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New CFIA / Merging Zones Technique for Determination of Captopril in Pure and Pharmaceutics Dosage forms through the Oxidation / Reduction Reaction of Drug with Cu(II)-Neocuproine Complex via Spectrophotometric Detection

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Abstract: ***A new simplicity, accuracy, rapid and sensitive batch and merging zones-flow injection analysis spectrophotometric ways for estimation of captopril in fine material forms and pharmaceutical formulations were suggested . The procedure was depended on the oxidation and reduction reaction of CPL with Cu(II) – Neocuproine complex as reagent in presence of acetate buffer solution (pH=5) as a medium to form colored complex Cu (I)-neocuproine chelate that was measured at 454 nm. The optimized FIA order was able to estimate of CPL. with a throughput 48 sample.h^{-1}, flow rate 1.4 mL/min was used distilled water as a carrier, 51.02 µL analyte volume (50 µg.ml^{-1} CPL) with acetate buffer (0.1 M sodium acetate and 0.1 M acetic acid) , 49.06 µL Neocuproine (2x10^{-3}M) , 43.175 µL Cu(II) nitrate.3H2O (8x10^{-3}M) for L1,L2 and L3, respectively. Open valve model for sample inject and chemicals that used in the work . Calibration curves of absorbance against concentration sign of Beer' s law is submitted to within the concentrations scope of 1-80 & 3-120 µg.mL^{-1} of captopril with detection limits 1.5x10^{-1} , 3.1x10^{-2} µg.ml^{-1} and quantification limits , 5x10^{-1} , 1.03x10^{-2} µg/mL of captopril for batch and CFIA systems, respectively . a correlation coefficient (r) were 0.9960 , 0.9981 and percentage linearity (r^2%) were 99.60% , 99.81% , repeatability (RSD%) (n=7) were 0.112 and 0.26 for estimation of captopril with concentration 30 & 70 µg.mL^{-1} . The suggested procedure carried out successfully for estimation of CPL. in pharmaceutical formulations and statistical analysis of the values comparing with results by United States Pharmacopoeia (USP) were also reported.***

Keywords: Captopril, Neocuproine, Cuppric ion, FIA - merging zones ; Spectrophotometric determination

1. Introduction

Captopril, (CPL.) 1-[(2S)-3-mercapto-2-methyl-L-oxopropyl]–L- proline figure (1), is an angiotensin - converting enzyme (ACE) preventer that refers to the class of anti-hypertensive medicines which impact the renin-angiotensin order . The drug interplays with angiotensin - converting enzyme (ACE) because its affinity with a dipeptide and the sulphydryl class as well as plays a serious part linking covalently to the Zinc atom in the enzyme plus situation [1,2]. It is a famous drug carried out in medication of hypertension coronary heart ailment and congestive heart failure coming after myocardial infarction also in diabetic nephropathy and congestive cardiac insufficiency[3,4]

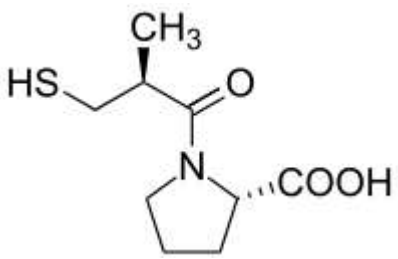


Figure 1: The chemical structure of captopril

Captopril was considered a development and ACE inhibitor both of because its modern mechanism of activity and also because of the revolutionary progression step[5,6].Several procedures for the estimation of Captopril which includes potentiometry[7,8] ،differential pulse polarography [9, 10] capillary electrophoresis [11, 12], capillary isotachophoresis[13], titrimetry[14], amperometry[15], conductometry[16], stripping voltammetry[17], gas chromatography[18] , HPLC[19-22], Flourimetry[23,24], flow injection analysis[25-30] , atomic absorption spectrometry[31,32] , chemiluminescence[33,34] , spectrophotometric[35-40] ,these methods include the use of reagents which interact with captopril to produce type with absorb in the visible version . Volumetric & HPLC ways for determination of drug in pure material and tablet preparations[41] , this method was time-consuming and use up large magnitudes of solvents. These hindrances delay the apply the methods for routine assay. These approaches depended on new instrumental systems ; in spite of sensitive; demand costly instruments and preservation and so include many manipulation moves and derivatization reactions ; many spectrophotometric ways undergo from deficiencies such as low sensitivity; demand prior extraction of the colored complex , long reaction period for color evolution (> 40min) or include boiling the reaction mixture for the long appoint . The formation of the charge transit colored product between copper(I) and neocuproine is the main of the existing spectrophotometric procedure for estimation of insignificant amounts of reducing agents (SH group)[42]. In the actual work , inexpensive, selective, reproducible, an ease and high sensitivity analytical method via FIA/ Merging zones

technique with spectrophotometric detection for indirect estimation of captopril in pure material and pharmaceutical preparations .This suggested FIA method offers a quite different approach , the manifold consisted in one channel and six-three way valves. The chemical process was proposed which involves the [Cu(II)-NC] complex is used as the reagent with thiol drugs (CPL) to produce an orange - yellow of [Cu(I)-NC] chelate is formed immediately takes few minutes to complete was measured at 454 nm of absorbance maximum. The proposed methods were designed in way that (CPL with Acetate buffer solution) , Neocuproine , Cu(II) were simply loaded in FIA system depended on the rule of merging zones through the homemade valves and distilled water as carrier (1.4 mL.min^{-1} (with no complicated extraction of samples, with no pretreatment, separation steps, time consuming and derivatization reagents were avoided.

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 absorbance measurements. A one channel manifold that 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 using for pump a carrier stream (distilled water) and solutions were passed the injection valve that (homemade); six-three way injection valve that contain three loops of (Teflon) where saddled with samples ; chemicals and reagents solutions which based on merging zones version. The injection valve that used to supplied suitable volumes that were injected of standard solutions and samples.

The tubes were made of flexible vinyl with 0.22 mm (I.D) using for the peristaltic pump ; mixing coil that was manufactured from glass with 2mm (I.D) . All of parts of the continuous flow injection analysis - merging zones technique was as shown as in Figure (8) with details. A carrier stream was distilled water that was joined with injected sample (captopril in acetate buffer solution at pH=5 in L1) and merged with the reagent (Neocuproine in L2) and Cu(II) nitrate.3H2O in L3). Then mixed it in mixing coil that it has length of 50cm , injection sample 51.02µL; flow rate of distilled water (carrier) 1.4 mL.min^{-1}. The maximum absorption was found under 454 nm as peak height in (mV) .

Chemicals and reagents

All the chemical materials and reagents employed were of analytical class and all the solutions preparing always used.

Captopril stock solution (M.wt=217.29 g.mole^{-1}, National Institute for the Control of Pharmaceutical and Biological Products (Beijing , China) (500 µg.mL^{-1} = 2.3x10^{-3}M): A 0.05 g amount of pure captopril was dissolving in distilled water then be consummated to 100 mL in standard flask with distilled water . More the diluted solutions preparing by adequate diluting of the stock standard solution with distilled water .

Neocuproine (M.wt=208.26 g.mol^{-1}, Merck) (5x10^{-3}M) : A 0.0521 g amount of neocuproine was dissolved in ethanol in 50 mL standard flask and dilution to the marked with ethanol.

