Indirect Way for the Assay of Captopril Drug in Dosage FormsUsing1,10-Phenanthroline as a Selective Spectrophotometric Agent for Fe(II) Via Homemade CFIA /Merging Zones Technique

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Abstract

A new speed and sensitive batch and merging zones-flow injection analysis spectrophotometric ways for estimation of captopril in a fine material and in pharmaceutical formulations were suggested. The procedure was depended on the decline of Fe(III) as $FeCl_3$ to Fe(II) by captopril in acetic acid as medium, the produced Fe(II) interplays with 1,10-phenanthroline to compose a soluble orange-red colored product that is determined at maximum wave length of 511nm. The manifold FIA system was able to determine of CPL. with a throughput 51 sample/h. Calibration curves of absorbance against concentration sign of that Beer، s law is submitted to within the concentration scale of 1-45 & 3-200 mg. L^{-1} of CPL. with detection limits 0.0962, 0.0157 mg. L^{-1} and quantification limits ,0.321, 0.0524 mg.L^{-1} of CPL. for batch and CFIA system, respectively . Repeatability (RSD%) (n=7) were 0.89 and 0.38 for estimation of CPL. with concentration 60 and 130 mg/L. The suggested procedure was carried out successfully for estimation of CPL. in pharmaceutical preparations, the values of the both procedures were compared with USP procedure.

Keywords: Spectrophotometric determination , $FeCl_3$, captopril , CFIA , 1,10-Phenanthroline , Merging zones method

1. Introduction

Arterial high blood pressure is one of the ailment with main diffusion in the world being caused by still unknown agents; some hazard agents partake of the developed of this pathology [1], like age, family history, sedentariness, obesity, stress, high salt intake and alcoholism. The first medication controlled to be used for arterial high blood pressure medication was captopril (CPL); 1-[(2S)-3-mercapto-2-methylpropionyl] Pyrrolidine –2 -Carboxylic acid (figure 1), that refers to the group of angiotensin-converting enzyme (ACE) preventers [2,3]. This medication interplays with(ACE) because of it's affinity with a dipeptide and the sulphydryl class plays a serious part, linkage covalently to the (Zn) atom in the enzyme plus situation. The medication is largely utilized major for arterial high blood pressure medication; but as well as in chronic congestive heart inability coming after myocardial infringement and in diabetic nephropathy [4-6].

Almost 60-75 percentage of a dosage of captopril is sucked up from the gastro-intestinal tract and pinnacle plasma concentration is in approximate 1h, while almost 30 percentage of the medication is going to plasma protein [7]. In order that assert the quality of CPL including pharmaceutical formulations, ease procedures for its analysis in technical and preparation grade sample are important for routine assay, including atomic absorption spectrometry [8,9] differential pulse polarography [10] high performance liquid chromatography [11-15] volumetric titration [16] voltammetry [17] capillary electrophoresis [18] conductometry [19] gas chromatography [20] chemiluminescence systems [21-24] diffuse reflectance spectroscopy [25] potentiometric titration [26-28] spectrophotometry [29-40] fluorimetry [41] amperometry [42-44] flow injection analysis [23,24,28,45-47] are suitable for use in daily assay in dosage forms specifity control laboratories because to the easiness, high repeatability and reproducibility with high sampling per hour, high analytical frequency and the result in diminishing reagents exhausting when compared with batch methods [28,30] . The united states Pharmacopeia [16] (USP) depictes a titrimetric method for (CPL.) estimation in dosage forms , but this method is so slow and laborious, therefore lower applied to wide-range assay and low sensitive method. In this paper, we presented a new flow injection analysis /merging zones technique for indirect estimation of CPL. in fine material and dosage forms using ferric chloride as an oxidizing agent, where ferric ion will be reduced to ferrous ion by thiol drugs (CPL.), the produced Fe(II) forms an orange-red colored product with 1,10-phenanthroline which is determined spectrophotometrically.

2. Experimental

Apparatus and manifold

All of spectral absorbance quantifications were applied on a Shimadzu UV-VIS 9200, Biotech engineering management CO, LTD, UK digital double beam that record spectrophotometer with (1cm quartz) cell. The flow cell (quartz, 1cm) with 100 µL internal volume is inside the detection unit and (1cm) an optical path length using for the absorption records. A one channel manifold that is used for the flow injection analysis- merging zones spectrophotometer estimation of captopril. A Power supply (Yaxun, 1501AD, China) with Peristaltic Pump (Master Flex C/L,USA) that is used for pumping the carrier stream (distilled water) and solutions were passed the injection valve that (homemade) six-three way injection valve (merging zones region) that steps at 90º and that contain three loops of

(Teflon) where saddled with samples, reactants and the reagents solution . The injection valve that is used to supply suitable volume that was injected of standard solutions and samples.
The tubes were made of flexible vinyl with 0.22 mm (ID) using for the Peristaltic pump and 0.5mm for manifold system, mixing coil that was manufactured from glass with 2 mm (I.D). All of parts of the continuous flow injection analysis-merging zones technique was shown as in Figure (8). A carrier stream was distilled water that was combined with injected sample of captopril as reducing agent in acetic acid in L1 and mixed with ferric chloride as oxidizing agent in L2 and the reagent of 1,10-phenanthroline in L3. After that combined it in reaction coil that it has length of 50 cm, volume that was injected of sample 56.91µL, flow rate of distilled water (carrier) 0.8 mL/min. The maximum absorption was found at 511 nm as peak height in mV.

Chemical and reagents

Each of the reactants and reagents were used of analytical class also each of the solutions preparing freshly were always used.

Captopril stock solution (M.wt=217.29 g.mole^{-1}, Beijing, China) (500 mg.L^{-1} = 2.3x10^{-3}M) : A 0.05 gm amount of fine captopril was dissolving in distilled water, then concluded to 100 mL in standard flask with distilled water . More dilute solutions were prepared by adequate diluting of the stock standard solution with distilled water.

1,10-phenanthroline (M.wt=180.2 g.mol^{-1}, Merck) (2x10^{-3}M) : A 0.0396 gm amount of 1,10-phenanthrolin was dissolved in deionized water in 100 ml standard flask and dilution to the marked with the deionized water .

Acetic acid solution (M.wt=60.05 g.mol^{-1}, BDH) (3x10^{-1}M) : Preparing acetic acid solution by transferring 6 ml from acetic acid stock solution (5M) .

$FeCl_3$ (M.wt=162.2 g.mole^{-1}, Merck) (1x10^{-3}M): Prepared by dissolving 0.0162 gm of $FeCl_3$ in 5ml of 0.3M acetic acid and 30ml distilled water in 100 mL standard flask and diluting to the marked.

Pharmaceutical preparations of captopril (500 µg. mL^{-1})

Pharmaceutical formulation was gained from trading sources obtainable tablet by choosing 10 tablets from six kinds of companies were assayed by the proposed procedures. Titles of the various providers included : (1) Rilcapton (25 mg) Medochemie Ltd., Limassol, Cyprus (EU) (2) Rilcapton (50 mg) M.A. Holder: Medochemie Ltd., Limassol, Cyprus (EU) (3) aceprotin (50 mg) Codalsynto Ltd, Limassol-Cyprus (EU) (4) Captopril (50 mg) PL Holder: Bristol Laboratories Ltd., Berkhamsted, Herts, HP4 1EG, UK (5) accord (25 mg) Healthcare, Ltd, Sage House, Middlesex, HA1 4HG, United Kingdom (6) accord (50 mg) Healthcare, Ltd, Sage House, Middlesex , HA1 4HF,United Kingdom .
The tablets were weighed exactly, exterminated and milled using motor up to become good powder. A 0.05 gm of each sample was weighting that be equal to 500 mg. L^{-1} solution of activated component for all dosage forms. This magnitude of captopril dissolving in distilled water and filtrated to remove the insoluble residue that effects on the response. The filtrate transferring in 100 mL standard flask and concluded to the marked with distilled water,

further solutions were diluted to prepare allot to the concentration inside of the straight line of the calibration graph.

General methods for calibration

Batch procedure

Transfer 4 mL of acetic acid (0.3M) into a set of 25 mL volumetric flask, after that add 10 mL of distilled water, after that add a growing concentration (1 - 45) mg/L of (50 mg/L) captopril and after that add 6 mL of FeCl3 $1x10^{-3}$M, then add 10 mL of 1,10-phenanthroline $2x10^{-3}$M and conclude the volume to the marked with distilled water. After 25 minute the maximum absorption of colored product was quantified under λmax 511 nm versus the reagent blank.

