المجلد (11) العدد (1)
لسنة 2019

المجلة العراقية
لبحوث السوق وحماية المستهلك

FLOW INJECTION ANALYSIS AND SPECTROPHOTOMETRIC DETERMINATION OF NIFEDIPINEIN PHARMACEUTICAL FORMULATION

http://dx.doi.org/10.28936/jmracpc11.1.2019.(8)

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تاريخ استلام البحث: 2019/3/12 تاريخ قبول النشر: 2019/4/15

ABSTRACT:

A new simple and sensitive spectrophotometric method is described for quantification of Nifedipine (NIF) and their pharmaceutical formulation. The selective method was performed by the reduction of NIF nitro group to yield primary amino group using zinc powder with hydrochloric acid. The produced aromatic amine was submitted to oxidative coupling reaction with pyrocatechol and ammonium ceric nitrate to form orange color product measured spectrophotometrically with maximum absorption at 467nm. The product was determined through flow injection analysis (FIA) system and all the chemical and physical parameters were optimized. The concentration range from 5.0 to 140.0 µg.mL^{-1} was obeyed Beer's law with a limit of detection and quantitation 1.48 and 4.96 µg.mL^{-1} respectively. Agoodprecision,low scattering point of the calibration graph and good accuracyin addition, FIA introduced a good linear range with acceptable sensitivity. High correlation coefficient (0.9996) was found. The proposed method was successfully applied to assay NIF and its pharmaceutical dosage also could be utilized for pharmaceutical routine analysis of the drug.

Key words: Nifedipine, Pyrocatechol, Oxidative coupling reaction, Spectrophotometry, Flow injection analysis

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التقدير الطيفي للنفديبين في المستحضر الصيدلاني من خلال منظومة الحقن الجرياني

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الخلاصة:

تم وصف طريقة طيفية جديدة بسيطة وحساسة للتقدير الكمي للنفديبين ومستحضره الصيدلاني، حيث انجزت من خلال اختزال مجموعة النايترو للنفديبين لتتحول الى مجموعة امينو اولية بتفاعله مع مسحوق الخارصين وحامض الهيدروكلوريك المركز، تم تفاعل الناتج مع كاشف البايروكاتيكول ونترات ثنائي امونيوم سيريوم الرباعي عن طريق تفاعل الازدواج التأكسدي، مما نتج عنه محلول ذو لون برتقالي تم قياسه طيفيا عند طول موجي 467 نانومتر وقد استعملت منظومة الحقن الجرياني لاجراء التقدير كما تم اختيار الظروف المثلى الكيميائية والفيزيائية، كان منحني المعايرة ضمن مدى 5.0 إلى 140.0 ميكروغرام/ مل مع حد الكشف والقياس الكمي 1.48 و 4.96 ميكروغرام/ مل على التوالي، وتم الحصول على دقة جيدة ونقاط تشتت منخفضة في الرسم البياني لمنحني المعايرة وضبط جيد كذلك قدمت تقنية التحليل بالحقن الجرياني مدى خطي جيد مع حساسية مقبولة، معامل الارتباط كان عاليا (0.9996)، ان الطريقة المقترحة استعملت بشكل ناجح في فحص وتقدير النفديبين وكذلك مستحضره الدوائي.

الكلمات المفتاحية: نفديبين، بايروكاتيكول ، تفاعل الازدواج التاكسدي، المقياس الطيفي،االتحليل بالحقن الجرياني.

INTRODUCTION

Nifedipine (NIF) is a member of 1,4-dihydropyridine (1,4-DHP) derivatives, it is a yellow crystalline substance, practically insoluble in water but soluble in ethanol (**Florey, 1989**). Chemically (3,5-dimethyl2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3,5-dicarboxylate) as in (Figure, 1), it is more sensitive to light when in solution than in thecrystalline form (**Al-Turk *et al.*, 1988**). NIF considered as one of calcium channel blockers (CCB) agents (**Tripati, 2008**) which is a group of drugs mainly used to treat high blood pressure by inhibits the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle **(Nelson, 2010).**

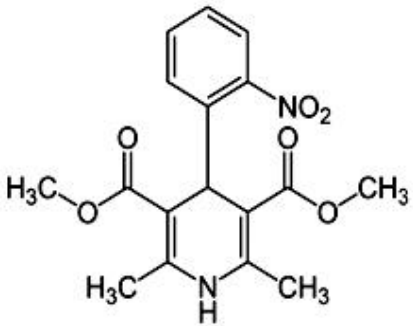

Figure (1): The chemical formula of Nifedipine.