Acetate buffer solution : prepared acetate buffer solution (pH=3) was dissolved 0.041gm of sodium acetate in distilled water in 50 ml volumetric flask to prepare (0.01M) and added (5.8 ml =0.58M) from acetic acid stock solution (5M) that preparing by diluting of concentrated 28.4 ml Acetic acid (BDH) with distilled water in 100 ml standard flask , preparing (pH=4) dissolving 0.41 gm of sodium acetate in 50 ml volumetric flask to prepare (0.1M) and added (5.8ml=0.58M) of acetic acid stock solution , preparing (pH=5) by dissolving 0.41gm of sodium acetate in 50 ml volumetric flask and added (1ml =0.1M) of acetic acid stock solution , preparing (pH=6) by dissolving 8.2 gm of sodium acetate in 50 ml volumetric flask to prepare (5M) and added (3ml=0.3M) of acetic acid stock solution.

Cu(II) nitrate.3H2O (M.wt=241.56 g.mole^{-1}, BDH) (1x10^{-2}M): preparing by dissolved 0.2416 gm of Cu $(NO_3)_2$.3H2O (BDH) in distilled water in 100 mL standard flask and diluting to the marked.

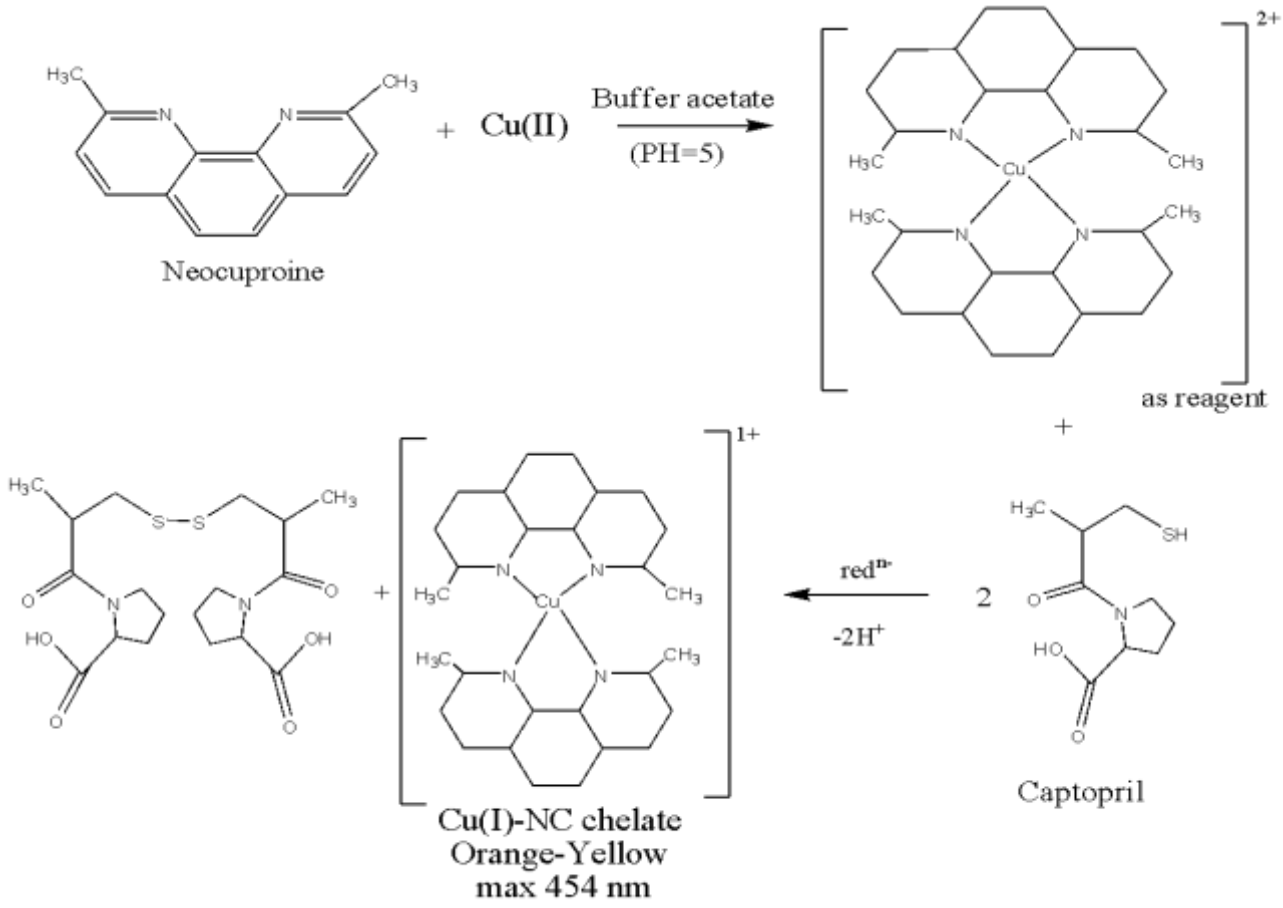
Pharmaceutical preparations of captopril (500 µg.mL^{-1})

Pharmaceutical formulations were gained from trading sources obtainable tablet by choosing 10 tablets from six kinds companies were assayed by the proposed procedures . Titles of the various providers were contain : (1) Rilcapton (25mg) MEDOCHEMI LTD, LIMASSOL-CYPRUS (EUROPE) (2) Rilcapton (50 mg) M.A. Holder: Medochemie Ltd., Limassol, Cyprus(EU) (3) aceprotin (50 mg) CODAL SYNTO LTD, LIMASSOL-CYPRUS (EUROPE) (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 the each sample was weighting that be equal to 500µg. mL^{-1} solution of activated component for each dosage forms. This amount of captopril dissolving in distilled water and filtrated to removal the insoluble residue that affect on the response. The filtrate transferring in 100 mL standard flask and concluded to the marked with the distilled water ; further solutions were diluted to preparing allot to the concentration inside of the linearity of the calibration graph.

3. Mechanism of the Reaction

The suggested mechanism of this reaction is an oxidation/reduction reaction[42] of captopril with [cupric ion-Neocuproine] complex as reagent that produced from reaction between Cu(II) with NC. The thiol drug (CPL.) was reduced the reagent to form a colored complex of [Cu(I)-NC] chelate complex immediately takes few minutes to complete . This colored product has the maximum absorption at 454 nm shown as in scheme (1):



Scheme 1: The suggested mechanism of the reaction between captopril with [Cu(II) - NC] complex

According to the suggested mechanism of the reaction; the number of moles of reactants that 1:2 (R/D), as shown in figure (2) .

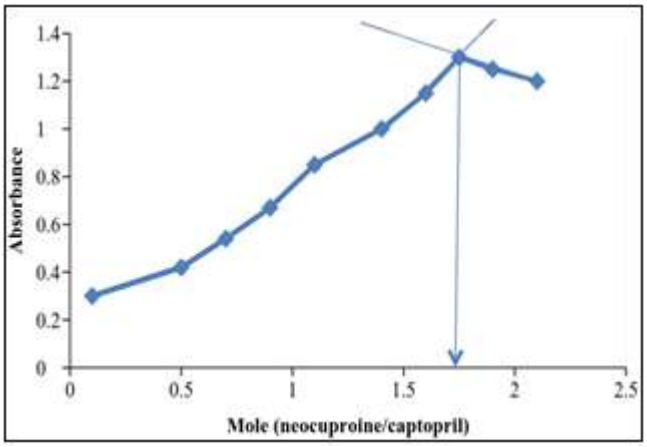


Figure 2: Mole ratio plot for the reaction of captopril with neocuproine at λmax 454 nm using batch procedure .