FIA-merging zones procedure

A captopril solution in the scale (3-200) mg/L preparing of the stock solution of 500 mg/L. A volume that was injected of 56.91 µL in L_1, 51.02 µL in L_2, 49.06 µL in L_3 consists of (Captopril and acetic acid 0.2M was loaded in L_1), while $1x10^{-3}$M $FeCl_3$ was loaded in L_2 and $1.5x10^{-3}$M 1,10-phenanthrolin was loaded in L_3. The sample and other reactants of each loops were injected with flow rate 0.8 $mL.min^{-1}$ as one channel with distilled water as carrier. The product absorption of the colored product was quantified under λmax 511nm and a calibration curve captopril (mg. L^{-1}) was constructed.

3. Results and Discussion

Batch spectrophotometric determination of captopril

Throughout the preliminary experiments on the reaction of captopril with $FeCl_3$ ($1x10^{-3}$M) as oxidizing agent, then Fe(II) was react with the reagent 1,10-phenanthroline ($2x10^{-3}$M) in acetic acid (0.3M) . The reaction occurs in 25°C, the orange-red colored product was composed and measured at a maximum absorbance of 511 nm opposition reagent blank and reagent blank opposition distilled water. Experiments were oriented for ideal of the experimental parameters in order to assemble the ideal parameters for quantitative and fast composition of the colored product with highest sensitivity and stability optimization of the experiential conditions. In subsequent experiments, 50 $mg.L^{-1}$ of captopril was used as shown in figure (2) .

The impact of the 1,10-phenanthroline concentration was investigated on the resulting absorbance of the orange-red complex. Variable concentrations of 1,10-phenanthroline reagent were used for the experiment. The absorbance increase with increasing1,10-phenanthroline concentration then absorbance begins decreasing with the increased concentration. A $2X10^{-3}$M of 1,10-phenanthroline concentration that gave the highest absorbance and selected to be optimum concentration of reagent for the composition of orange-red complex. The effect of 1,10-phenanthroline concentration is as shown in figure (3).

Effect of $FeCl_3$ concentration

The impact of $FeCl_3$ concentration was studied on composition of colored product, it has been observed that the absorbance increase with the increase of $FeCl_3$ concentration but a high concentration of $FeCl_3$, the absorbance decreased therefore $1x10^{-3}$ is chosen to be ideal concentration of oxidizing agent for the drug to form the colored complex, as shown in figure (4) .

Effect of acetic acid concentration

The impact of acetic acid concentration was observed carefully because it directly effects on the absorbance of the colored complex**.** **V**ariable concentrations of acetic acid were used for the experiment. A 0.3M of acetic acid was selected to be ideal concentration for the formation of colored product. as shown in figure (5).

Effect of order addition

NC-Cu(II)-Buffer-CPL is the ideal series of addition, other orders gave less absorbance results at the same experimental conditions as shown in table (1).

Calibration curve of classical method

Transfer a series of volumetric flask 50mL containing 4 mL of acetic acid 0.3M and added 10 mL of distilled water. Then added increasing concentrations (0.125-22.5 mL) standard solutions of captopril (100 mg. L^{-1}) . The solutions were diluting to be marked with distilled water. Then the reaction mixture stands for 25min and measures the absorbance of the colored product under maximum wave length 511 nm versus a reagent blank prepared in the same way without captopril. Each measurement was repeated three times. The standard curve was constructed and linear range (1-45) mg. L^{-1} for the estimation of captopril as shown in figure (6).

Accuracy and Precision

At the ideal conditions described in the established method , accuracy and precision was studied through measuring three different concentrations of captopril , and according to the results that have been reached as shown in table (2) indicate that the classical method have a good with high accuracy and precision , each measurement are repeated for five times .
Calculated stability constant [48] for the proposed interaction (CPL.:1,10-phenanthroline) was calculated depending on the two groups of solutions were prepared , first group of solutions were placed to include stoichiometric lot of captopril to the reagent 1,10-phenanthroline (As) , while the second group were placed to include fivefold excess of 1,10-phenanthroline (Am) as shown in table (3) **.** The stability constant may be written as follows:

$$K=1-\alpha/4\alpha^3C^2$$

While α (degree of dissociation) written as follows:

$$\alpha=Am-As\ /\ Am$$

Stiocheoimetry of the formed product

To identify the ratio of complexation of captopril to 1,10-Phenanthroline in the formed product**,** the continuous variation (Job's method) methods were applied on the formed complex**.** The job's method was carried out by transfer 4 mL of acetic acid (0.3M) in to a set of 25 mL volumetric flask, after that added 10 mL of distilled water, after that added an increasing volumes (0.1-0.9 mL) of captopril (139.36mg.L^{-1}) ($2x10^{-3}$M) , then added 6 mL of $FeCl_3$ $1x10^{-3}$M, after that added a decreasing volumes (0.9-0.1mL) of $2x10^{-3}$M 1,10-Phenanthroline ^{3}M and conclude the volume to the marked with distilled water. After 25min, the maximum absorption was quantified under λmax 511 nm versus the reagent blank. The result obtained were plotted as shown in figure (7) and indicating the existence of 1:2, (CPL.: 1,10-Phenanthroline) .

Mechanism of the reaction

The mechanism of this reaction is based on an oxidation/reduction reaction[42] of captopril with ferric chloride to produce Fe(II), then Fe(II) interplays with 1,10-phenanthroline as reagent to compose a colored complex was founded under λmax 511 nm as shown in scheme (1) according to the suggested mechanism of the reaction, the number of moles of reaction that is (1:2) (Reagent: Drug) .

Flow injection / merging zones spectrophotometric determination

After selecting the optimum conditions of redox reaction of captopril with Fe(III) – 1,10-phenanthroline in acetic acid for the classical spectrophotometric method. The spectrophotometric reaction was automated with flow injection-merging zones technique to study the best practical parameters and to obtain spectral automated with fast way for determination of captopril. So the batch procedure for estimation of captopril was employed as a base to develop flow injection analysis method.

The manifold of flow injection system

After installing the system and linked portions, we study optimal design of system. The developed system shown in Figure (8) is composed of one line supplied the distilled water (carrier) under flow rate 0.8 mL/min leading to the injection valve , which contains three loops (different loop lengths with 0.5mm I.D.) that filled with the sample and reagents according to the order (captopril with acetic acid, L_1) ,($FeCl_3$, L_2) and (1,10-phenanthroline , L_3) .

Optimization of experimental parameters

The flow injection manifold as shown in figure (8) a, b was employed for the ideal of chemical and physical parameters to get the ideal variables for the order. All the parameters were investigated by making all factors stable and change one each at time (single varied optimization)

Effect of chemical variables

The influence of 1,10-phenanthroline, acetic acid and $FeCl_3$ concentration on the analytical signal were studied to obtain ideal chemical conditions depicted, peak height expressed as mV. was differ in absorbance (extreme height of peak with the best baseline).

Effect of acetic acid concentration

The impact of acetic acid concentration on the sensitivity was observed using optimum concentration of 1,10-phenanthroline $1.5x10^{-3}$ M. Series of diluted solution of acetic acid concentration (0.1M - 0.5M) was prepared, 56.91µL sample volume (50mg.L^{-1}CPL.) $FeCl_3$ was $1x10^{-3}$ M as reaction medium was used and the data obtained were plotted as shown in figure (9) . 0.2M of acetic acid was chosen as the best value to complete the reaction.

Effect of 1,10-phenanthroline concentration

A series of solutions ($1x10^{-3}$- $3x10^{-3}$ M) were prepared of 1,10-phenanthroline using flow rate 0.8 mL.min^{-1}, with 51.02 µL of 50 mg. L^{-1} captopril as injected sample volume. All measurements were repeated for three successive times. Table (4) and figure (10) shows that $1.5x10^{-3}$M of 1,10-phenanthroline,$1x10^{-3}$ M of $FeCl_3$ and 0.2M of acetic acid was selected as the optimum concentration.

Effect of $FeCl_3$ concentration

Various concentrations ($1x10^{-4}$- $1x10^{-2}$M) of $FeCl_3$ were examined on the analytical signal. The oxidizing power of FeCl3 for thiol drug in a solution containing 1,10-phenanthroline is dependent on the ease of formation of $[Fe(C_{12}H_8N_2)_3]^{+2}$ (referred in batch procedure) . The values show that the best concentration of $FeCl_3$ was $8x10^{-3}$M. Therefore, the ideal $FeCl_3$ concentration was selected to be $8x10^{-3}$M as shown in figure (11).

