on the response intensity

[NaOH] M	*av. pkh (n=3) $\bar{X}$, mV	*σ_{n-1}	%RSD*	CL* $\bar{x} \pm t_{0.05}$ SEM*
0.1	430	1.61	0.37	430 ± 1.15
0.3	580	2.09	0.36	580 ± 1.49
0.5	840	1.84	0.22	840 ± 1.32
0.7	890	0.72	0.08	890 ± 0.51
1	830	1.35	0.16	830 ± 0.96
1.2	760	1.22	0.16	760 ± 0.87
1.5	640	2.1	0.33	640 ± 1.50

*av. pkh; average peak height, *σ_{n-1}; standard deviation,
*CL; Confidence interval of the mean,
%RSD*; Relative standard deviation,
*SEM; Standard Error Mean.

Table (2):Effect of sodium hypochlorite concentration on peak height in (mV)

[NaOCl] M	*av. pkh (n=3) $\bar{X}$, mV	*σ_{n-1}	%RSD*	CL* $\bar{x} \pm t_{0.05}$ SEM*
0.01	150	0.25	0.167	150 ± 0.178
0.03	220	0.55	0.25	220 ± 0.393
0.05	330	1.02	0.31	330 ± 0.73
0.07	530	0.07	0.013	530 ± 0.05
0.1	600	2.01	0.335	600 ± 1.44
0.3	650	1.09	0.167	650 ± 0.78
0.5	720	0.03	0.004	720 ± 0.021
0.7	820	0.06	0.007	820 ± 0.005
1	900	1.03	0.114	900 ± 0.081
1.2	925	1.01	0.11	925 ± 0.079
1.5	875	0.2	0.023	875 ± 0.016
1.7	702	0.57	0.081	702 ± 0.058

Table (3):Effect of 1-naphthol on peak height in (mV)

[1-naphthol] M	*av. pkh (n=3) $\bar{X}$, mV	*σ_{n-1}	%RSD*	CL* $\bar{x} \pm t_{0.05}$ SEM*
$1x10^{-4}$	100	0.02	0.02	100 ± 0.014
$3x10^{-4}$	220	1.02	0.46	220 ± 0.73
$5x10^{-4}$	320	0.035	0.011	320 ± 0.025
$9x10^{-4}$	400	0.06	0.015	400 ± 0.043
$1x10^{-3}$	501	0.079	0.015	501 ± 0.057
$1.5x10^{-3}$	652	1.03	0.158	652 ± 0.74
$1.9x10^{-3}$	710	1.21	0.170	710 ± 0.87
$2x10^{-3}$	830	0.061	0.0073	830 ± 0.044
$2.5x10^{-3}$	950	0.081	0.0085	950 ± 0.058
$3x10^{-3}$	804	1.021	0.13	804 ± 0.73
$3.5x10^{-3}$	700	1.01	0.144	700 ± 0.72

Table (4):ANOVA for linear equation result obtain from calibration graph for the determination of metformin .HCl using FIA-merging zones technique [3-,32]

Source	Sum of squares	D_f	Mean square	$F_{stat.}=S_1^2/S_2^2$
Regr. $(\hat{y}_{i-}\bar{y})$	988362.1	1	988362.1	845.5631
Error $(\bar{y}\text{-}\hat{y}_i)$	8182.162	7	1168.88028	
Total $(y_i\text{-}\bar{y})$	996544.262	8		

Table (5):Limit of detection of metformin .HCl at ideal conditions for [MTF- Naocl- α-naphthol – OH] system

Minimum concentration in calibration graph	The value of slope $x=\frac{3SB}{slope}$	Linear equation ($\hat{y}(mV) = y_B + 3S_B$)
0.01µg.mL^{-1}	2.2x10^{-4} µg.mL^{-1}	65.187 µg.mL^{-1}

y_B = average response for (the blank solution) (equivalent to intercept of straight line in equation , S_B= standard deviation of blank solution (0.002) , x = value of LOD based on slope .