General procedures for calibration

Batch method

A 2mL of neocuproine (5 x 10^{-3}M) was transferred into a set of 25 mL standard flask ; after that were added 1mL of Cu(II) nitrate.3H2O (1 X 10^{-2}M), 3mL of acetate buffer solution pH=**5**. Then an increasing concentration (1-80) µg.mL^{-1} Captopril were prepared in a set of 25mL standard flask and consummate the volume of the solutions to the mark with distilled water. Stand for 150 sec ; the maximum absorption of the orange - yellow complex was found at λmax 454 nm opposition reagent blank.

FIA - merging zones procedure

The captopril solutions within the concentration range (3-120) µg .mL^{-1} preparing of stock solution 500 µg .mL^{-1}. The volumes of sample ; reagent and Cu(II) that were injected were 51.02 µL in L1 , 49.06 µL in L2 , 43.175µL in L3 are consist of captopril with acetate buffer solution (pH=5) was loaded in L1 , while neocuproine (2x10^{-3}M) was loaded in L2 , Cu(II) nitrate.3H2O (8x10^{-3}M) was loaded in L3 . A carrier stream was (distilled water) ; the analyte and other chemicals of loops injecting with flow rate 1.4 mL.min^{-1} as one channel. The product absorbance of colored complex was quantified at λmax 454 nm and the calibration graph were established of captopril µg/mL .

4. Results and Discussion

Batch spectrophotometric determination

Throughout preliminary experiments on the reaction of captopril with Cu(II) nitrate .3H2O (1x10^{-2}M) in the presence of neocuproine reagent (5x10^{-3}M) in acetate buffer solution at (pH=**5**) [42] . This reaction accurs in 25°C, the yellow-orange chelate complex was composed of and measured at λmax 454 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 complex with highest sensitivity and stability ideal of experiential conditions. In subsequent experiments, 50 µg. mL^{-1} of captopril was used and the absorbance that resulting of colored complex was found at λmax 454 nm opposition reagent blank , as shown in figure (3).

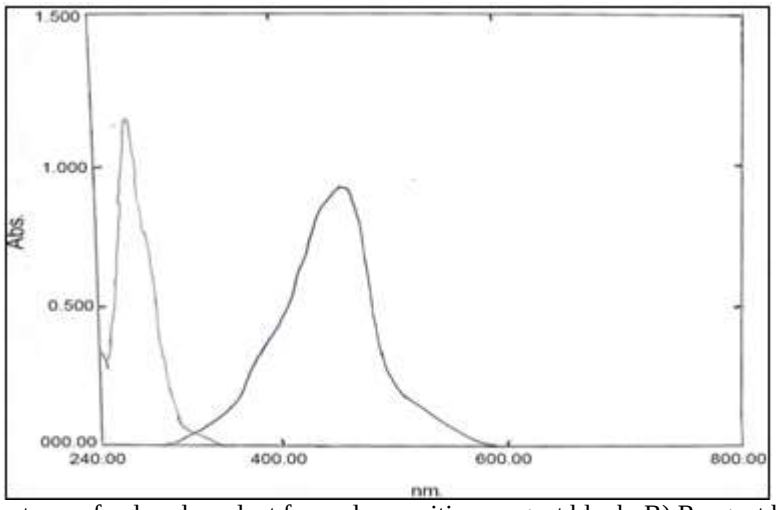


Figure 3: A) UV-VIS spectrum of colored product formed opposition reagent blank, B) Reagent blank opposition distilled water. (50 µg.mL^{-1} of CPL)

Effect of Cu (II) nitrate.3H2O

The effect of Cu (II) nitrate .3H2O concentration was examined. It has been monitored that the absorbance increase with increase of Cu (II) nitrate .3H2O concentration. The more than enough of Cu (II) can expose an affinity for neocuproine thereby inhibiting the favored quantitative composition of $[Cu(NC)2]^{+}$. The over plus of Cu(II) contends with Cu(I) for colored product composition with neocuproine. The $1x10^{-2}$ M of Cu (II) nitrate .3H2O was selected for subsequent experiences, as shown in figure (4).

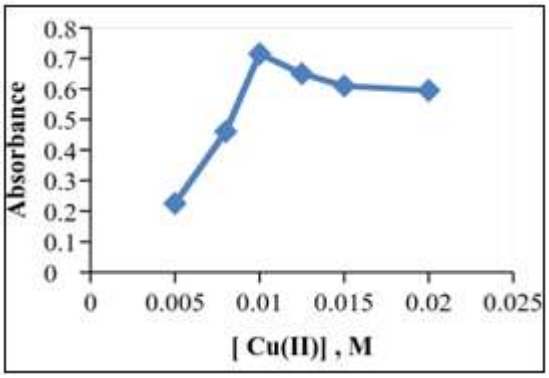


Figure 4: The effect of Cu (II) nitrate.3H2O concentration

Effect of medium acidity

The influence of medium acidity was studied carefully due to it was directly effect on the reduction of Cu(II) by CPL. and composition of [Cu (I)-NC] colored product . A series of solutions (pH=**2-6**) of acetate buffer solution were used for the experiment in the presence of 2 mL neocuproine ($5x10^{-3}$M) , 1mL of Cu(II) nitrate .3H2O ($1x10^{-2}$M) and suitable volume of captopril. The absorbance increase with observed pH up to 4.0 and remains stable to pH 5.0. A pH=**5** was chosen the optimum buffer solution as a suitable medium for the formation of colored product, as shown in figure (5).

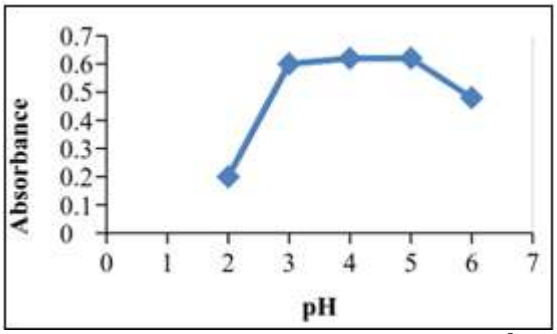


Figure 5: The effect of medium acidity at 25°C

Effect of Neocuproine (NC)

The influence of NC reagent was studied on the absorbance of the complex in the range $1x10^{-3}$ to $1x10^{-2}$M. In high concentration of NC, the absorbance due to Cu(I)-NC colored product decreases that the high concentration of neocuproine would result in a constructive interference of Cu(II) which could have appear insufficient transition of Cu(I) into Cu(I) - NC colored product through merged ligand complex formation [42,43]. A $5X10^{-3}$ M of NC concentration that gave the best absorbance and selected to be optimum concentration of reagent for the formation of colored product, as shown in figure (6)

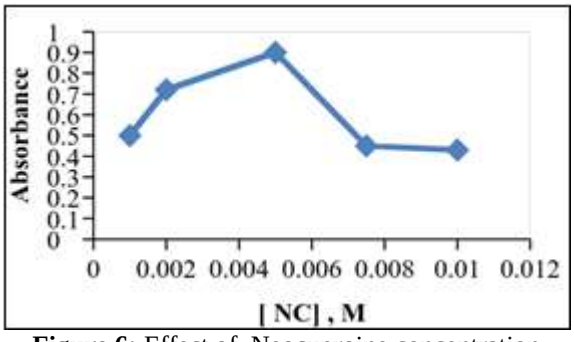


Figure 6: Effect of Neocuproine concentration

Effect of sequences of addition

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

Table 1: The sequence of addition

Reaction components	*Absorbance*
NC-Cu(II)-Buffer-CPL	0.534
CPL-NC-Cu(II)-Buffer	0.294
CPL-Buffer-NC-Cu(II)	0.267
NC-CPL-Cu(II)-Buffer	0.252
CPL-Buffer-Cu(II)-NC	0.421
NC-CPL-Buffer-Cu(II)	0.273