Manifold variables

The effect of variables like injected volume of sample, reagent volume, flow rate, purge time and reaction coil length on the analytical response was observed. The peak height based on the stay time of the sample in the order which was conducted with lengths for reaction coil and flow rate. The physical variables were investigated at the ideal concentration of the reactants, 1,10-phenanthroline ($1.5x10^{-3}$M), acetic acid (0.2M), $Fecl_3$ ($8x10^{-3}$M) and primary concentration of captopril (50 mg. L^{-1}).

Effect of flow rate

The influence of the flow rate was observed at the ideal chemical parameters. These values obtained show that the optimum flow rate of pump of sample with least dispersion will be in 0.8 mL.min^{-1}. In the lower flow rate, a dispersion will be the highest level while under more flow rate, the reaction may be not complete as shown in figure (12) and table (5).

Effect of sample and reagents volumes

The injected volume of sample and reagents were investigated by using various sample and reagents volumes. (56.91, 51.02, 49.06, 43.175 and 39.25) µL, using open valve mode .The values obtained show which injected volumes of 56.91 , 51.02 and 49.06 µL for sample volume (50 mg.L^{-1} captopril) with acetic acid (0.2M) in $\mathbf{L_1}$, $8x10^{-3}$M of $FeCl_3$ in $\mathbf{L_2}$ and $1.5x10^{-3}$M of 1,10-phenanthroline in $\mathbf{L_3}$ respectively were optimum volumes that gave the maximum response as shown in figure (13) (a , b , c).

Purge Time

Purge time for the sample segment to be injected via a carrier stream (distilled water) was investigated, using the optimum chemical and physical parameters were studied previously, (40,45,50,55,60) sec and open valve (injected mode) were used for this study. The purge time more than 60 sec giving a highest response intensity with less dispersion, we calculated by the period time between the sample that injected and inception of the end of the signal. Open valve was selected as ideal injection time to conclude transportation of sample from sample loop to flow cell, as shown in figure (14) and table (6). The reaction period of each sample was 70 secs, so the sample throughput was 51 sample / h.

Effect of reaction coil

The influence of different reaction coil lengths (50, 100, 150, 200, 250) cm with (ID 2mm) which was placed after injection valve straight in flow technique (figure8). This ideal concentration using for redox reaction of captopril (50mg.L^{-1}) in acetic acid 0.2M with $FeCl_3$ $8x10^{-3}$M and 1,10-phenanthrolin $1.5x10^{-3}$M on the reaction of captopril was examined. It was found that the peak height was decreased with the reaction coil length up to 50 cm as shown in figure (15). A sharp decline in the peak height was observed above this value because of the dispersion phenomena. Therefore, a 50 cm presented the highest peak height and using in all subsequent experiments.

Calibration Graph

Data processing using the equation of a straight line

A set of captopril solutions (3-200 mg.L^{-1}) was prepared by a suitable dilution of stock solution. All chemical and physical parameters were fixed at their optimum values .Each measurement was recurrent three times, the response which represented as peak height (mV.) plotted against the concentrations of captopril (mg.L^{-1}) .The results obtained were summarized in table (7) and displayed in figure (16) that offers the contrast of response with concentration of captopril . Data were processed mathematically [49,50] and will clarify the method was used to calculate the linear equation of the class ($y = bx + a$)**.**

Repeatability

To investigate the efficiency of the suggested procedure in the estimation of captopril by repeating injection process and measurement for multiple times using two concentrations of captopril (60,130) mg.$L^{-1}$and calculated standard deviation and relative standard deviation for both concentrations that were studied, as shown in table (8) .

Analysis of variation (ANOVA)[51,52] for the linear equation

Calculate the sum of the squares of the different values y_i (response) from $\hat{y}i$ (appraiser response), (imply error) and called (about regression) to obtain $\Sigma (y_i - \hat{y}_i)^2$ for(n-2) of degrees of freedom to get the sum of squares $(S_0)^2$.

Calculate the sum of squares of different values $\hat{y}_i$ from average value $\bar{y}$ (due to regression) to obtain $\Sigma(\hat{y}_i - \bar{y})^2$ and for (1) of degrees of freedom to obtain sum of squares $(S_1)^2$, when dividing the $(S_1)^2$ on $(S_0)^2$ we get the value (F) as shown in Table (9) .

Analytical parameters

The analytical characteristics such as linear range, detection limit, correlation coefficient and relative standard deviation of each procedure were determined [50,51] under the optimized conditions, as shown in table (10) . A calibration graph was constructed figure (16) for a set of captopril standard solution and the main analytical figure of merits of the proposed method. Statistical assessment of regression line offered a result of standard deviation for residuals ($S_{y/x}$) , slope (S_b) and intercept (S_a) under 95% confidence levels for (n-2) freedom degrees were clarificated in the table . The small subjects showed the high repeatability reproducibility of the proposed flow injection analysis compared with the batch method. The flow injection analysis / merging zones was easier than first procedure because

that was rapid (sample throughput of 51 sample.h $^{-1}$) , larger straight line scale of calibration curve were gotten .

Pharmaceutical Applications

The suggested methods were carried out to the assay of some pharmaceutical formulations containing captopril. The standard addition method was applied by preparing a series of solution from each pharmaceutical drug by transferring 2 ml of 500 mg.L^{-1} of pharmaceutical drug to each of the seven standard flask (25 mL) , followed by the addition of (0.0 , 0.83 , 1.66 , 2.50 , 3.33 , 4.16 and 5.00) ml from 150 mg.L^{-1} of captopril solution in order to have the concentration range from (5-30 µg.mL^{-1}) . Six types of pharmaceutical formulations were analyzed and results were mathematically treated. These presented gave a good accuracy and replicable as shown in table (11).

Assessment of developed procedure

To assesst the success and efficiency of the proposed procedure, the values obtained by the CFIA technique were compared with those gottent by standard procedure [41]. Pharmaceutical preparations were assayed by standard procedure, the values that got by both methods were statistically compared, using variance ratio F-test and the student t – test at confidence limit 95% in each states [48,49] . The computed F- and t- results did not extend the theoretical results which showed that there was not considered variations between both of the procedures in accuracy and precision for estimation of captopril in dosage forms. The values were shown in table (12).

4. Conclusion

The proposed homemade CFIA /merging zones analytical procedure are fast, cheap and sensitive for the spectrophotometric of (CPL.) in fine form and pharmaceutics formulation. These methods can be used for the estimation of mg.L^{-1} amount of CPL. without the need for previous separation steps, temperature or pretreatment of sample and solid phase extraction. The main benefits of the methods are its large dynamic range, adequate sensitivity and its suitable for applying in daily assay in pharmaceutics specifity control laboratories because of their facility and their result in diminishing reagents consumption when compared with batch methods [42] and low limit of detection compared with the referenced method (USP) [16]. The procedures have good linearity, high analytical frequency with throughput 51 sample /h. In addition, the wide applicability of developed method for analyzing the assay of CPL. at concentration of trace levels in pharmaceutical preparations.

Table (1): The sequence of addition

Reaction components	Absorbance
Ac - CPL - Fe(III) – 1,10-Phen.	0.684
Ac – CPL - 1,10-Phen. - Fe(III)	0.541
CPL – Ac – Fe(III) - 1,10-Phen.	0.417
1,10-Phen. – CPL - Fe(III) - Ac	0.278
CPL - Fe(III) – Ac - 1,10-Phen.	0.492
1,10-Phen. – CPL –Ac – Fe(II)	0.324

Table (2) Accuracy and precision of the classical procedure

Captopril conc.mg.L^{-1}		Error	Rec %	Erel %	RSD %
Present μ	Found $\bar{x}$				
10	9.67	0.33	99.67	-0.33	0.00
20	19.80	0.20	99.80	-0.20	0.02
40	39.95	0.05	99.95	-0.05	0.07

*Average of five determinations

Rec % (the recovery) = 100 + E_{rel} %; Erel % (relative error) = [(x -μ) / μ] x100
RSD % (relative standard deviation) = $(\frac{\sigma_{n-1}}{\bar{x}})$x100

Table (3): Stability constant of colored complex of captopril with [Fe(II)-1,10-phenanthroline] in acetic acid as acidic medium

captopril	Am*	As*	α	C(M)	K(L^2.mol^2) or (M^{-2})
	0.126	0.102	0.190	3.4x10^{-6}	2.6x10^{10}