Table (6):Repeatability of metformin . HCl results

metformin concentration µg.ml^{-1}	Number of measuring (n)	$\bar{y}_i$ (n = 10) mV	Standard deviation σ_{n-1}	Repeatability RSD%	CL* $\bar{y}_i \pm t_{0.05,n-1} SEM^*$
8	10	334	1.53	2.22	334 ±1.09
12	10	390	1.50	0.207	390 ±1.07

*$t_{tab.}$ = 2.26 at 95% confidence limit for (n-1) .

Table(7): Results obtained for the estimation of metformin .HCl in tap water samples and pharmaceutical preparations

Pharmaceutical preparation and tab water samples	Conc. of metformin. HCl µg.mL^{-1}		E_{rel} %	Rec%	RSD%
	Present	Found			
Glucosam tablet (NDI) Iraq 500mg	3	3.005	0.166	100.166	0.2
	25	25.07	0.28	100.28	1.05
Glucosam tablet (NDI) Iraq 850mg	3	3	0.00	100	0.5
	25	24.995	-0.02	99.98	0.021
Metformal (SPA-Italy)	3	3	0.00	100	0.1
	25	25	0.00	100	1.67
Tap water sample 1	3	3.02	0.66	100.66	0.23
	25	25.1	0.4	100.4	2.2
Tap water sample 2	3	3	0.00	100	1.62
	25	24.99	-0.04	99.96	2.1
Tap water sample 3	3	3.1	3.3	103.3	2.09
	25	25.09	0.36	100.36	1.20

Table (8):The comparison of the proposed FIA-merging zones method with standard spectrophotometric method using F &t- statistical test

Pharmaceutical preparation and tap water samples	Proposed methods FIA		[33] Official method		S	Value	
	Rec%	$(x_i-\bar{x})^2_1$	Rec%	$(x_i-\bar{x})^2_2$		t_{cal}** (critical.)	F_{cal}*** (critical.)
Glucosam tablet (NDI) Iraq 500mg	100.2	0.06	99.75	0.27	0.187	1.808 2.179	1.156 4.284
Glucosam tablet (NDI) Iraq 850mg	99.99	0.21	100.25	0.00026			
Metformal (SPA-Italy)	100	0.2	99.80	0.217			
Tap water sample 1	100.5	0.0030	101	0.54			
Tap water sample 2	99.98	0.216	99.5	0.59			
Tap water sample 3	102	2.42	101.3	1.07			
	$(\bar{x}_1)$ = 100.445	$\Sigma(x_i-\bar{x})^2_{1=}$ 3.109	$(\bar{x}_2)$ =100.266	$\Sigma(x_i-\bar{x})^2_{2=}$ 2.69	$n_1+n_2-2=12$	$n_1-1=6$ $n_2-1=6$	