Because of the importance and widely clinical uses of NIF enhanced the development of many analytical methods for its determination in pharmaceutical formulations and biological fluids **(Mostafa, 2015)**. Various techniques such as; spectrophotometry **(Esfahani, 2008; Rahman, 2005; Rahman, 2006; Mahadik *et al.*, 1991; Askal *et al.*, 2010; Sayed *et al.*,2012; Eihamd, 2013)**, Spectrofluorimetry **(Eihamd *et al.*, 2009; Ahadbavili, 2007; Walash *et al.*, 2009; Al-Ghannam, 2008)**, electrochemical methods **(Xiaofeng *et al.*, 2014; Baghayeri *et al.*, 2013; Xia *et al.*, 2007)**, Voltammetry **(Jara *et al.*, 2012; Wirzal *et al.*, 2015; Gaichore, 2013)**, liquid chromatography **(Vertzoni *et al.*, 2006; Wang *et al.*, 2007; Abou-Auda, 2000; Nassar, 2003; Zendelovska**, **2006)**, gas chromatography **(Locati, 1986; Martens, 1994)** were reported. Determination of NIF also performed by flow-through detector using either amperometrically, or voltametrically under continuous flow operation **(Richter, 1997)**.

Spectrophotometric measurements and their applications are considered as a routine analytical methods in most quality control laboratories, based on formation of colored complex with reagents such as bromocresol green, bromophenol blue, bromothymol blue and eriochrome black-T **(Rahman, 1997)**, Reduction of the NIF nitro group with Zn/NH_4Cl to hydroxyamino derivatives then coupling with N-methyl-1,4- benzoquinoneimine, to produce colored product **(Rahman, 1999)**, other reactionincluded reducing nitro group to yield free primary aromatic amine which diazotized and coupled to give red azo-dye and a violet with Bratton marschal and β-naphthol, respectively **(El-ghadafi, 2002)**. All literature survey showed no research for determination of this drug using different systems of flow injection analysis (FIA) with spectrophotometric technique.

This study suggested a developed,simpleand sensitive method for determination of NIF in pure and pharmaceutical formulation. The method based on reduction NIF nitro group by using Zn/HCl then coupling with pyrocatechol which oxidizedby $(NH_4)_2[Ce(NO_3)_6]$ to produce colored product measured spectrophotometrically with flow injection system **(Solich, 2001)**.

MATERIALS AND METHODS

Apparatus

A digital double beam spectrophotometer a type of Shimadzu UV-VIS 260 (Shimadzu, Kyoto-Japan) was used for spectral and absorbance measurements with FIA system. All the absorbance measurements were performed using 1 cm path length of quartz flow matched cells (Cecil, 50 lL internal volume).

*A peristaltic pump of six channels (Ismatec, Labortechnik-Analytic, type CH-8152, Glatbrugg Zurich-Switzerland) to pumpthe solutions of reagents.

*A 6-ways injection valve with different loops (Rheodyne, Altex 210, Supelco-USA) used for injecting samples.

*A flexible tubs (0.8 mm i.d.) was used for the peristaltic pump and a teflontubes (0.5mm i.d.) was used for made different lengths of reaction coil.

*Y-link was used to mix two streams of reagents.

Materials

All the reagents and chemicals used were of analytical grade.

-Zinc powder was obtained from BDH (Poole, UK) 90%.

-Hydrochloric acid solution fromThomas Baker 37% ≈ 11.97M.

-Pyrocatechol were supplied from BDH (Poole, UK) 98%.

-$(NH_4)_2[Ce\ (NO_3)_6]$ from BDH (Poole, UK) 99%.

-Ethanol solution analytical grade.99.9%.

-Pharmaceutical grade NIFwas supplied from sigma chemical co. (Germany).