*Average of three determinations

Table (4): Effect of 1,10-phenanthroline concentration on the response measured as peak height (mV.) for [captopril-1,10-phenanthroline-Fe(III)] system

[1,10-phen.] M	Absorbance as peak height ($\bar{x}$) (n=3) mV	Standard deviation σ_{n-1}	Repeatability %RSD	Confidence interval of the mean $\bar{X} \pm t_{0.05} \frac{\sigma n-1}{\sqrt{n}}$
1x10^{-3}	140	0.23	0.16	140 ± 0.57
1.5x10^{-3}	340	0.00	0.00	340 ± 0.00
2x10^{-3}	290	1.25	0.43	290 ± 3.11
2.5x10^{-3}	270	0.17	0.06	270 ± 0.42
3x10^{-3}	180	0.00	0.00	180 ± 0.00

Table (5): Influence of flow rate on value of measurement of peak height for [captopril - $FeCl_3$ - 1,10-Phenanthroline] system

Pump speed indication	Flow rate (mL/min)	Average Peak height (n=3) mV	Standard deviation σn-1	%R	Confidence interval of the mean $\bar{X} \pm t_{0.05} \frac{\sigma n-1}{\sqrt{n}}$
1	0.2	60	0.2	0.33	60 ± 0.5
1.5	0.4	90	0.00	0.00	90 ± 0.00
2	0.6	125	0.14	0.11	125 ± 0.35
2.5	0.8	180	1.2	0.67	180 ± 2.98
3	1	165	0.00	0.00	165 ± 0.00
3.5	1.2	140	1.6	1.14	140 ± 3.98

***R% is RSD% (Repeatability)**

Table (6): Effect of purge time on peak height in (mV)

Purge Time (sec)	Average Peak height (n=3) mV ($\bar{x}$)	Standard deviation σn-1	Repeatability %RSD	Confidence interval of the mean $\bar{X} \pm t_{0.05} \frac{\sigma n-1}{\sqrt{n}}$ for n-1
40	75	0.14	0.19	75 ± 0.35
45	135	0.00	0.00	135 ± 0.00
50	160	0.7	0.44	160 ± 1.74
55	210	0.23	0.11	210 ± 0.57
60	225	0.00	0.00	225± 0.00
Open Valve	250	1.2	0.48	250 ± 2.98

Table (7): Summary of linear calibration graph for the estimation of captopril via CFIA/ Merging zones system

Linear range of Captopril ($mg.L^{-1}$)	Average Peak height (n=3) mV ($\bar{x}$)	Standard deviation □□□□□□□ □n-1	Repeatability %RSD	Confidence interval of the mean $\bar{X} \pm t0.05 \frac{\sigma n-1}{\sqrt{n}}$ for n-1
3	35	0.07	0.2	35 ± 0.17
5	55	0.00	0.00	55 ± 0.00
10	70	0.014	0.02	70 ± 0.03
15	80	1.2	1.5	80 ± 2.98
20	90	0.25	0.28	90 ± 0.62
30	115	0.00	0.00	115 ±0.00

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35	125	1.4	1.12	125 ± 3.48
40	145	0.7	0.48	145 ± 1.74
50	160	1.26	0.79	160 ± 3.13
60	180	0.00	0.00	180 ± 0.00
70	200	1.23	0.62	200 ± 3.1
80	225	0.05	0.02	225 ± 0.12
90	245	0.3	0.12	245 ± 0.75
100	270	0.16	0.06	270 ± 0.40
110	290	0.00	0.00	290 ± 0.00
130	320	0.23	0.07	320 ± 0.57
150	377	0.4	0.11	377 ± 0.99
170	425	1.2	0.28	425 ± 2.98
190	450	0.25	0.06	450 ± 0.62
200	490	0.00	0.00	490 ± 0.00

Table (8): Repeatability of consecutive measurement for captopril

Captopril mg.L^{-1}	**Number of measuring (n)**	**$\bar{y}$ (n = 7) mV**	**Standard Deviation σn-1**	**Repeatability RSD%**	**Confidence interval of the mean $\bar{y} \pm t0.05 \frac{\sigma n-1}{\sqrt{n}}$ for n-1**
60	7	180	1.6	0.89	180 ± 3.98
130	7	320	0.7	0.38	320 ± 1.74

Table (9): ANOVA of equation of the straight line.

Source	**Sum of squares**	**Df**	**Mean square**	**Fstat. =S_1^2/S_2^2**
Regression	**$\Sigma(\hat{y}_i-\bar{y}_i)^2$=58328.4**	**V_1= 1**	**58328.4**	**1407.980**
Error	**$\Sigma(\bar{y}_i-\hat{y}_i)^2$= 455.7**	**V_2= 11**	**41.427**	
Total	**58784.1**	**12**		

$F^{V1}{}_{V2} = F^{V1}{}_{V11} = 5.117 << Fstat. = 1407.980,$ therefore, it may be complete which there is an important relation between the response was gotten and the concentration of captopril.

Table (10): Analytical characteristics and regression parameters of the developed procedure for estimation of captopril

Parameters	**FIA procedure**	**Batch procedure**
Linear range (mg.L^{-1})	3-200	1-45
Regression equation y = b x +a ;	Y=2.1176x+52.583	Y=0.0293x0.0051

y = absorbance, x = concentration (µg. mL^{-1})		
Correlation coefficient.r^2 $r = \Sigma i[(xi - \bar{x})(yi - \bar{y})][(\Sigma i (xi - \bar{x})^2)(\Sigma i (yi - \bar{y})^2)]^{0.5}$	0.9973	0.9958
Linearity (r^2%)	99.73	99.58
Intercept (a = y– b x)	52.583	0.0051
Slope (b) ,(ml.µg^{-1}) $b = \Sigma i [(xi - \bar{x})(yi - \bar{y})] / \Sigma i (xi - \bar{x})^2$	2.1176	0.0293
Standard deviation of the residuals, $Sy/x = [\Sigma i (yi - \hat{y}i)^2 / (n - 2)]^{0.5}$, $\hat{y}i = b\ xi + a$	21.74	0.2118
Standard deviation of the intercept, (Sa) $Sa = Sy/x [\Sigma i\ xi^2 / (n \Sigma i (xi - \bar{x})^2)]^{0.5}$	2.127	$8.4x10^{-4}$
Standard deviation of the slope, (S_b) Sb $= Sy/x / [\Sigma i (xi - \bar{x})^2]^{0.5}$	0.0117	$1.7x10^{-4}$
Confidence limit of intercept(a) = a $\pm t S_a$	52.583 ± 27.03	$0.0051 \pm 2.2x10^{-3}$
Confidence limit of slope (b) =b $\pm t S_b$	2.1176 ± 0.15	$0.0293 \pm 1.1x10^{-2}$
(LOD)	0.017	0.086
(LOQ)	0.055	0.287
Sample throughput (h^{-1})	51	6
Molar absorptivity (ε) ($L.mole^1.cm^{-1}$) ε = $b \times M \times 1000$	485013.009	5692.998
Sandal's sensitivity (µ$g.cm^{-2}$) , S = M/ ε , M=M.wt of drug	0.000448	0.03816

Table (11): Application of the developed procedure for estimation of captopril in pharmaceutical formulations

CFIA / Merging Zones technique						Batch method				
Pharmaceutical preparation	**Present conc. mg.L^{-1}**	**Found**	***Rec%**	E_{rel}%	***RSD %**	**Present conc. mg.L^{-1}**	**Found**	**Rec%**	E_{rel}%	**RSD %**
Rilcapton (25mg) MEDOCHEMIE LTD,LIMASSOL -CYPRUS (EUROPE)	20	20.05	100.25	0.25	0.1	15	14.98	99.88	-0/12	0.05
	40	39.92	99.80	-0.20	0.088	35	34.96	99.92	-0.08	0.25
Rilcapton (50mg) M.A. Holder: MEDOCHEMIE LTD,LIMASSOL -CYPRUS (EUROPE)	20	19.98	99.90	-0.1	0.05	15	15.03	100.2	0.20	0.2
	40	40.10	100.25	0.25	0.014	35	35.04	100.12	0.12	1.76
aceprotin (50 mg) CODAL SYNTO LTD,LIMASSOL -CYPRUS (EUROPE)	20	20.10	100.5	0.5	0.664	15	15.02	100.20	0.2	0.014
	40	39.84	99.60	-0.40	0.267	35	34.92	99.84	-0.16	1.2
Captopril (50 mg) PL Holder: BristolLaboratories Ltd., Berkhamsted, Herts, HP4 1EG, UK	20	19.95	99.75	-0.25	0.014	15	14.96	99.76	-0.24	0.082
	40	40.10	100.25	0.25	0.1	35	34.90	99.80	-0.20	0.292
Accord (25mg) Healthcare,LTD, Sage House, Middlesex, HA1 4HG, United Kingdom	20	19.96	99.80	-0.2	0.08	15	14.95	99.70	-0.30	0.07
	40	39.90	99.75	-0.25	0.05	35	35.02	100.06	0.06	0.23
accord (50 mg) Healthcare,LTD, Sage House, Middlesex , HA1 4HF,United Kingdom	20	20.05	100.25	0.25	0.07	15	14.97	99.80	0.00	0.1
	40	40.05	100.125	0.125	0.29	35	34.98	99.96	-0.04	0.014