t** calculate, F** calculate

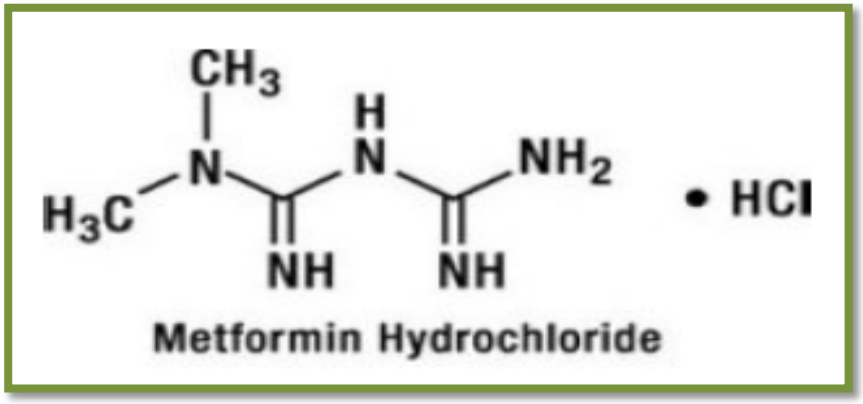


Figure (1) : Chemical structure of Metformin-HCl

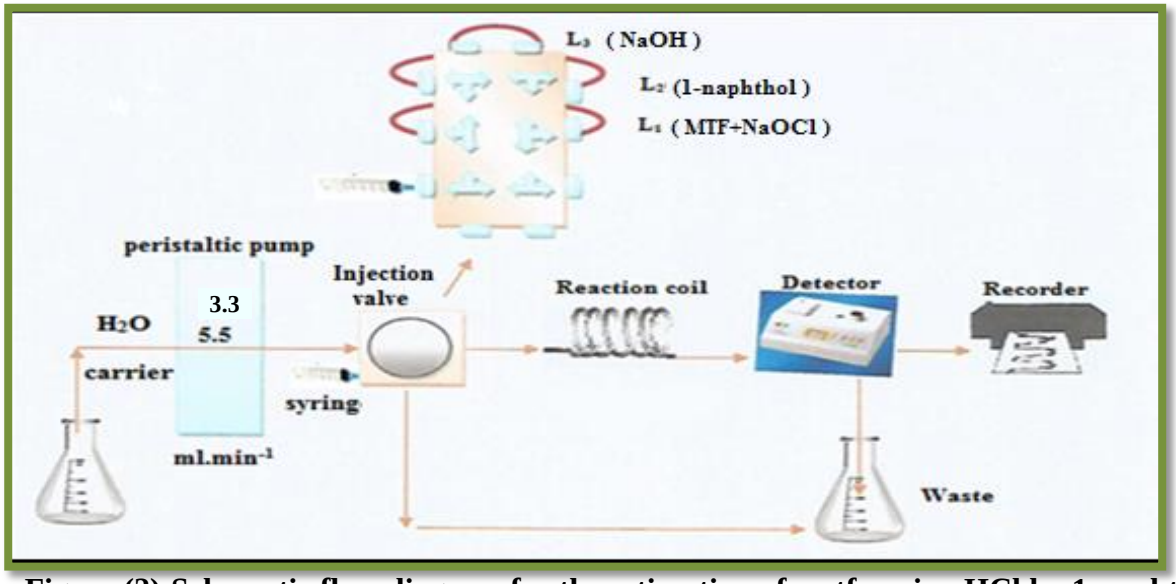


Figure (2):Schematic flow diagram for the estimation of metformin . HCl by 1-naphthol as a reagent in basic medium