-NIF tablets (Adalat®LA) contained 30 mg of nifedipine per tablet (Payer, Pharma AG, Germany).

Reagents

-Stock solution of pyrocatechol (PC) 0.01Mwas prepared by dissolving 0.1101g with distilled water to 100 volumetric flask.

-PC solution $2x10^{-4}$M.

Freshly prepared by dilute 2mL of stock solution and completed to 100mL volumetric flask with distilled water.

-Stock solution of $(NH_4)_2[Ce\ (NO_3)_6]$ (Ce (IV)) 0.1M was prepared by dissolving 5.4823g with 0.1MHNO_3 to 100mL volumetric flask.

-Ce (IV) solution 0.008M.

Freshly prepared by dilute 8mLof stock solutionand complete to 100mL volumetric flask using 0.1M HNO_3.

Procedure

Reduction nitro group to amino groupin Nifedipine.

Reduction solution of NIF was performed by dissolving 50mg of NIF in 50mL of ethanol,then transferred into 150mLbeaker and added 20 mL of distilled water followed by 20 mL of conc. HCl≈11.97 M and 3 g of zinc powder. Allowed the solutionto stand for 15 min at room temperature (25°C) to complete the reduction then filtered into 100 mL volumetric flask, and completed with distilled water **(Abdullah, 2017)** .Finally, the reduced stock solutions ($500\mu g.mL^{-1}$) were subjected to the general recommended procedure.

Preparation of pharmaceutical dosage form samples.

Twenty tablets of commercial NIF (adalat-30mg) were accurately weighted then grinded. An amount of powdered equivalent to 50 mg NIF were taken and dissolved in 30 mL of ethanol. The solution was filtered into a 50 mL volumetric flask, washed and completed the volume with ethanol. This solution was transferred into 150 mL beaker and reduced as

previously described. Appropriate solutions of pharmaceutical tablets were made using distilled water.

RESULTS AND DISCUSSION

The reduction of NIF with zinc in hydrochloric acid converted the nitro group into the corresponding amino group (**Solomons, 1996**). When a solution of reduced NIF was mixed with PC reagent and oxidized with Ce (IV) solution, an orange color formed immediately. The solution has a maximumabsorption at wave length 467nm (Figure 2) which was chosen to fulfil all subsequent experiments.

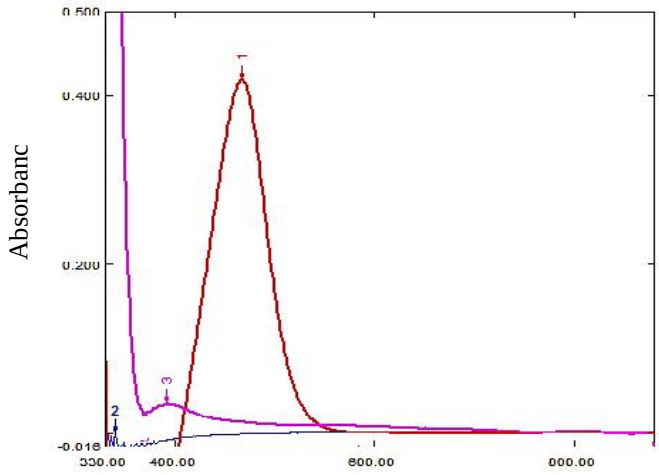

Figure (2): Absorption spectra of 75µg.mL^{-1} of reduced NIF with (2x10^{-4}M) PC and (0.008M) Ce(IV)measured against reagent blank[1], the reagent blank[2] and reduced NIF measured against distilled water [3].

Optimization of Reaction Conditions

Order of Addition

Three paths of reactions were suggested for two-channel manifold was utilized for normal flow injection analysis (nFIA) system to determine NIF drug. As shown in A, B and C baths (Figure 3A), the solution of drug (80µg.mL^{-1}) was injected with 100 µL sample volume by using the injection valve, the reagents PC (1x10^{-4} M)and Ce(IV)(8mM) were pumped with flow rate 3.1ml/min and 50 cm reaction coil.It was found that C path when flow stream solutions were R1and R2 after injected reduced NIFgave the maximum absorbance (Figure 3B) and it was established to all experience.