Calibration curve of classical method

Transfer a series of volumetric flask (50 mL) containing 2mL of Neocuproine ($5x10^{-3}$M) , then added 1mL of Cu(II) nitrate .3H2O ($1x10^{-2}$M) and 3mL of acetate buffer solution (pH=**5**) .Then an increasing volumes (0.125 – 45 mL) standard solutions of captopril (100µg.ml^{-1}) . The solutions had been diluted to the marked with distilled water. Then the reaction mixture to stand for 150 sec and measure the maximum absorption of the colored product at 454 nm against reagent blank prepared in same way without captopril . Each measurement repeated three times .The standard curve was constructed and linear range (1-80) µg.mL^{-1} for the estimation of captopril, as shown in figure (7)

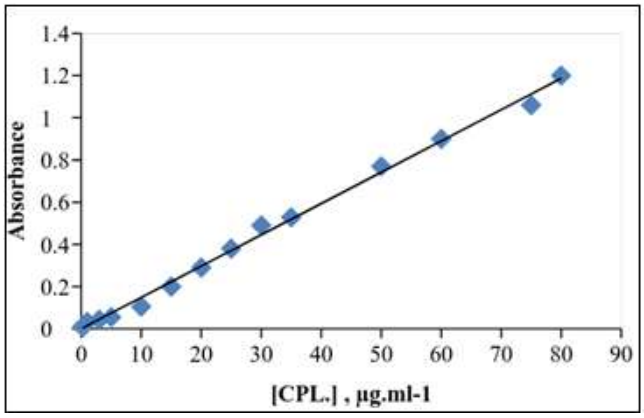


Figure 7: Calibration graph of Captopril

Accuracy and precision

Under the ideal conditions described in 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) show that the classical method have good with high accuracy and precision; each measurement are repeated for three times .

Table 2: Accuracy and Precision of the classical procedure

Captopril conc. µg.ml^{-1}		Error	*Rec %	Erel %	*RSD %
Present µ	found $\underline{x}$				
10	9.95	-0.05	99.500	-0.500	0.04
25	24.86	-0.14	99.440	-0.560	0.00
50	50.09	0.09	100.18	0.180	0.14

*Average of three determinations

Rec % (the recovery) = 100 + Erel %; Erel % (relative error) = [($\underline{x}$ -µ) / µ] x100 RSD % (relative standard deviation) = ($\frac{\sigma n-1}{\underline{x}}$) x 100 ; (standard deviation) = $\frac{\sigma n-1}{\underline{x}}$ = [$\frac{\sum(xi-\underline{x})^2}{n-1}$]$^{0.5}$; $\underline{x} = \sum xi/n$

Calculations of stability constant

Calculated static stability[44] for the proposed interaction (CPL. : neocuproine) was calculated depending on the two groups of solutions were prepared ; first group of solutions were placed to include stoichiometric lot of captopril to reagent neocuproine , while the second group were placed to include fivefold excess of neocuproine **.** According to the proposed mechanism and stoichiometry ratio between reagent and drug (1:2**)**. The stability constant can be wrote as follows:

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

While (α) (degree of dissociation) can be wrote as follows:

α = Am - As /Am

Where Am ; As are the values of absorbance of the aqueous solution including a more than enough and stoichiometric amount of the reagent (neocuproine) as shown in Table (3)

Table 3: Stability constant of yellow-orange complex of captopril with [Cu(II)-NC] in acetate buffer (pH=5)

captopril	Am*	As*	α	C(M)	K ($L^2.mol^{-2}$) or (M^{-2})
	0.554	0.486	0.123	$2.3x10^{-4}$	$2.24x10^{10}$

*Average of three determinations

Flow injection/ Merging zones spectrophotometric determination

After selecting the optimum conditions of redox reaction of captopril with Cu(II) -NC as a reagent in acetate buffer at pH=**5** in 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 to estimate captopril. So the batch procedure for estimation of captopril was employed as a basis to develop flow injection analysis method.

Manifold of flow injection system

After installing the system and linked portions , been the study of optimal design of system . The developed system shown in Figure (8) is composed of one line supplies the distilled water (carrier) under flow rate 1.4 mL/min leading to the injection valve ; which contain three loop (different loop length with 0.5mm I.D.) that fills by the sample and reagents according to the order (captopril with acetate buffer solution, L1), (Neocuproine , L2) and (Cu(II) nitrate.3H2O , L3) .