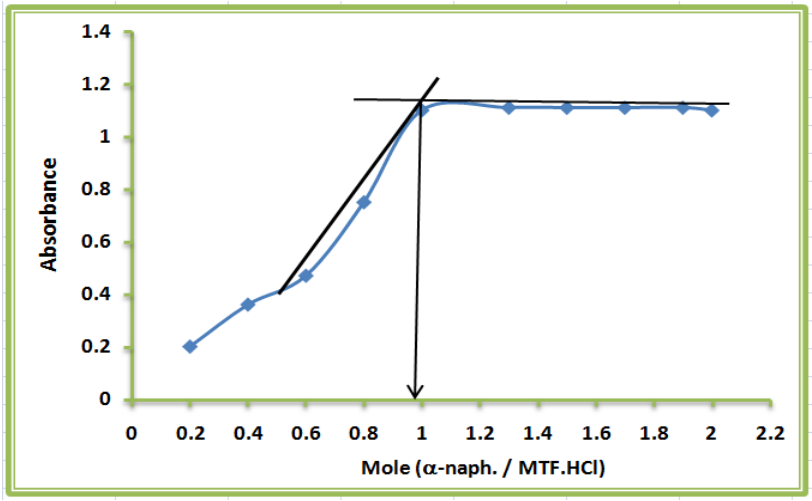


Figure (3): Mole ratio

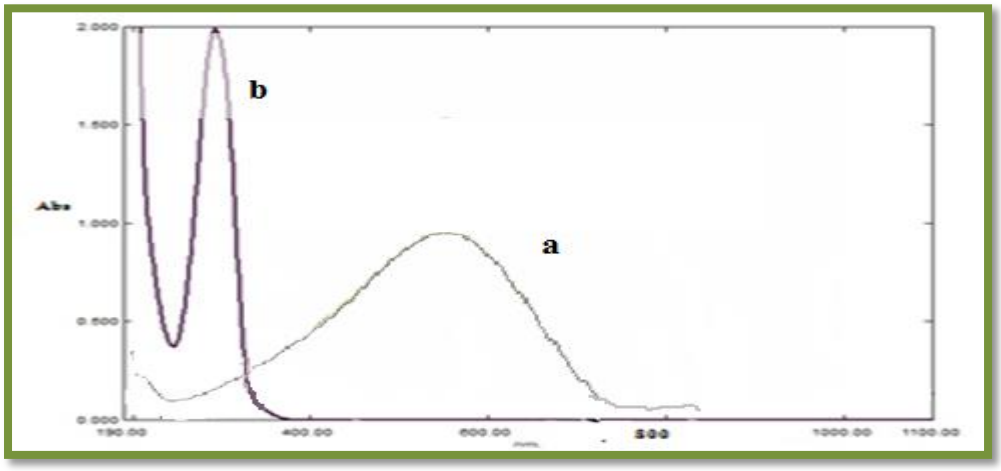


Figure (4): Absorption spectrum of the complex formed against reagent blank (a) and blank against distilled water (b)