*Mean of six measurements of each method

Table (12): comparison of the suggested method with official method

Pharmaceutical preparation	suggested procedure CFIA/ Merging Zones procedure		Official method		S	Value	
	*Rec%	$(xi-\bar{x})_1^2$	*Rec%	$(xi-\bar{x})_2^2$		tcal*	Fcal**
Rilcapton (25 mg) MEDOCHEMIE LTD,LIMASSOL-CYPRUS (EUROPE)	99.96	0.01	100.1	0.06	0.089	0.89	1.25
Rilcapton (50 mg) M.A. Holder: MEDOCHEMIE, LTD.,LIMASSOL -CYPRUS (EUROPE)	100.1	0.07	99.94	0.02			
aceprotin (50 mg) CODAL SYNTO LTD, LIMASSOL-CYPRUS (EUROPE)	99.98	0.09	100.2	0.14			
Captopril (50 mg) PL Holder: Bristol Laboratories Ltd., Berkhamsted, Herts, HP4 1EG, UK	99.70	0.03	99.60	0.01			
accord (25 mg) Healthcare, LTD, Sage House, Middlesex, HA1 4HG,United Kingdom	100.3	0.06	100.1	0.02			
accord (50 mg) Healthcare, LTD, Sage House, Middlesex , HA1 4HF,United Kingdom	99.95	0.14	99.70	0.07			
	$(\bar{x}_1)$ = 99.99	$\Sigma(xi-\bar{x})_1^2$ = **0.4**	$(\bar{x}_2)$ = 99.94	$\Sigma(xi-\bar{x})_2^2$ = 0.32	$n_1+n_2-2=10$	$n_1-1=5$ $n_2-1=5$	

Theoretical results at (95%) confidence level; $n_1 = n_2 = 6$; t = 2.23 Where t has v = n_1+n_2-2 freedom degrees = 10 ; F=5.786 Where F has $v_1 = n_1-1$; $v_2 = n_2-1$ freedom degrees = 5

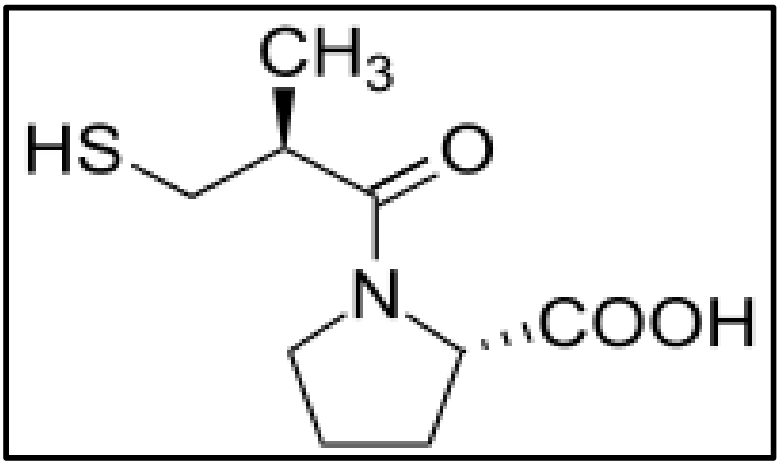

Figure (1): The chemical composition of captopril

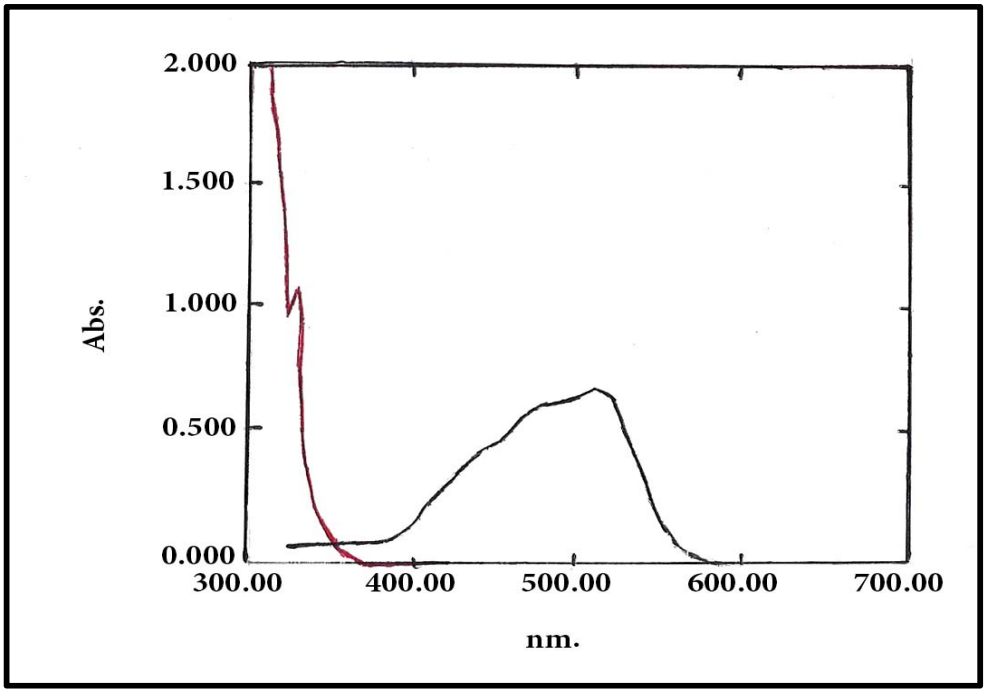

Figure (2): A - UV-VIS spectrum of colored product opposition reagent blank & B - Reagent blank opposition distilled water [(50 mg/L of CPL.)