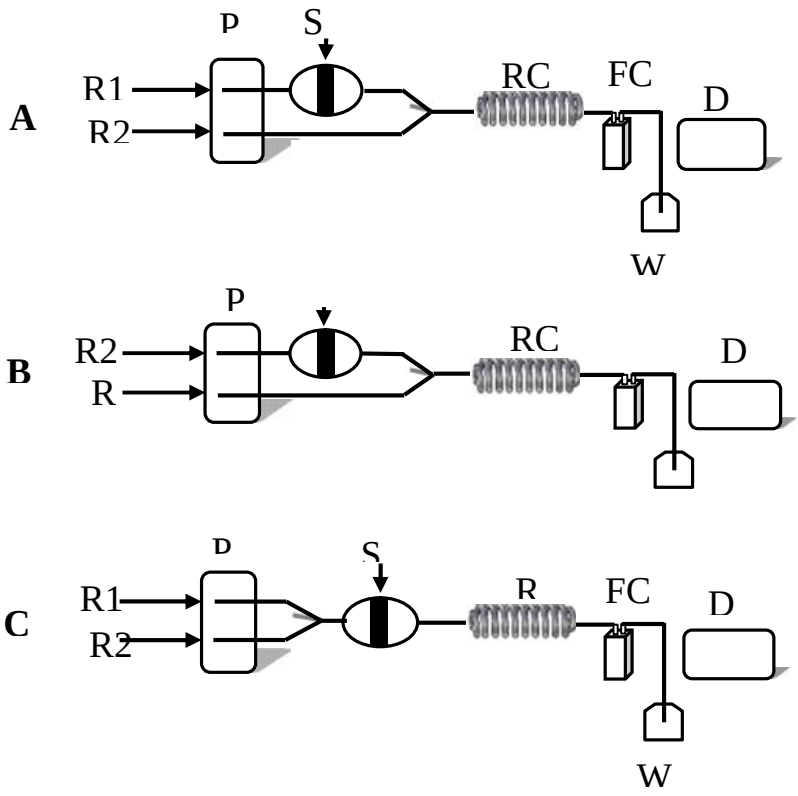

Figure (3A): nFIA Manifold estimated for determination of NIF A, B and C. S: sample (NIF); R1: PC; R2: Ce (IV); P: peristaltic pump; V: injection valve; RC: reaction coil; FC,: flow cell; D: detector; W:waste.

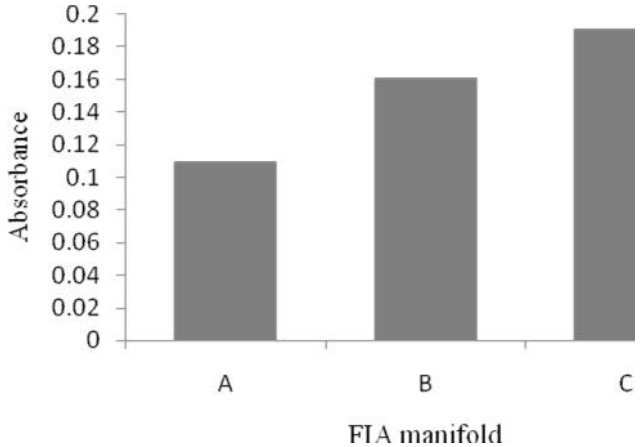

Figure (3B): The effect of variation in FIA manifold

Effect of The Chemicals Variables

It was important to study the effect of various concentrations for PC and Ce(IV) solutions on the sensitivity of the submitted study and then select the optimum concentration.

Effect of Pyrocatechol Concentration

Range of PC concentrations ($0.5x10^{-4}$–$3.5x10^{-4}$M) was studied. The results revealed that the absorbance and the sensitivity increased with increasing PC concentration till to the concentrationof 2 $x10^{-4}$M, then the absorbance was decreased slightly, thus 2 $x10^{-4}$M was chosen as an optimum concentration as shown in(Figure4).