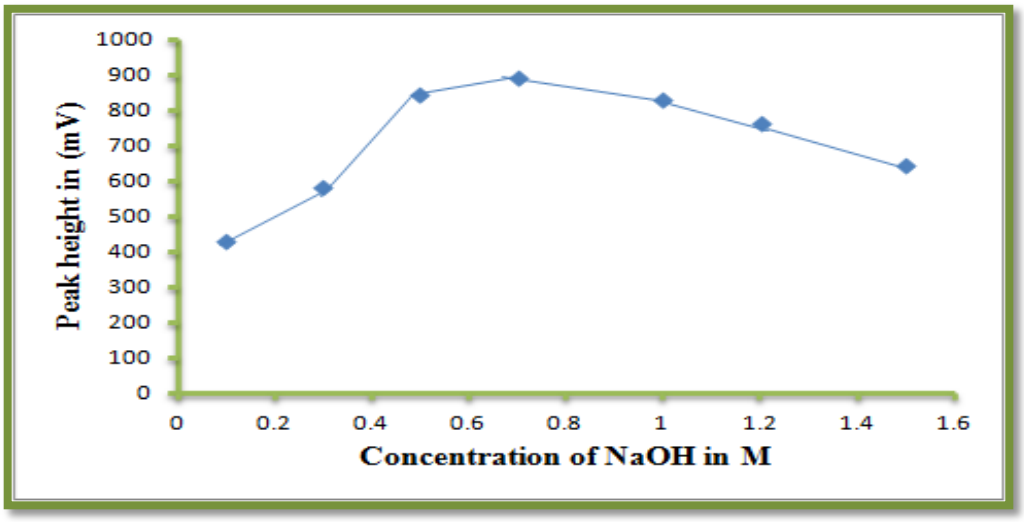


Figure (5): Influence of NaOH concentration

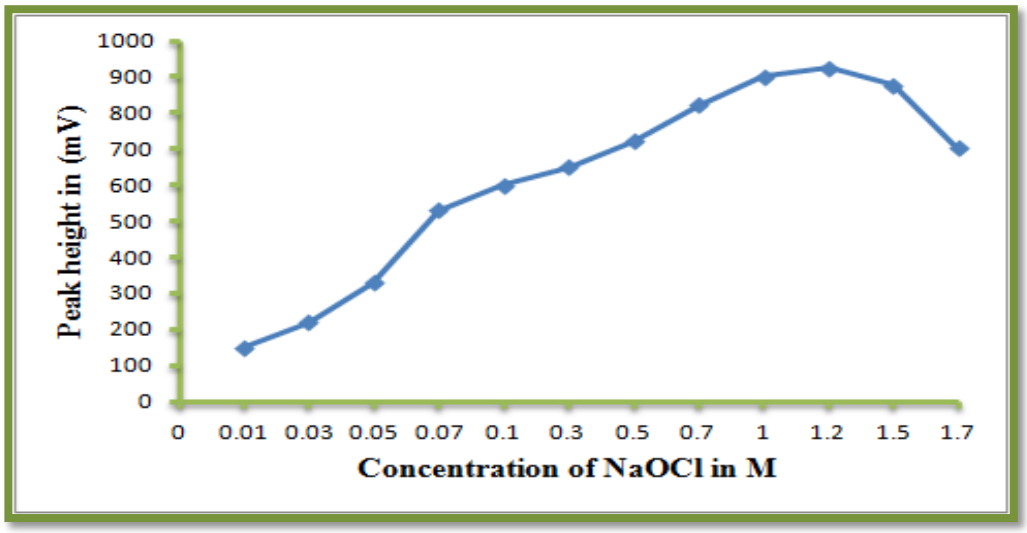


Figure (6): Influence of NaOBr concentration

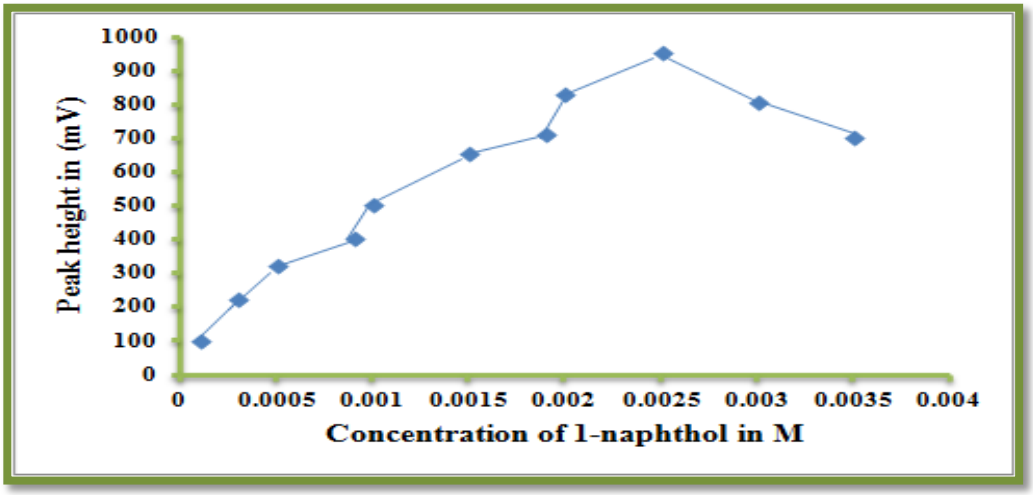


Figure (7): Effect of 1-naphthol concentration

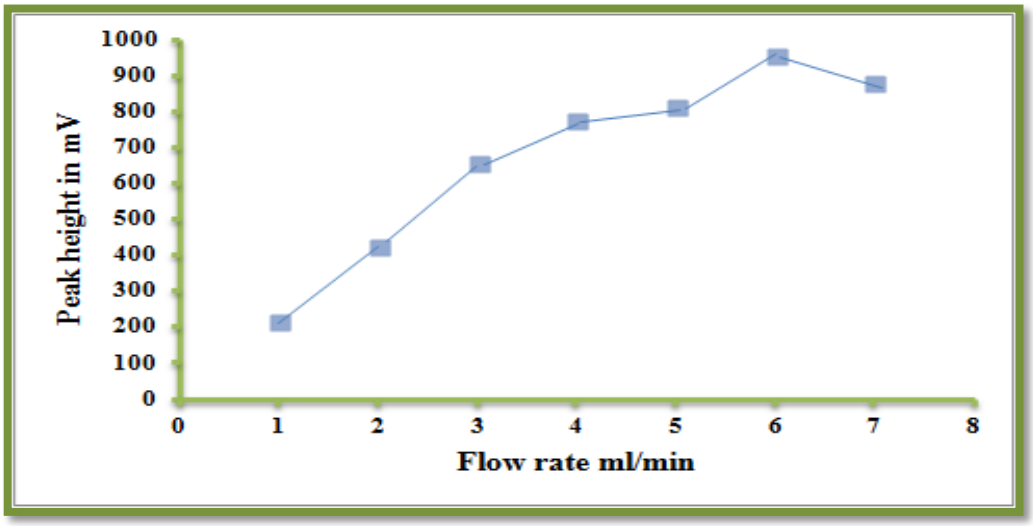


Figure (8): A plot variation of response versus flow rate (ml.min^{-1})