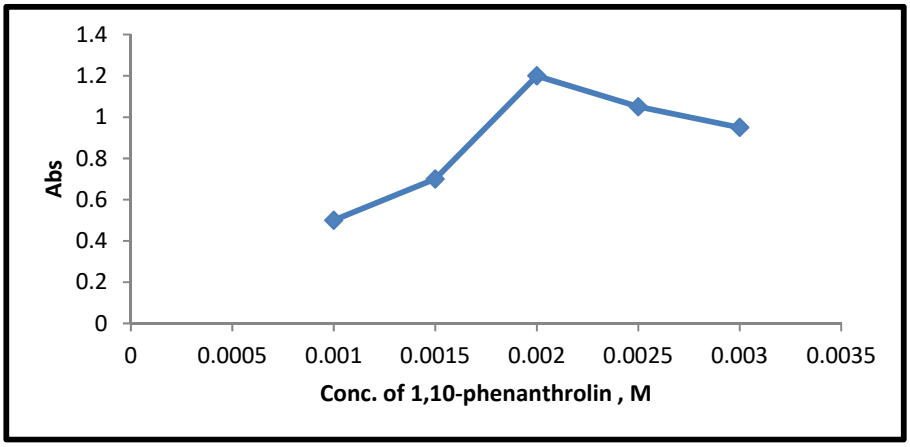

Figure (3): Effect of Conc. of 1,10-phenanthroline

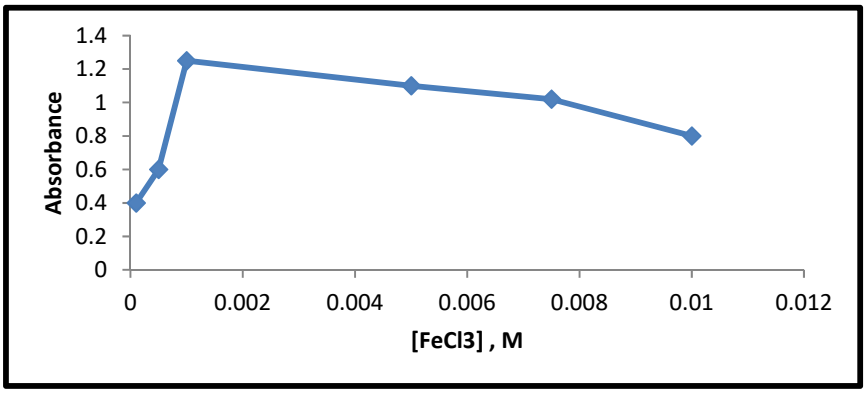

Figure (4): Effect of Conc. Of $FeCl_3$

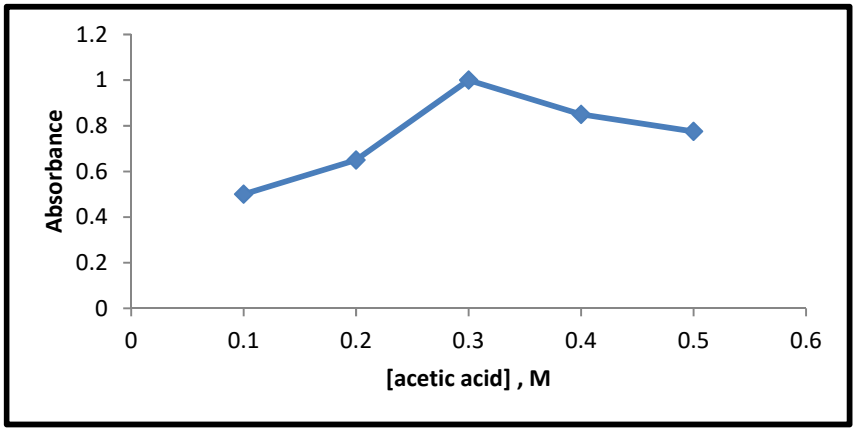

Figure (5): Effect of acetic acid concentration

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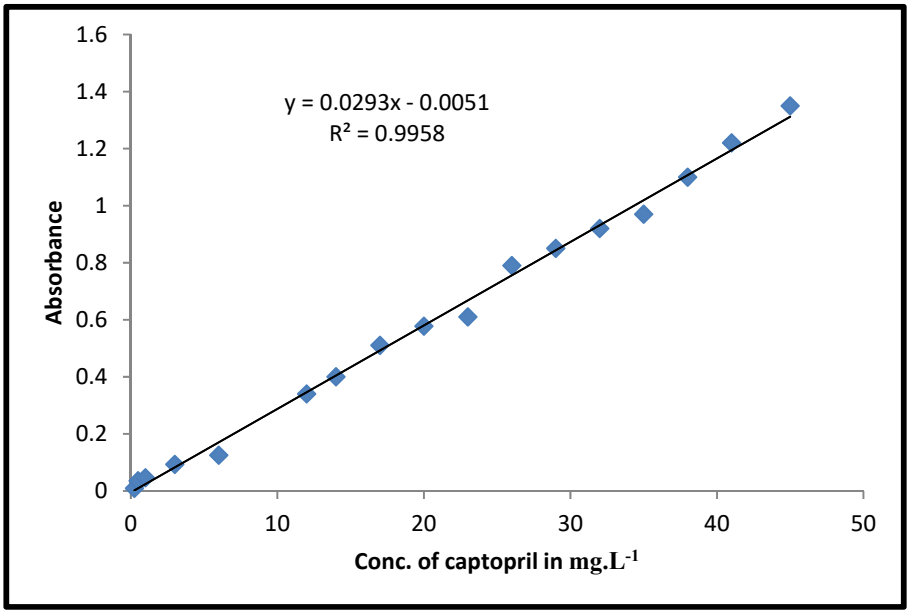


Figure (6): Calibration curve of CPL.

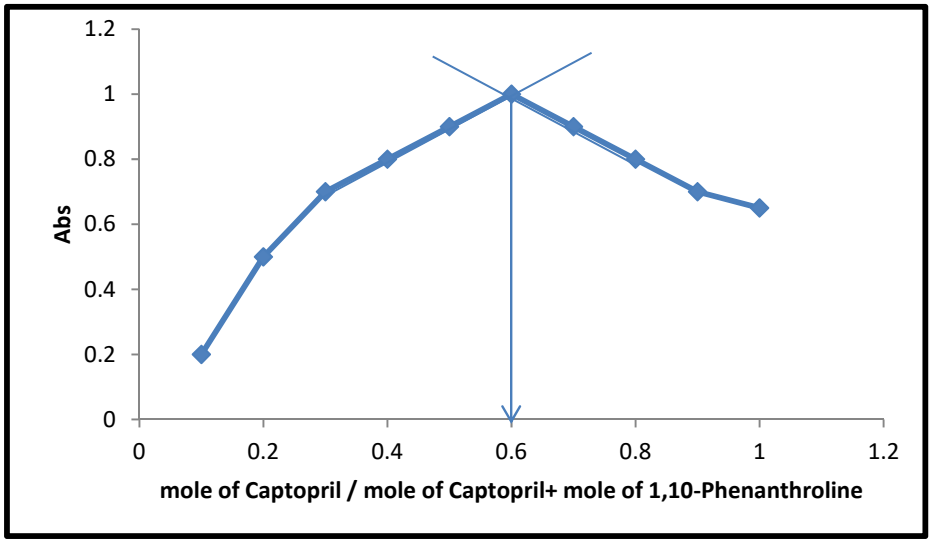
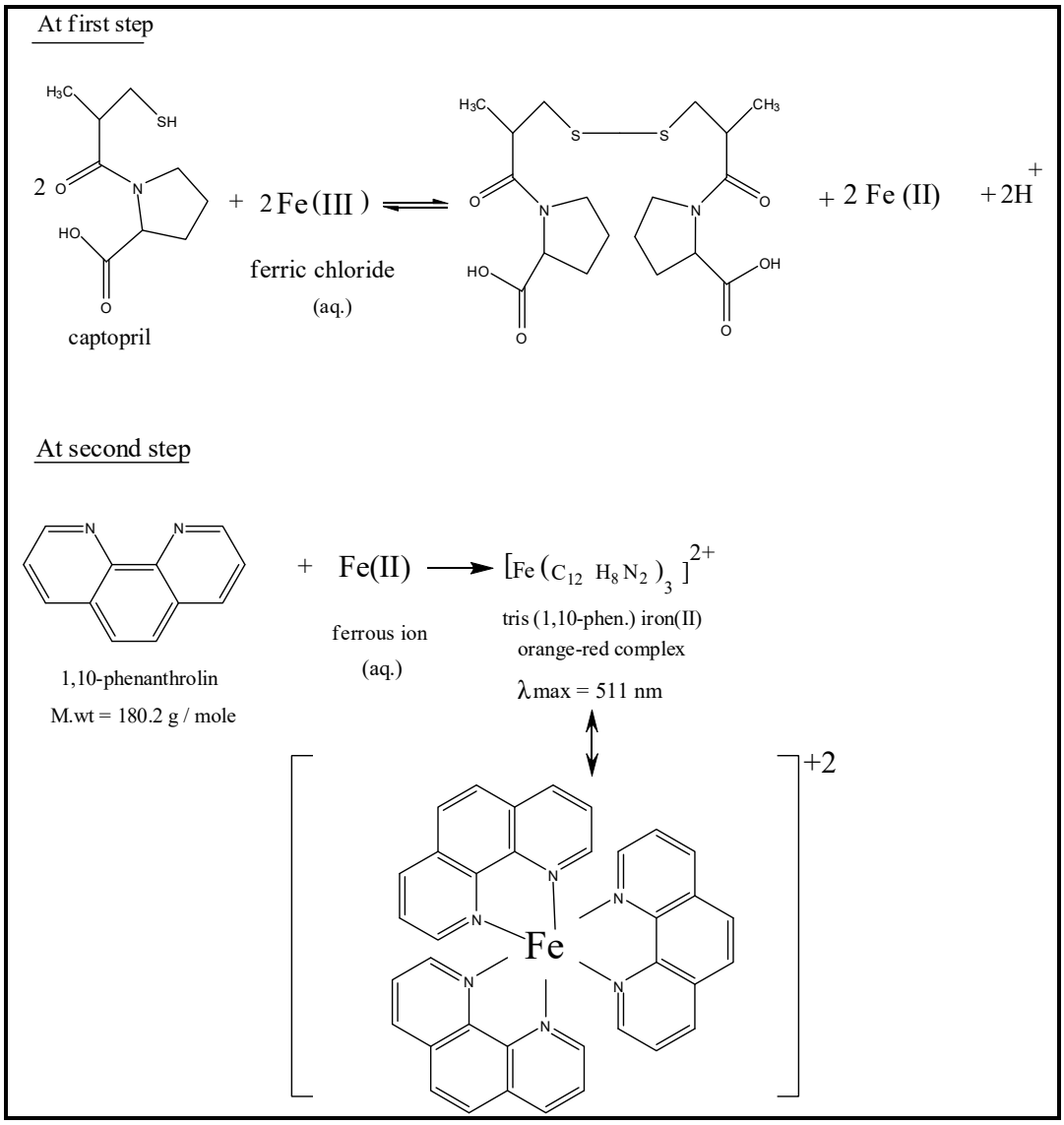