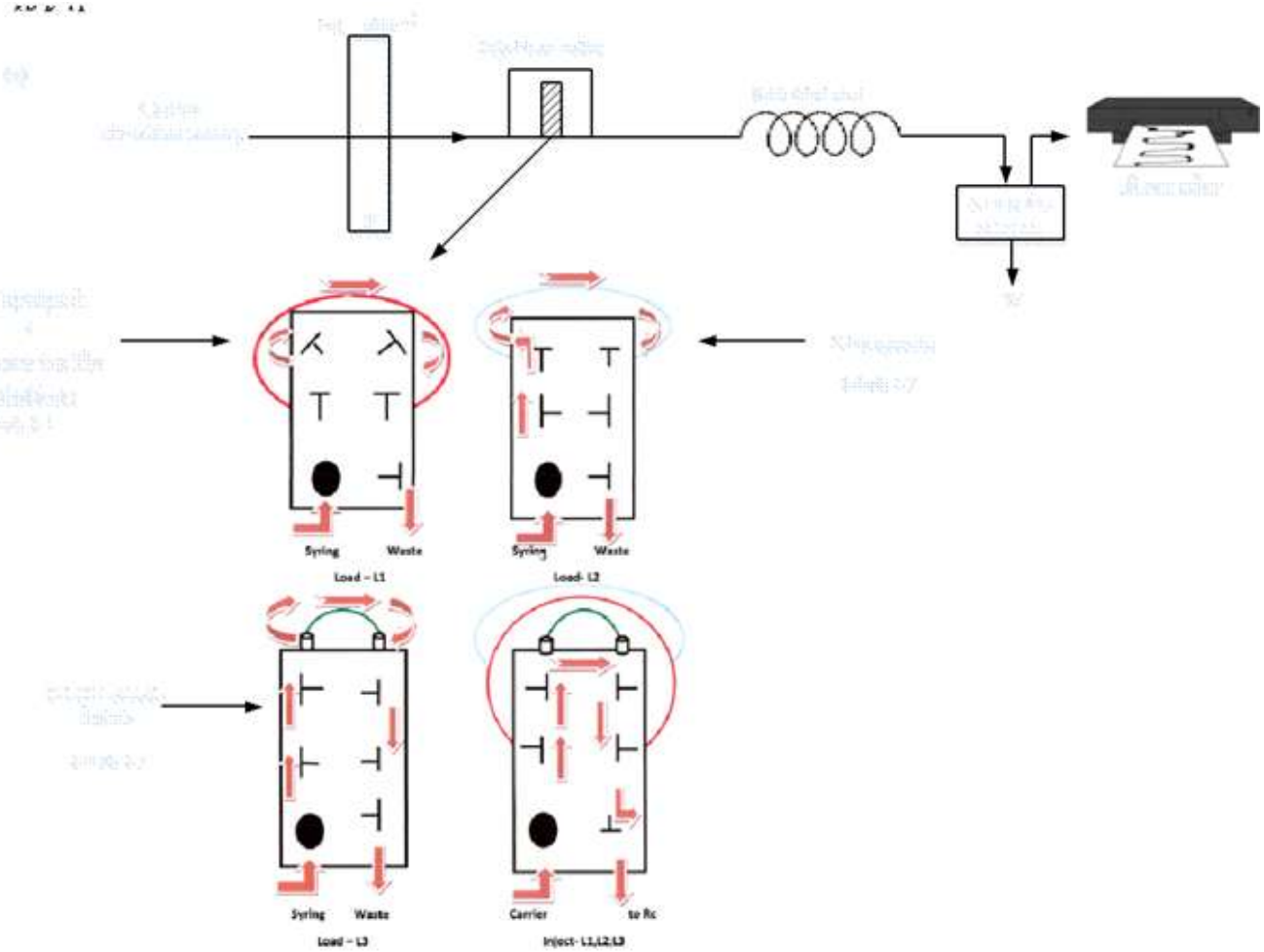


Figure 8: (a)The diagram of merging zones - flow injection analysis technique, Where Sp via FC , Spectrophotometry via flow cell; p; peristaltic pump; w; waste (b) I.V; injection valve (scheme by details of six-three way injection valve load and inject to the developed FIA-system.

Optimization of Experimental parameters

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

Effect of Chemical variables

The influence of neocuproine , acetate buffer and Cu(II) concentrations 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 neocuproine concentration

A series of solutions ($1x10^{-3}$ – $1x10^{-2}$ M) were prepared of neocuproine using flow rate 1.4 mL/min; with 51. 02 µL of 50 $\mu g.mL^{-1}$ captopril as injected sample volume . All measurements were repeated for three successive times. Table (4) and figure (9) shows that $2x10^{-3}$M of neocuproine is the optimum concentration because it can be seen ; in high concentrations of (NC) ; the absorbance due to Cu(I)-NC colored product diminish , it was used in subsequent work.

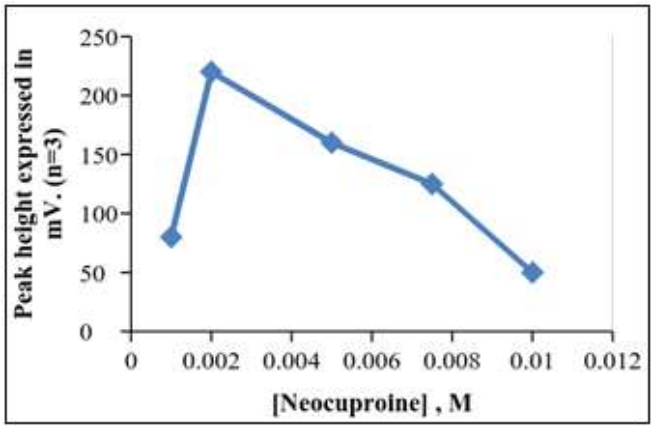


Figure 9: Effect of neocuproine concentration

Table 4: Effect of Neocuproine concentration on value of measurement of peak height for [Captopril-Neocuproine-Cu(II)] system

[NC] , M	Absorbance as peak height ($\underline{x}$) (n=3) mV.	Standard deviation σn-1	Repeatability %RSD	Confidence interval of the mean $\underline{x} \pm t0.05\frac{\sigma n-1}{\sqrt{n}}$ for n-1
$1x10^{-3}$	80	1.00	0.52	80 ± 2.49
$2x10^{-3}$	220	0.00	0.00	220 ± 0.00
$5x10^{-3}$	160	1.24	0.49	160 ± 3.08
$7.5x10^{-3}$	125	1.70	0.81	125 ± 4.23
$1x10^{-2}$	50	0.00	0.00	50 ± 0.00

Effect of buffer solution

The impact of acetate buffer solution concentration on the analytical response was studied using ideal concentration of neocuproine $2x10^{-3}$M. Series of diluted solution of acetate buffer solution (pH=**2-6**) were prepared, 51.02µL sample volume (50µg.mL^{1}CPL.) was used and the data obtained were plotted as shown as in figure (10), pH=5 of reaction medium was chosen as the best value to complete the reaction .

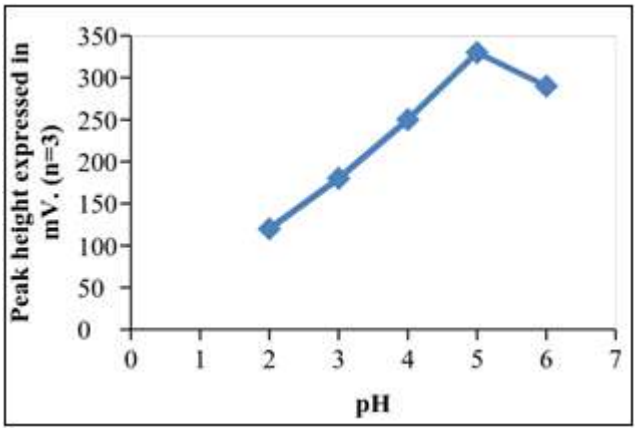


Figure 10: Effect of buffer acetate solution

Effect of Cu(II) concentration

Various concentrations ($5x10^{-3}$- $2x10^{-2}$M) of Cu(NO3)2.3H2O was examined on the analytical signal. The oxidizing force of Cu(II) in the solution including Neocuproine is reliance on ease of composition of $[Cu(NC)]^{+}$ (referred in batch procedure). The values show that best concentration of Cu(II) is $8x10^{-3}$M . Therefore , the ideal Cu(II) concentration was chosen to be $8x10^{-3}$M as shown as in figure (11) .

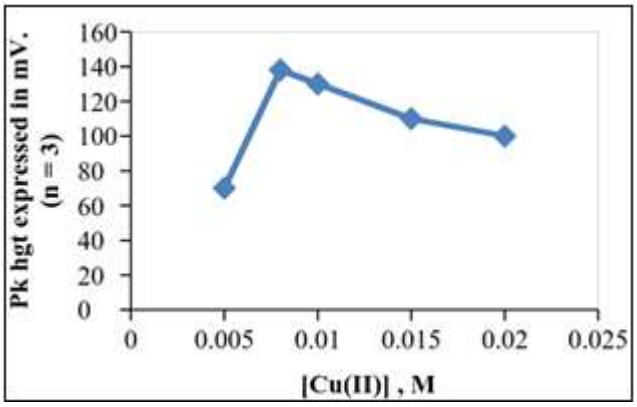


Figure 11: Effect of Cu(II) nitrate.3H2O concentration

Effect of manifold variables

The effect of variables like injected volume of sample , reagent volume , reaction coil length , purge time and flow rate on the analytical response was observed . This peak height based on stay time of the sample in the system which was conducted with lengths for reaction coil and flow rate. The physical variables were studied under the ideal concentration of the reactants ; neocuproine ($2x10^{-3}$M), acetate buffer (pH=**5)** , Cu(II) nitrate.3H2O ($8x10^{-3}$ M) and primary concentration of captopril (50 µg.ml^{-1}).