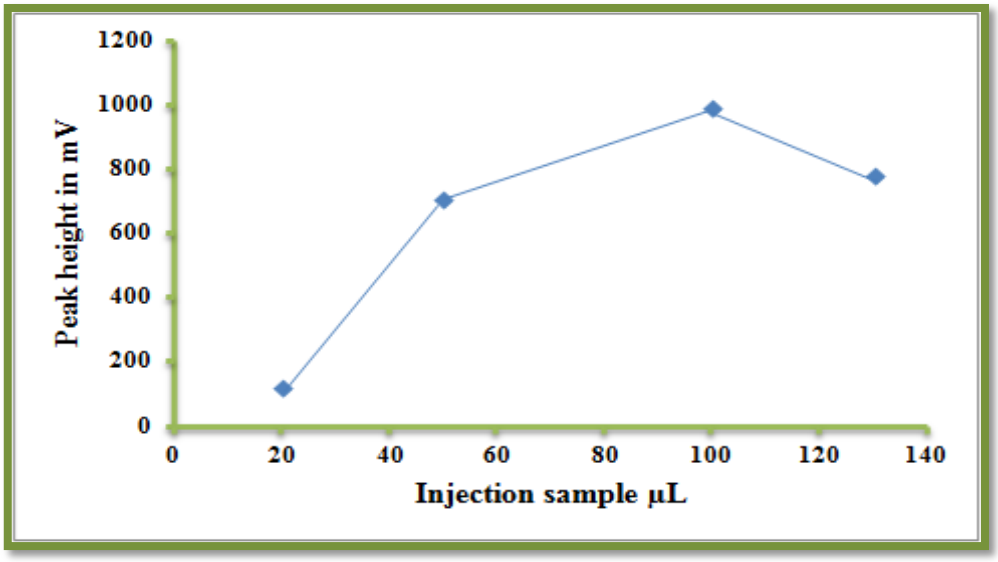


Figure (9): Influence of injection sample

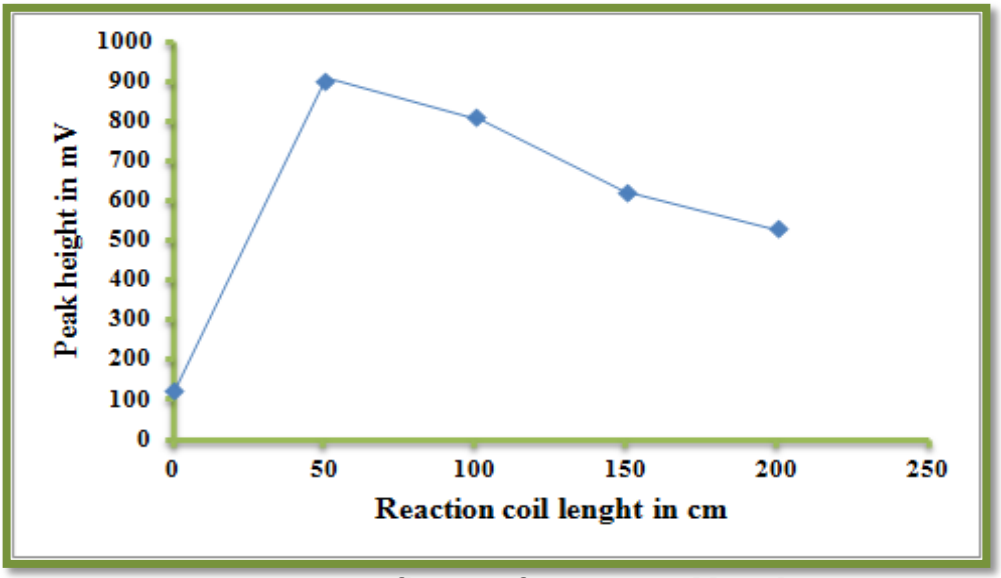


Figure (10): Influence of reaction coil length

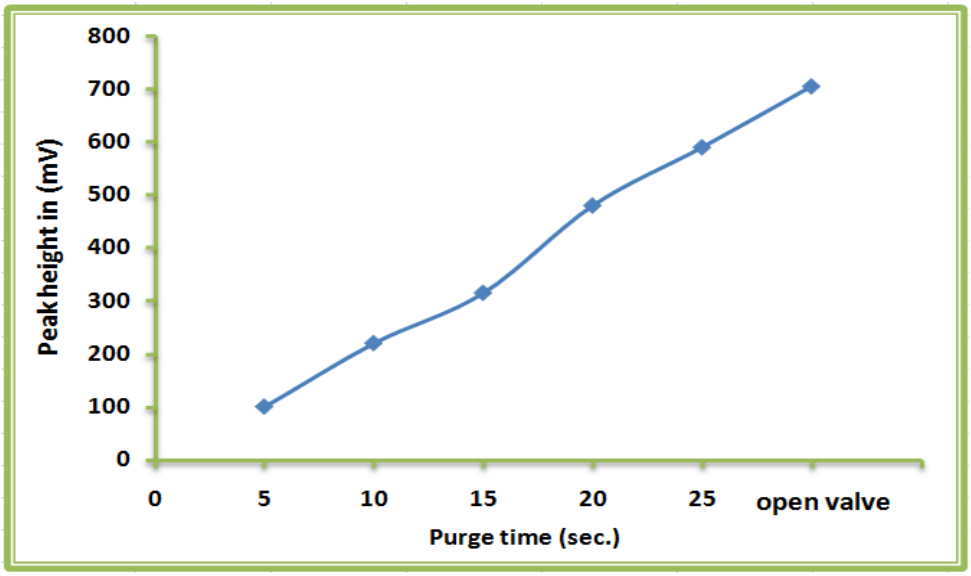