Figure (7): Continuous variance plot of the reaction between captopril and 1,10 Phenanthroline at λmax 511nm using batch procedure



Scheme (1): The proposed mechanism of the reaction between captopril with [$FeCl_3$ – 1,10-phenanthroline] complex

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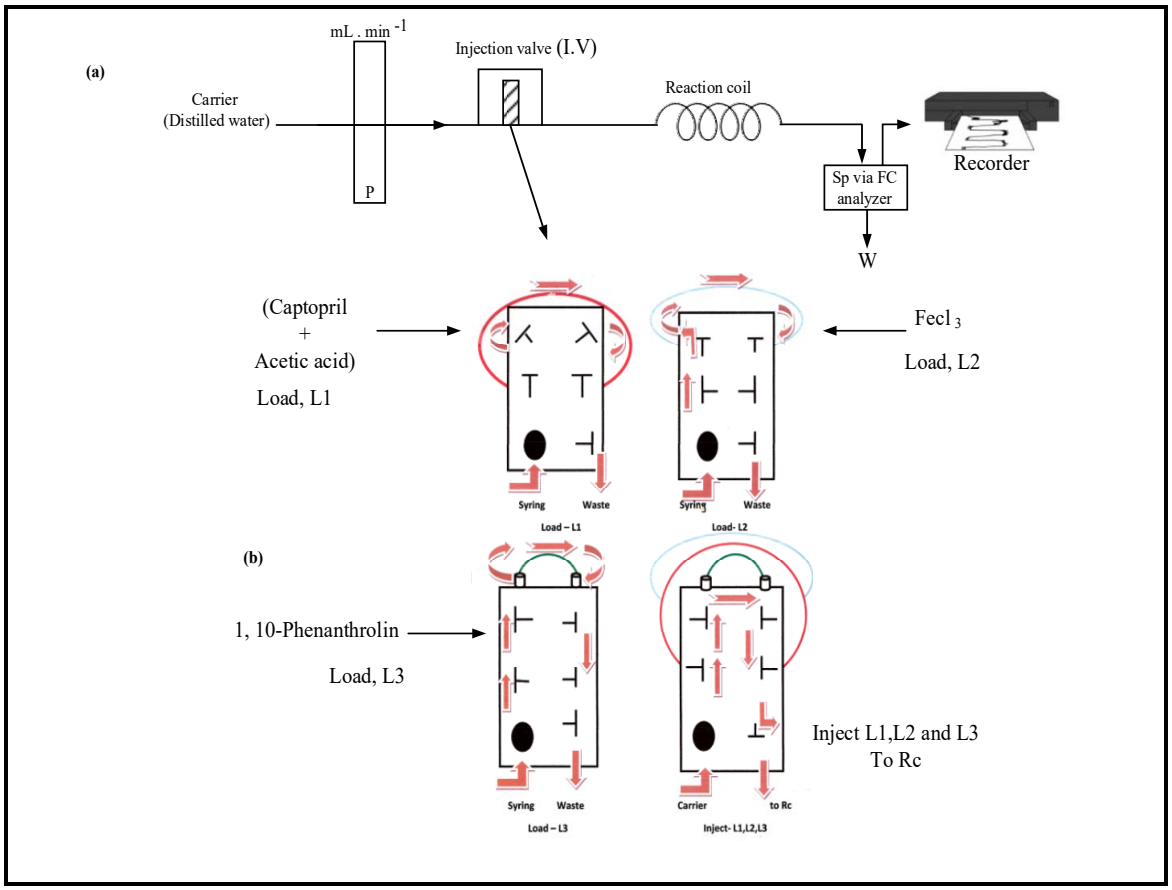

Figure (8): a/ The diagram of merging zones – flow injection analysis technique , Where Sp via FC , Spectrophotometry via flow cell; p; peristaltic pump, w; waste. b/ I.V; injection valve (scheme by details of six-three way injection valve load and inject to the developed FIA-system

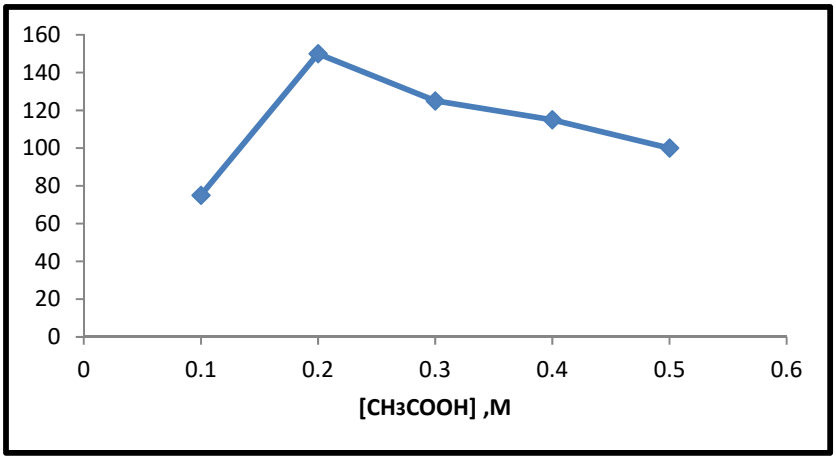

Figure (9): Effect of acetic acid concentration

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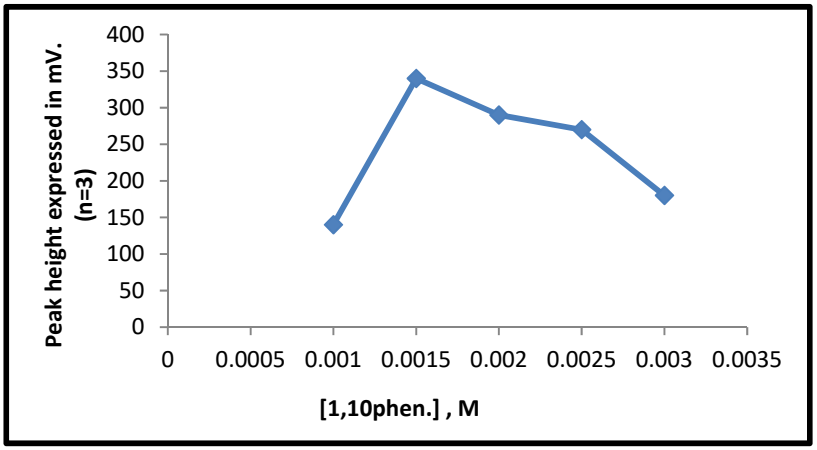