Effect of flow rate

The influence of the flow rate was observed under ideal chemical parameters. These values obtained shows which the optimum flow rate of pump of sample with least dispersion will be in 1.4 mL.min^{-1}. In the lower flow rate, a dispersion will be the highest level while in the greater flow rate and the reaction may be not complete, as shown as in figure (12) and Table (5)

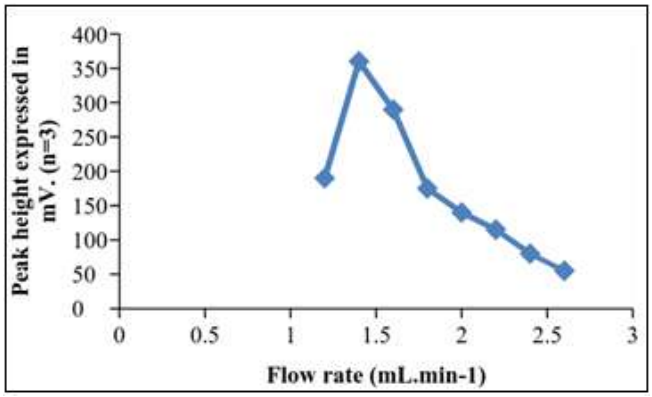


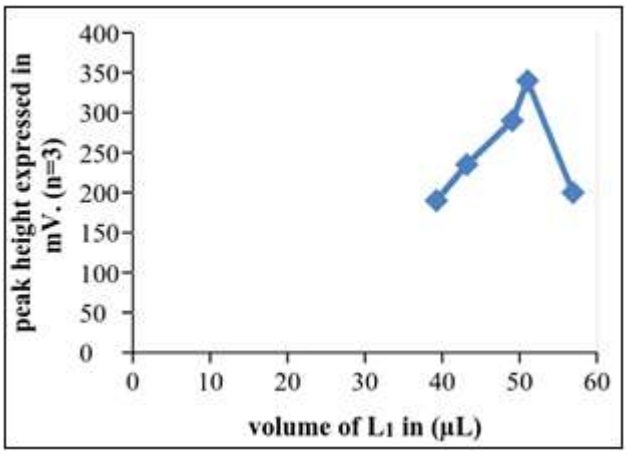
Figure 12: Influence of flow rate of distilled water

Table 5: Influence of flow rate on value of measurement of peak height for [captopril – neocuproine – Cu(II)] system
*R% is RSD% (Repeatability)

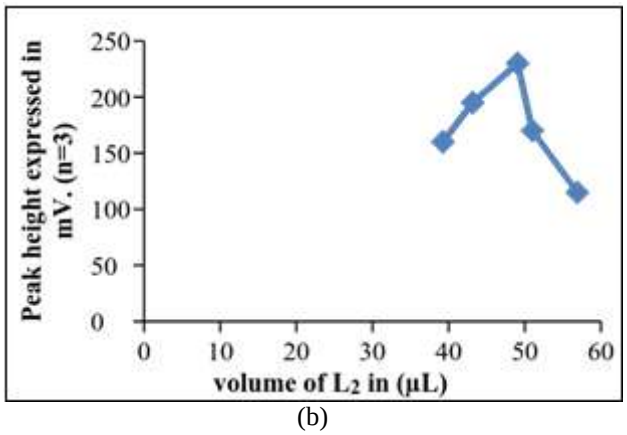
Pump speed Indication approximate	Flow rate (ml.min^{-1}) (carrier)	Average Peak height (n=3) mV ($\underline{x}$)	Standard deviation σn-1	R%	Confidence interval of the mean $\underline{x} \pm t0.05\frac{\sigma n-}{\sqrt{n}}$ for n-1
3.5	1.2	190	0.00	0.00	190 ± 0.00
4	1.4	360	0.14	0.04	360 ± 0.35
4.5	1.6	290	0.21	0.07	290 ± 0.52
5	1.8	175	0.28	0.11	175 ± 0.70
5.5	2.0	140	0.00	0.00	140 ± 0.00
6	2.2	115	0.07	0.04	115 ± 0.17
6.5	2.4	80	0.03	0.02	80 ± 0.07
7	2.6	55	0.00	0.00	55 ± 0.00

Effect of sample and reagents volumes

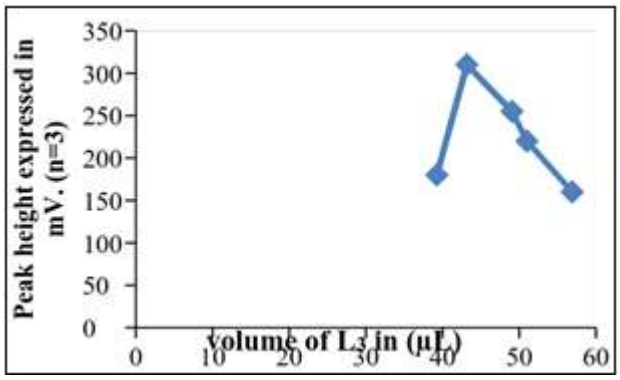
The injected volume of the sample and reagents were observed by using various sample and reagents volumes. (56.91, 51.02, 49.06, 43.175and 39.25) µL, respectively using open valve mode. The values obtained shows which injected volumes of 51.02, 49.06 and 43.175 µL for sample volume (50 µg.mL^{-1} captopril) with acetate buffer solution (pH=**5**) in **L1** , 2x10^{-3}M of neocuproine in **L2** and 8x10^{-3}M of Cu(II) nitrate.3H2O in **L3** respectively were optimum volumes that presented the maximum signal as shown in figure 13 (a , b , c)



(a)



(b)



(c)

Figure 13: Effect of sample and reagents volumes
a) vol . of sample, b) vol . of reagent, c) vol . of Cu(II)

Effect of reaction coil

The influence of different reaction coil lengths (50, 100, 150, 200, 250) cm which has (I.D. 2mm) which was placed after injection valve directly in the flow technique (figure8). This ideal concentration using for redox reaction of captopril (50µg.ml^{-1}) in acetate buffer (pH=**5)** with neocuproine (2x10^{-3}M) and (8x10^{-3}M) cuppric nitrate 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 (14). A sharp decline in the peak height was observed above this value because of the dispersion phenomena Therefor, a 50 cm gave the maximum peak height and was used in all subsequent experiments.

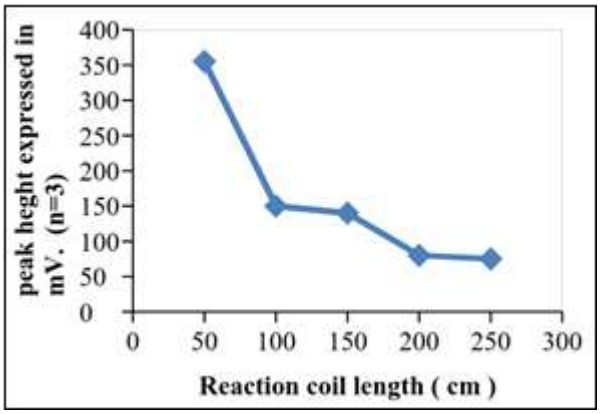


Figure 14: Effect reaction coil (cm)

Purge Time

Purge time of the sample segment to injecting with a carrier (distilled water) was investigated , using optimum chemical and physical parameters were studied previously ,

(5,10,15,20,25,30,35) sec and open valve (injected mode) were used for this study . The purge time more than 35 sec giving a highest response intensity with less dispersion , we calculated by the period time between the analyte injection and inception of the end of the analytical signal. The Open valve was chosen ideal injection time to conclude transport the sample from sample loop to flow cell , as shown in figure(15) and table (6). The reaction period of the each analyte was 75 sec , so the analyte throughput was 48 sample / h .