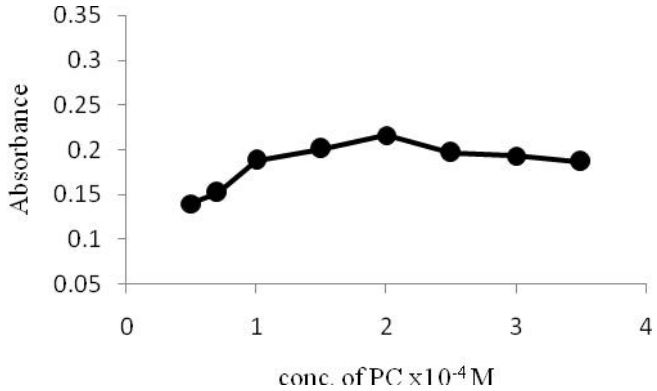

Figure (4): The effect of PC concentration

Effect of Ce (IV) Concentration

It was important to study the effect of Ce (IV) concentration, so arange of Ce (IV) concentrations from 1to 20mM were chosen. The maximum absorbance was detected in 8mM then gradually decreased with increasing the concentration, thus the optimized concentration selected was 8mM (Figure 5).

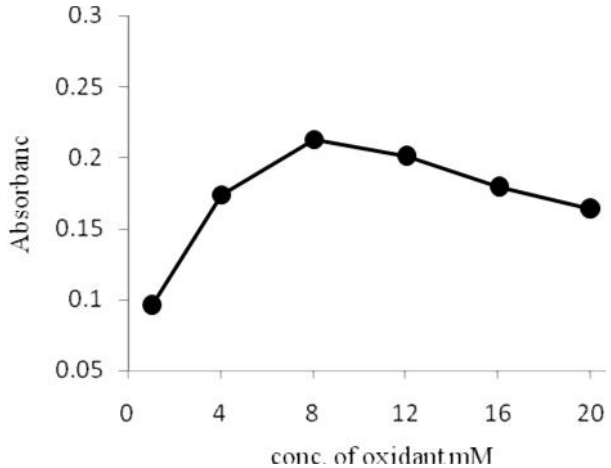

Figure (5): The effect of Ce (IV) concentration

The effect of physicalvariables

Effect of the flow rate

The study includedthe effect of the flow rate started from 0.35to 4.2 mL.min^{-1}viacollecting flow stream of the two channels of reactants from the waste (see figure3A-C). Thehighest sensitivity and maximumabsorbance showed in1.4mL.min^{-1}which means this flow rate was suitable to complete the reaction between the reagents and reduced NIF while increasing the flow rate may lead to incomplete reaction as well as more dilutingfor the sample (NIF) (Figure 6).

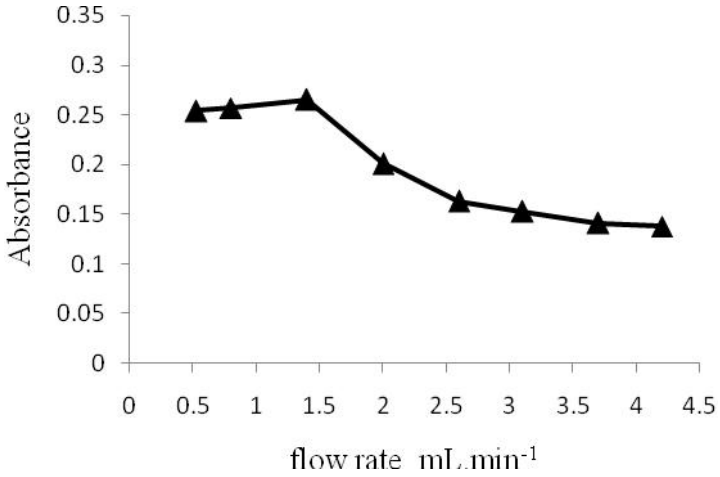

Figure (6): The Effect of flow rate.

Effect of reaction coil length

Various reaction coil tubing lengths between 25 and 250 cm were selected forchoosing thebest reaction coil that produced maximum absorbance. It was found 75 cm coil length gave thehighestabsorbance whereas increasing coil length may cause increasing in dispersion which results from spending more time in the coil and that lead to increasing analysis timetherefore 75 cm was selected as optimumreaction coil (Figure 7).