Figure (11): Effect of purge time

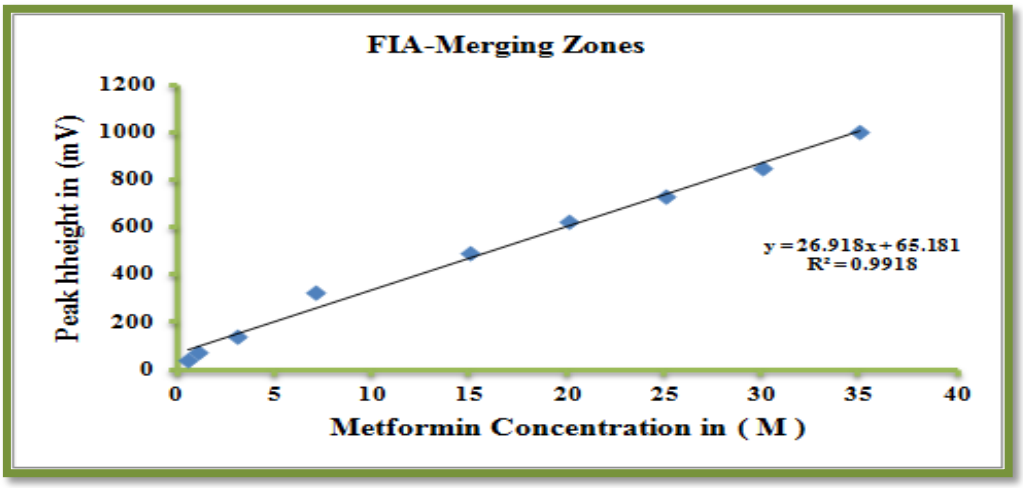


Figure (12): Calibration Curve for determination of metformin hydrochloride by FIA – merging zones technique