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

Purge Time (sec)	Average Peak height (n = 3) mV	Standard deviation σn-1	R%	Confidence interval of the mean $\underline{x} \pm t0.05\frac{\sigma n-1}{\sqrt{n}}$ for n-1
5	15	0.4	2.67	15 ± 0.99
10	44	0.00	0.00	44 ± 0.00
15	52	1.00	1.92	52 ± 2.49
20	85	0.14	0.16	85 ± 0.35
25	110	0.00	0.00	110 ± 0.00
30	150	1.2	0.8	150 ± 2.98
35	178	0.21	0.12	178 ± 0.52
Open valve	260	0.00	0.00	260 ± 0.00

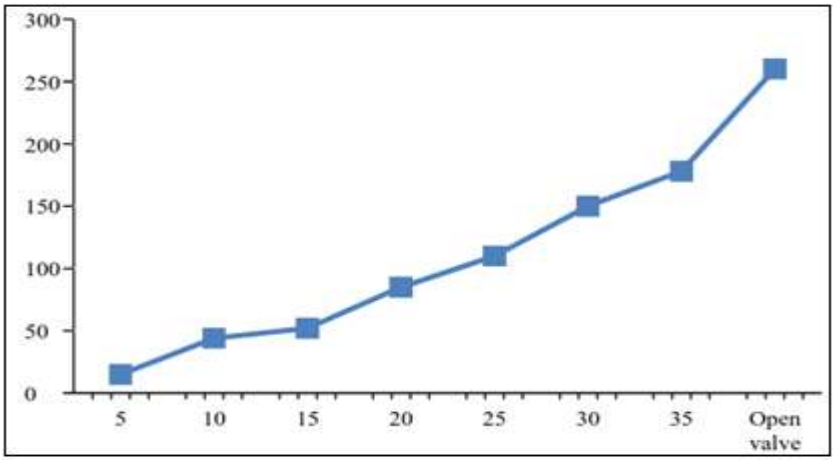


Figure 15: Effect of purge time

Calibration Graph

Data processing using the equation of a straight line

A set of captopril solutions (3-120 µg . mL^{-1}) preparing by a suitable dilution of the stock solution . All of the 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 (µg.ml^{-1}) . The results obtained were summarized in table (7) and displayed in Figure (16) that offers contrast of response with concentration of captopril . Data were processed mathematically[45,46] and will clarify the method was used to calculate the linear equation *of the class* (*y* = bx+a)

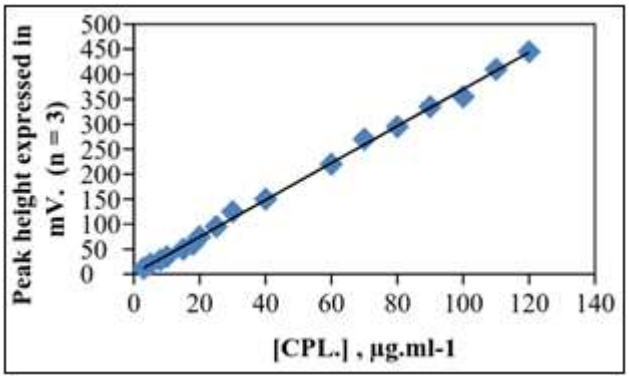


Figure 16: Linear calibration curve for determination of Captopril using CFIA merging Zones system

Table 7: Summary of linear calibration curve for the determination of captopril via CFIA/ Merging zones system

Linear range of Captopril (µg.ml^{-1})	Average Peak height (n=3) mV $\underline{x}$	Standard Deviation σn-1	R%	Confidence interval of the mean $\underline{x} \pm t0.05\frac{\sigma n-1}{\sqrt{n}}$ for n-1
3	12	0.00	0.00	12 ± 0.00
5	20	0.21	0.87	20 ± 0.39
8	28	0.14	0.43	28 ± 0.26
10	35	1.00	2.63	35 ± 1.84
15	50	0.00	0.00	50 ± 0.00
18	60	0.40	0.57	60 ± 0.73
20	75	1.20	1.46	75 ± 2.20
25	95	0.00	0.00	95 ± 0.00
30	125	0.14	0.112	125 ± 0.13
40	150	0.021	0.01	150 ± 0.04

60	220	0.014	0.006	220 ± 0.03
70	270	0.7	0.26	270 ± 0.65
80	295	0.30	0.10	295 ± 0.55
90	335	0.00	0.00	335 ± 0.00
100	355	0.14	0.04	355 ± 0.26
110	410	0.21	0.05	410 ± 0.39
120	445	0.00	0.00	445 ± 0.00

Repeatability

To study the efficiency of the suggested procedure in estimation of captopril by repeated of injection process and measurement for multiple times using two concentrations of captopril (30,70) µg.mL^{-1}and calculated of standard deviation and relative standard deviation for both concentrations that were studied ,as shown as in table (8).

Table 8: Repeatability of consecutive measurement for Captopril t_{tab} = 2.45 at 95%confidence limit for (n-1)

Captopril µg.ml^{-1}	Number of measurements (n)	$\underline{y}$ (n =7) mV	Standard deviation σn-1	Repeatability R%	Confidence interval of the mean $\underline{y} \pm t0.05\frac{\sigma n-1}{\sqrt{n}}$ for n-1
30	7	125	0.14	0.112	125 ± 0.13
70	7	270	0.7	0.26	270 ± 0.65

Analysis of variation(ANOVA) [47,48] of the equation linear.

Calculate sum of squares of the difference of values y_i (response) from ŷi (appraiser response), (imply error) and called (about regression) to obtain Σ $(y_i - \hat{y}_i)^2$ for (n-2) freedom degrees to get sum of squares $(So)^2$. Calculate the sum of squares of the variance of values y^8_i from average value ȳ (due to regression) to obtain $\Sigma(\hat{y}_i - \bar{y})^2$ and for (1) of degrees of freedom to obtain sum of squares $(S1)^2$, when dividing the $(S1)^2$ on $(S0)^2$ get the value(F) as showed in the table (9) .

Table 9: ANOVA for the equation of a straight line values

Source	Sum of Squares	Df	Mean square	Fstat. $=S1^2/S2^2$
Regression	$\Sigma(\hat{y}i- \bar{y}i)^2$=344543.05	V1 = 1	344543.05	670.80
Error	$\Sigma(\bar{y}i-\hat{y}i)^2$ = 3595.42	V2 = 7	513.63	
Total	348138.47	8		

F_{v2}^{v1}=Fv_7^{v1}= 5.591 << Fstat. = 670.80 , so it may be complete which there is an important relation between the concentration of captopril and the signal got.

Analytical parameters

The analytical characteristics just as correlation coefficient (r) , detection limit , linear range and relative standard deviation of each procedure were estimated[45,46] at the improved conditions ;as shown in the Table (10) . A calibration curve was constructed (fig. 16) for a set of captopril standard solution and the basic analytical figure of deserts of the proposed method . Statistical assessment of regression line presented result of standard deviation for residuals (Sy/x) ; intercept (S_a) and slope (S_b) under 95% confidence limits for (n-2) freedom degrees were clarified in the table . The small subjects were 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 48 sample/h); large linear scale of calibration curve were gotten.