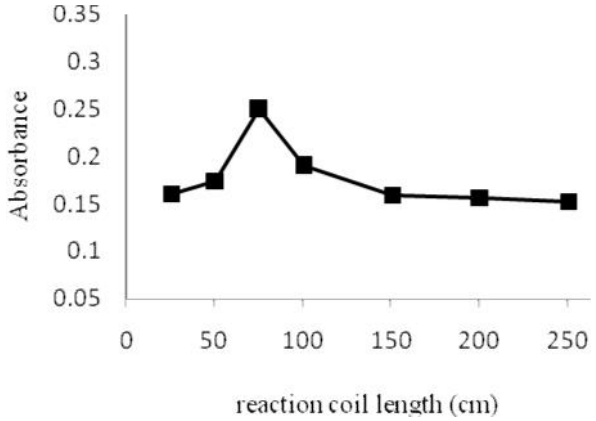

Figure (7): The effect of reaction coil length.

Effect of injected sample volume

The injection volume effect was studied. A sufficient amount of sample should be necessary to allow effective reaction, produced good sensitivity and high accuracy. The absorbance was investigated by injecting the NIF solution (80 µg. mL^{-1}) with volumes ranging from75to 250 µL. It was found that 150 µL injected sample volume measured maximum absorbancetherefore it selected for further experiences.Increasing sample volume effectedon dispersion of the sample zone (NIF) and may be caused no intermixing with the reagents streams leaded to loss of sensitivity and sampling rate (Figure 8).

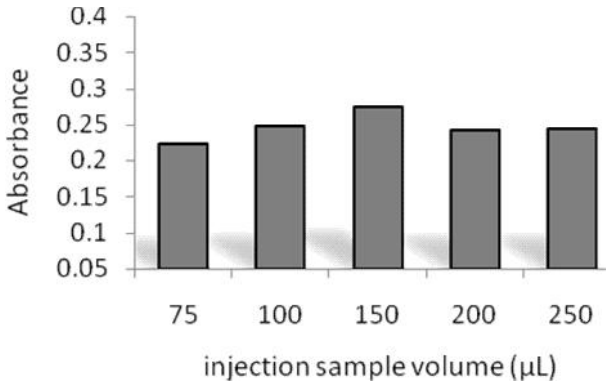

Figure (8): The effect of injected sample volume.

Effect of temperature

Temperature take an importantinfluence on several reactions consequently, the present studycarried out underthree temperature 5, 25 and 45°C. All the solutions (NIF, PC, Ce (IV)) were placed in 5 and 45°C water bath. The results showed a high absorbance at ambient temperature (25°C), less at 5° and 25°C temperature. That may be due to decrease the coupling affinity between the reactants so this temperature established for all parameters (Table1).

Table (1): Effect of temperature

Temp. (˚C)	**5**	**25**	**45**
Abs	**0.274**	**0.299**	**0.260**

Stoichiometry of the formed product

The stoichiometry of the formed product was investigated by continuous variation (Job's method)(**Hadjiioannou, 1993**). The job's method was applied by placing 0to10 mL of $4x10^{-4}$M NIFsolutionsinto a series of 25mL volume flasks, mixing with 10 to 0 mLof $4x10^{-4}$M reagent (PC) which flow as stream solution R1 (Figure 3A)and8mM Ce (IV) as stream solution R2. It was found that the ratio was 1:1(NIF: PC) seen in (Figure 9).

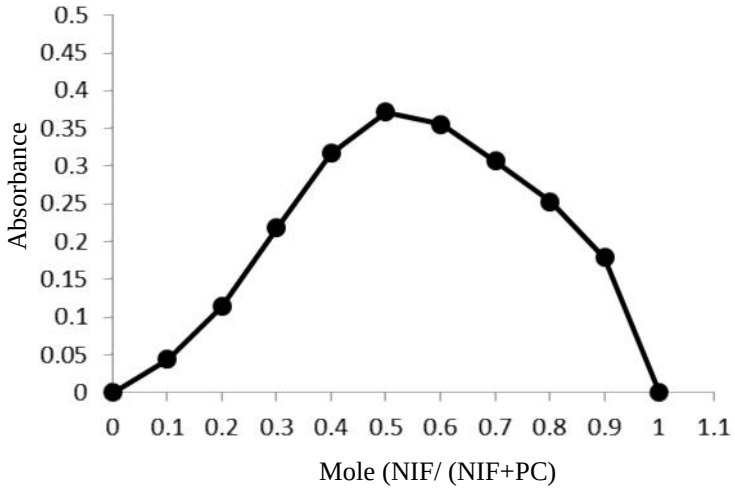

Figure (9): The Job's method.