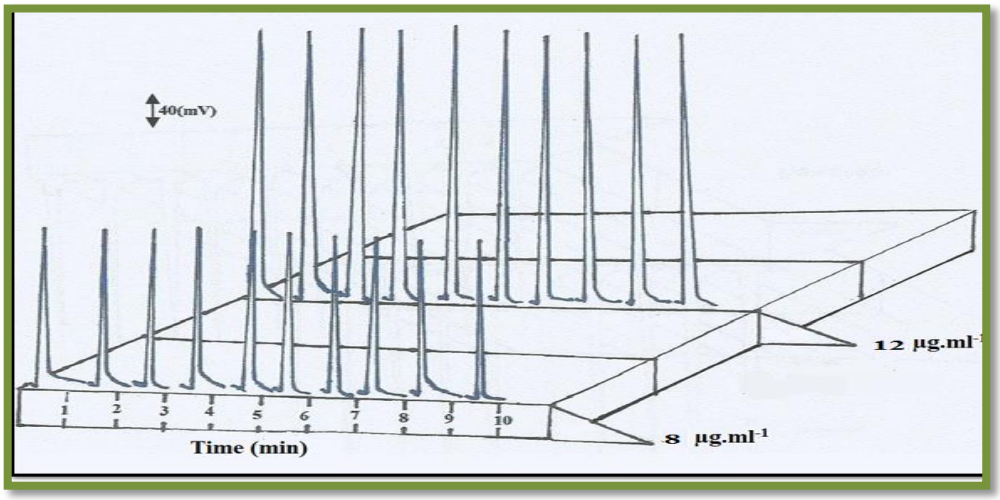
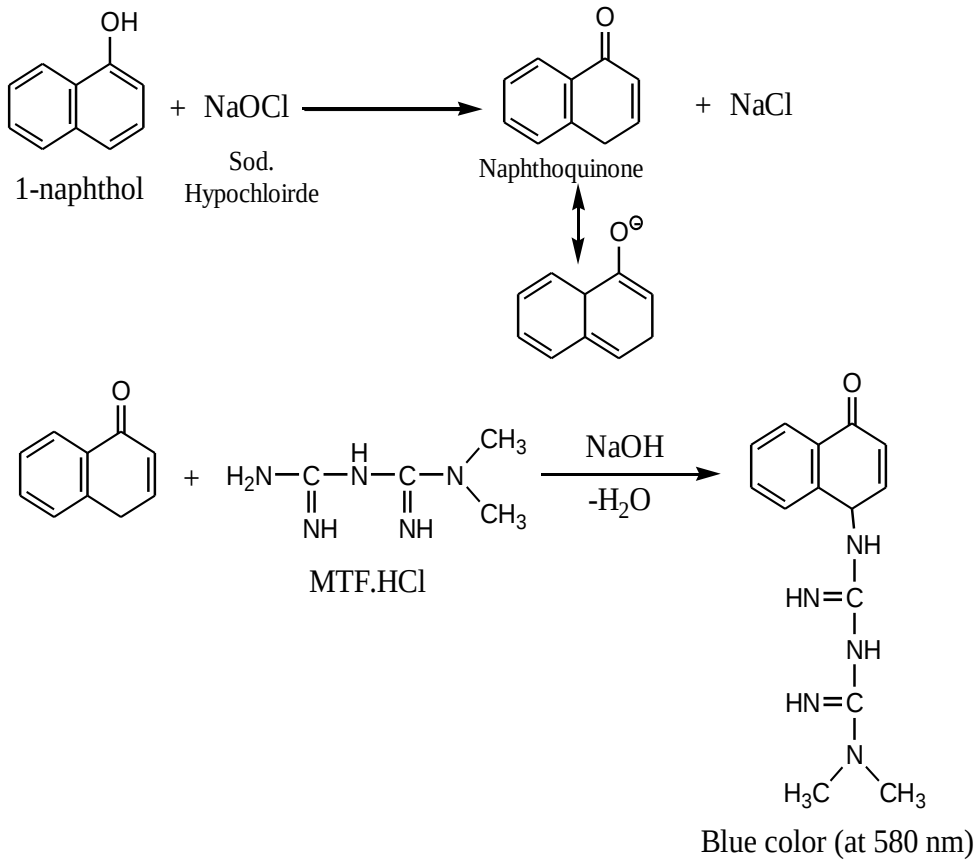


Figure (13): Response profile of repeatability measurement of metformin .HCl in FIA-merging zones technique



Scheme (1): The proposed mechanism of reaction for the determination of metformin by oxidative coupling reaction with 1-naphthol