Table 10: Analytical characteristics and regression parameters of the suggested procedure for estimation of captopril

Parameters	*FIA procedure*	*Batch procedure*
Linear range (µg. ml^{-1})	3-120	1-80
Correlation Coefficient / r^2 $r=\Sigma i [(xi - \underline{x})(yi - \bar{y})][(\Sigma i(xi - \underline{x})^2)(\Sigma i(yi - \bar{y})^2)]^{0.5}$	0.9981	0.9960
Regression equation y = b x+ a; y = absorbance ; x = concentration (µg. mL^{-1})	y = 3.693x+ 0.65	y = 0.0148x+ 0.0001
Linearity (r^2%)	99.81	99.60
Slope (b) ; (mL.µg^{-1}) $b = \Sigma i [(xi - \underline{x})(yi - \bar{y})]/\Sigma i(xi - \underline{x})^2$	3.693	0.0148
Intercept;(a) ; (a = y– b x)	0.65	0.0001
Standard deviation of the residuals; $Sy/x = [\Sigma i (yi - \hat{y}i)^2 /(n - 2)]^{0.5}$; ŷi = bxi+a	26.477	0.2961
Standard deviation of the slope (Sb) $Sb = Sy/x /[\Sigma i(xi - \underline{x})^2]^{0.5}$	0.0382	$7.4x10^{-4}$
Standard deviation of the intercept (Sa) $Sa = Sy/x[\Sigma i xi^2 / (n\Sigma i(xi - \underline{\bar{x}})^2)]^{0.5}$	$3x10^{-2}$	$1.6x10^{-6}$
Confidence limits of Intercept, $a = a \pm t S_a$	0.65 ± 0.129	0.0001 ± 0.00032
Confidence limits of Slope, $b = b \pm t S_b$	3.693 ± 0.1643	0.0148 ± 0.000007
Limit of Detection (LOD)	$3.1x10^{-2}$	0.15
Limit of Quantification (LOQ)	$1.03x10^{-2}$	0.5
Molar absorptivity (ε) (L / mole . cm) $\varepsilon = b \times M \times 1000$	$8.0x10^5$	$3.2x10^4$
Sandell's sensitivity (S) (µg. cm^{-2}) S =M / ε ; M=M.wt of drug	$2.7x10^{-4}$	$6.7x10^{-3}$
Sample throughput (h^{-1})	48	7

Pharmaceutical Applications

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

Table 11: Application of the developed procedure for estimation of Captopril in pharmaceutical formulations

CFIA / Merging Zones technique						Batch method				
Pharmaceutical preparation	**Present conc.** µg.ml^{-1}	**found**	***Rec%**	E_{rel}%	***R %**	**Present conc.** µg.ml^{-1}	**found**	**Rec%**	E_{rel}%	**R%**
Rilcapton (25mg) MEDOCHEMIE LTD,LIMASSOL-CYPRUS (EUROPE)	10 30	9.88 29.90	98.80 99.66	-1.20 -0.34	1.00 0.07	15 40	14.99 39.82	99.93 99.55	-0.07 -0.45	1.45 0.06
Rilcapton (50mg) M.A. Holder: MEDOCHEMIE LTD,LIMASSOL-CYPRUS (EUROPE)	10 30	9.94 28.95	99.40 96.50	-0.60 -3.50	0.03 0.02	15 40	14.94 40	99.60 100	-0.40 0.00	2.5 0.00
aceprotin (50 mg) CODAL SYNTO LTD,LIMASSOL-CYPRUS (EUROPE)	10 30	9.97 28.90	99.70 96.34	-0.30 -3.66	0.73 1.40	15 40	15.05 39.91	100.3 99.78	0.3 -0.22	0.033 1.71
Captopril (50 mg) PL Holder: BristolLaboratories Ltd., Berkhamsted, Herts, HP4 1EG, UK	10 30	10 30.06	100 100.2	0.00 0.2	0.00 0.20	15 40	14.75 39.99	98.34 99.98	-1.66 -0.02	0.02 0.14
accord (25 mg) Healthcare, LTD, Sage House, Middlesex, HA1 4HG,United Kingdom	10 30	10.01 30	100.1 100	0.1 0.00	1.25 0.00	15 40	14.87 40.05	99.14 100.125	-0.86 0.125	0.04 0.06
accord (50 mg) Healthcare, LTD, Sage House, Middlesex , HA1 4HF,United Kingdom	10 30	9.86 29.55	98.60 98.50	-1.40 -1.50	0.03 0.73	15 40	15 39.85	100 99.63	0.00 -0.37	0.00 0.07

*Mean of six measurements of each method

Erel % = [($\bar{x}$ -µ) / µ] x100 , Rec % =100+Erel %

Assessment of developed procedure

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

Table 12: Comparison of the suggested method with official method

Pharmaceutical preparation	proposed method CFIA/ Merging Zones method		official method		S	Value	
	*Rec%	$(xi-\underline{x})_1^2$	*Rec%	$(xi-\underline{x})_2^2$		tcal*	Fcal**
Rilcapton (25 mg) MEDOCHEMIE LTD, LIMASSOL-CYPRUS (EUROPE)	99.75	0.06	98.80	0.14	0.145	0.435	1.60
Rilcapton (50 mg) M.A. Holder: MEDOCHEMIE, LTD., LIMASSOL-CYPRUS (EUROPE)	99.80	0.17	98.95	0.01			
aceprotin (50 mg) CODAL SYNTO LTD, LIMASSOL-CYPRUS (EUROPE)	99.75	0.02	100	0.17			
Captopril (50 mg) PL Holder: Bristol Laboratories Ltd., Berkhamsted, Herts, HP4 1EG, UK	100.2	0.07	99.90	0.00			
accord (25 mg) Healthcare, LTD, Sage House, Middlesex, HA1 4HG,United Kingdom	100.1	0.09	100.5	0.01			
accord (50 mg) Healthcare, LTD, Sage House, Middlesex , HA1 4HF,United Kingdom	98.60	0.15	99.85	0.02			
	$(\underline{x}1) = 99.7$	$\Sigma(xi-\underline{x})_1^2 = 0.56$	$(\underline{x}2) = 99.66$	$\Sigma(xi-\underline{x})_2^2 = 0.35$	n1+n2-2=10	n1-1=5 n2-1=5	

Theoretical results under 95% confidence level ; $n_1=n_2 = 6$; t = 2.23Where t has v = n_1+n_2-2 freedom degrees = 10 ; F=5.786Where F has v1 = n1-1 ; v2 = n2-1 freedom degrees = 5

5. Conclusion

The proposed homemade CFIA /Merging zones analytical method are fast, cheap and sensitive for the spectrophotometric of CPL. in fine forms and pharmaceutical formulations . These methods can be used for the estimation of mg. L^{-1} amount of CPL. without need for previous separation steps, temperature or pretreatment of sample and solid phase extraction. The main advantages of the methods are its large dynamic range ; adequate sensitivity and its suitable for apply in routine assay in pharmaceutics specifity control laboratories due to 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) [41]. The procedures have good linearity, high analytical

frequency with throughput 48 sample.h^{-1}. In addition, the wide applicability of developed procedure for analyzation the examine of CPL at concentration of microgram level ($\mu g.mL^{-1}$) in pharmaceutical preparations and biological samples.

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