Mechanism of the reaction

Pyrocatechol was found to be a useful reagent for oxidative coupling reaction, this reagent is easily to obtain and solve in water. Reduced NIF reacts as nucleophilic coupled with electrophilic PC which is oxidized by Ce (IV) (**Nair, 2007; Al-Abachi, 2003**). The result of this oxidative coupling was colored product as shown in (Figure 10).

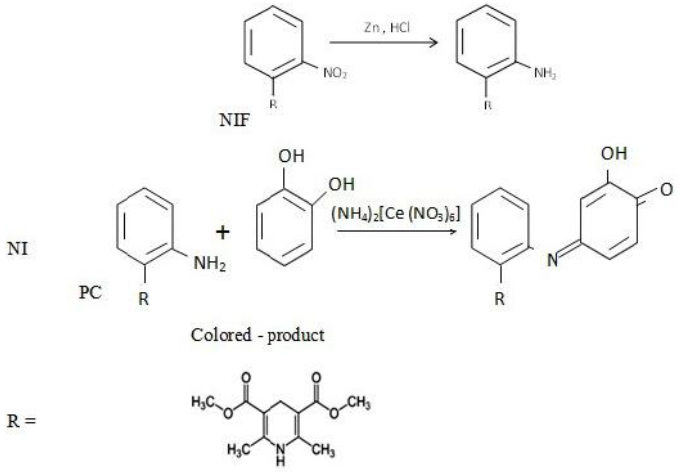

Figure (10): Reaction scheme

Table (2): The optimum conditions

Parameters studied	Range	Optimum
Concn. of ceric (IV),mM	1-20	8
Concn. of pyrocatechol, x10^{-4} M	0.5-3.5	2
Total flow rate, mL min^{-1}	0.53-4.2	1.4
Reaction coil length, cm	25-250	75
Injected sample volume,µL	75-250	150
Temperature (°C)	5-45	25

Dispersion

Two streams were passed through the flow system, the first stream containing 80 μg.mL^{-1} of NIF in 2x10^{-4} M of PC, and the second containing 8mM of Ce(IV). A continuous response was obtained for a constant concentration of NIF and no dispersion from diffusion and convection was occurred and the absorbance was measured (A_o). In another experiment, the same concentration of NIF was injected as in the previous experiments and the absorbance was measured (A_{max}). The dispersion was calculated using:

A_o= 0.450, A_{max}= 0.270

D= A_o / A_{max}; A_o and A_{max} = Absorbance for undispersed and a dispersed sample respectively.

D= 0.450/0.270= 1.666

Sampling frequency

After optimum conditions done (Table 2), number of samples could be frequented. This could be known when recorded the time from injection the sample, it was 62 sec., so the sampling frequency 58 per hour.

Linearity

The linearity of the calibration graphs, for nFIA, was studied. A series of solutions containing 5to140 μg.mL^{-1} of NIF were prepared through diluting stocksolution (500 μg.mL^{-1}) which was prepared previously. By using optimum conditions, calibration graph of determination NIF were obtained (Figure 11). All the analytical figures of FIA procedureare summarized in (Table 3). The statistical treatments for calibration graph are also reported. Values of the Sy/x, Sa, and Sb (small values) were indicated a good "precision" of the current methods, and low scattering of points of the calibration graph and good accuracy. In addition FIA methods gave a good linear range with acceptable sensitivity.