Figure (10): Effect of 1,10-phenanthroline concentration

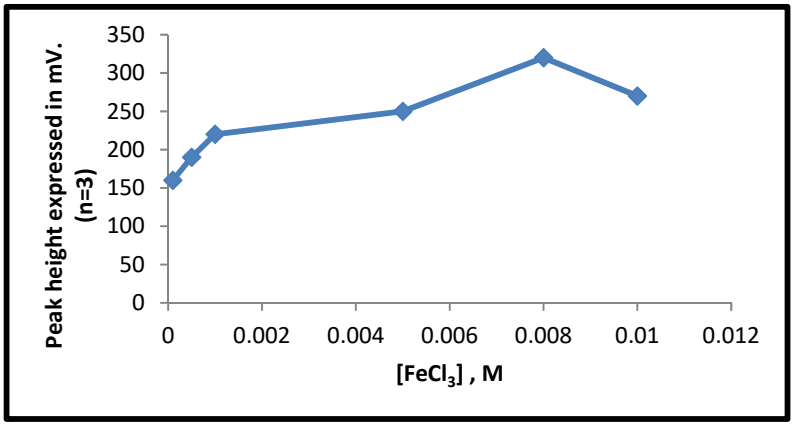

Figure (11): Effect of $FeCl_3$ concentration

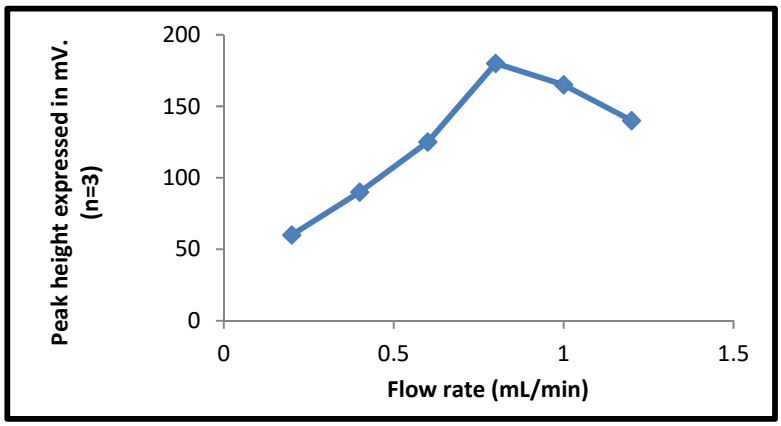

Figure (12): Influence of flow rate of distilled water

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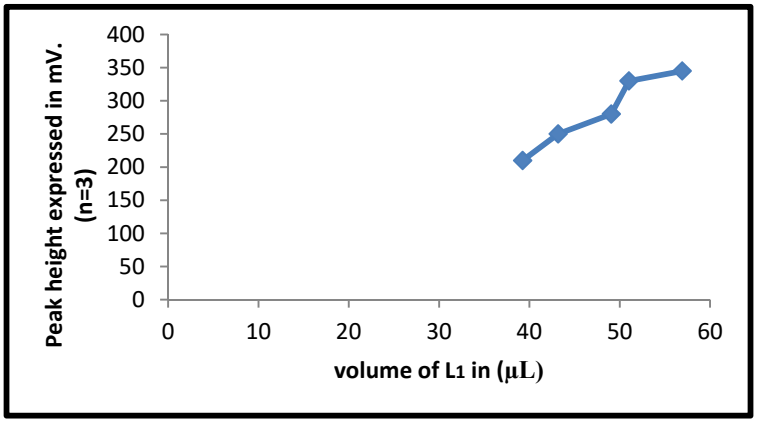
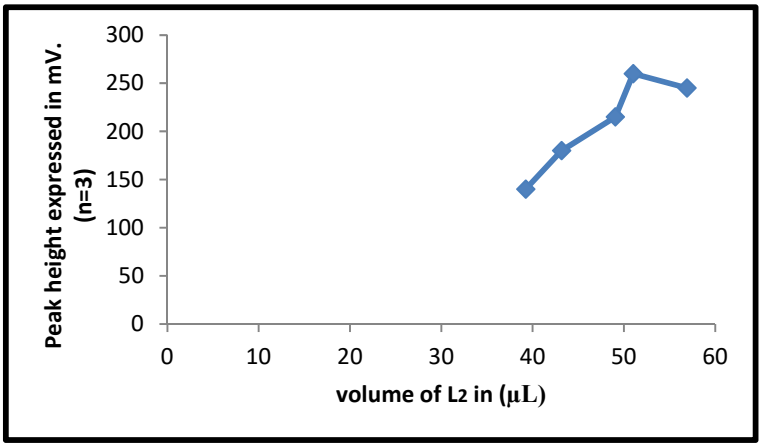
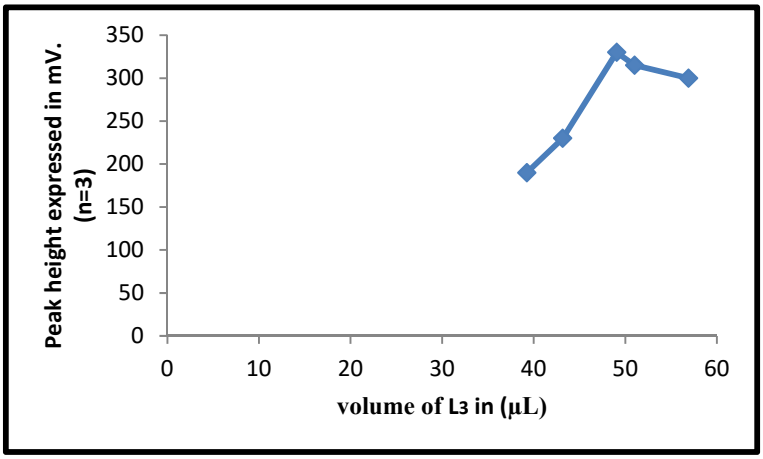


(a)



(b)



(c)

Figure (13): Effect of sample and reagents volumes (a) vol . of sample, (b) vol . of $FeCl_3$ & (c) vol . of reagent

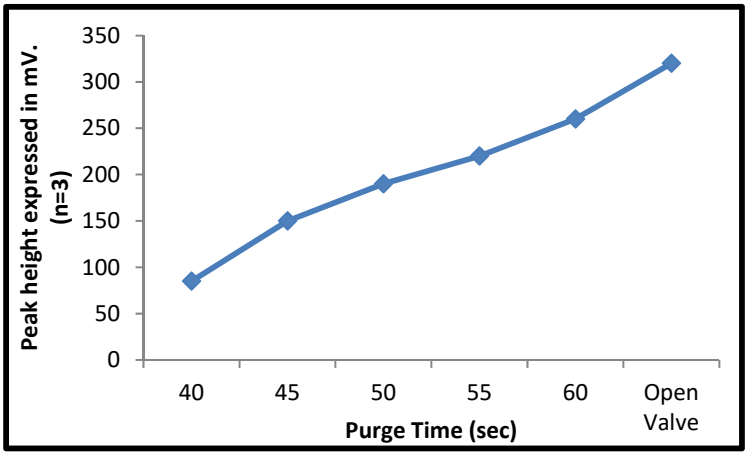


Figure (14): Effect of purge time

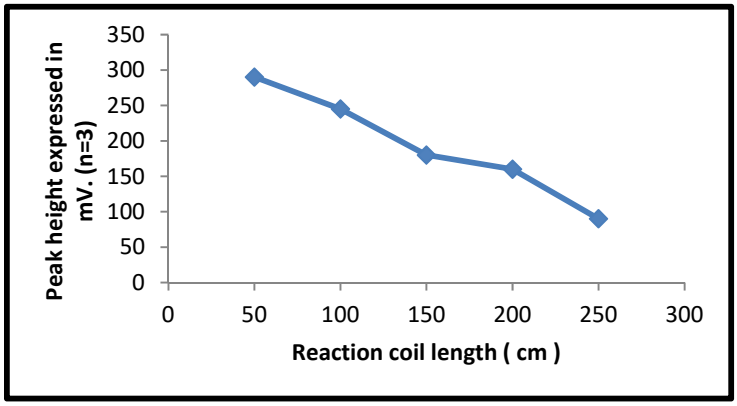


Figure (15): Effect of reaction coil (cm)

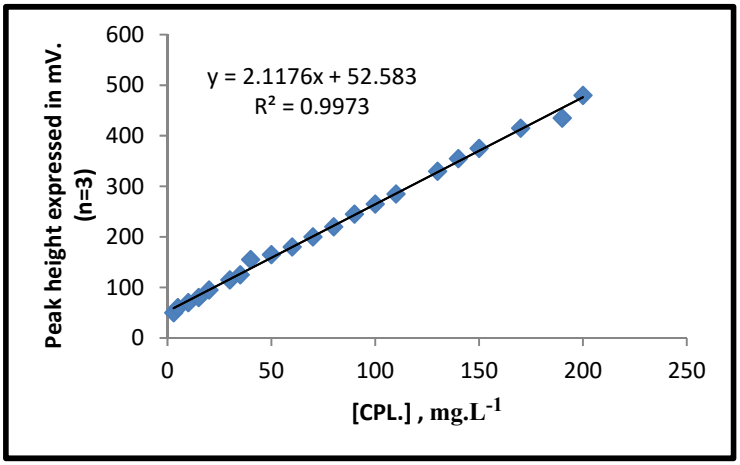


Figure (16): Linear calibration graph for estimation of captopril via CFIA merging Zones system

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