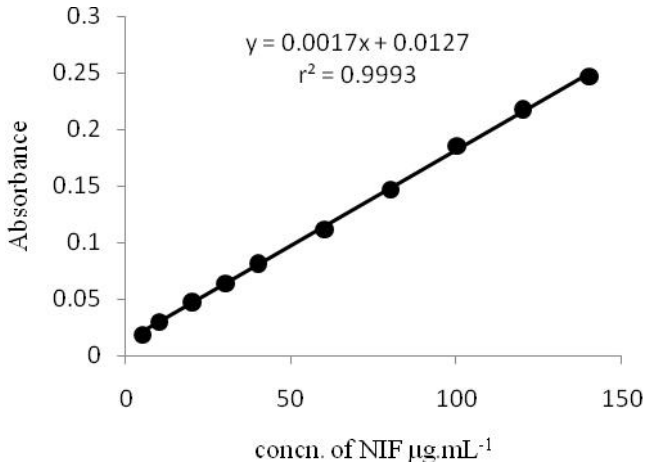

Figure (11): calibration graph of NIF

Table (3): Analytical values of statistical treatments for the calibration graph-nFIA

Parameter	Value
Regression equation	y = 0.0017x + 0.0127
Correlation coefficient, r	0.9996
Linearity percentage, % r2	99.9255
Dynamic range, $\mu g.mL^{-1}$	5-140
Molar absorptivity, ε ,$L.mol^{-1}cm^{-1}$	588.71
Slope, b, $mL.\mu g^{-1}$	0.0017
Intercept, a	0.0127
Sy/x	0.0052
Sb	$3.6345x10^{-5}$
Sa	0.0027
LOD ,$\mu g.mL^{-1}$	1.4881
LOQ ,$\mu g.mL^{-1}$	4.9604

Accuracy and precision

Accuracy and precision of the proposed method were calculated under optimum conditions using three different concentrations of standard NIF (20, 40 and 60) $\mu g.mL^{-1}$ in five replicate. (Table 4) summarized the values of E%, Rec%, and RSD% for the chosen concentrations. The acceptable values for the RSD and the relative error indicated a good accuracy, and acceptable precision of the current methods.

Table (4): The accuracy and precision of determination of NIF using n-FIA

Concn.of NIF, $\mu g\ mL^{-1}$		E%	REC%	RSD%*
Present	found			
20	19.24	-3.82	96.18	2.51
40	39.94	-0.15	99.85	1.88
60	60.529	0.88235	100.88	1.79

∗Average of three determinations, RSD%<5

Pharmaceutical applications

In this study, it was successfully applied to determine the commercial dosage in pharmaceutical formulation. The solutions of pharmaceutical formulation were prepared as described previously. The proposed method was applied for the determination of NIF in tablets by selected of three concentrations of sample using the recommended procedure. The results obtained shown in Table 5.

Table (5): Application of the proposed method for determination NIf in pharmaceutical formulation.

Pharmaceutical formulation	Conc.of NIF ($\mu g.mL^{-1}$)		E%	REC%	RSD%*
	present	found			
NIF Tablets (adalat-30mg)	20	20.07	0.36	100.36	1.08
	30	29.18	-2.72	97.28	0.72
	60	61.75	2.91	102.91	1.14

∗Average of five determinations

The statistical comparison between proposed and standard methods using the student t- and F-test indicated that the calculated values were less than the theoretical one, which referred to the insignificant difference between both methods in accuracy and repeatability.

Pharmaceutical formulation	Proposed method	Standard method	Value	
	Rec.%*	Rec.%	t	F
NIF Tablets (adalat-30mg)	100.18	101.03	0.347	3.591
Theoretical value			2.776	19.000

∗Average of three determinations

CONCLUSION

Flow injection system is proposed as a technique lent a significant contribution in quantification of pharmaceuticals performed with spectrophotometry method; it was done by use of simple or even rather complicated derivatization routines leading to the formation of colored reaction products "on-line" in the flow system. This study submitted developed, sensitive, simple and accurate method for estimation NIF in pharmaceutical form by using oxidative coupling reaction between the drug and PC (available, soluble in water and unexpansive reagent) in the presence of Ce(IV) oxidant (low toxicity, ease of handling, experimental simplicity, and solubilityin water). The proposed method had low scattering of points of the calibration graph anddoes not need critical conditions such as heating or extraction, also frequency of samples could be used in routine analysis.

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