DOI: https://doi.org/10.53350/pjmhs22168622

ORIGINAL ARTICLE

Determination of Nifedipine drug in Pure and Pharmaceutical Preparations by Normal Flow Injection Analysis using Sulfamethaxazole Drug as Chromogenic Reagent

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ABSTRACT
A highly sensitive and accurate spectrophotometric method including Technique (Normal Flow injection analysis have been used for determined Nifedipine (NIF) drug in pure and dosage forms. This approach based on the response of drug with diazotized sulfamethoxazole (SMZ) to form colored product after diazotization of reagent and coupling reaction with drug The coloured chromogen suggests absorption maximum at 496 nm. The method of the line conditions of response had been investigated by (1) invariable chemical approach, by optimizing the impact of experimental variables (special bases, reagent awareness, and reaction time), (2) physical methods including the effect of three experimental factors (injection loop length , reaction coil ,flow rate and reaction time). The results gave Beer's law is obeyed over the awareness range 2-180 µg. ml-1 with an excellent determination coefficient (r^2= 99.97) and molar absorptivity 3428.714. The stoichiometry of the resulting azo dye has been also worked out and it is found to be 1:1 Nif: SMZ. This method has been carried out successfully for the determination of Nifedipine in pharmaceutical preparations (tablets).
Keywords: Nifidipine , flow injection analysis, spectrophotometry, Sulfamethoxazol ، diazotization.

INTRODUCTION
Nifedipine the abbreviation of the word is (NIF) used have an prolonged release tablet dosage shape of the calcium channel blocker nifedipine.. Nifedipine is three,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-four-(2-nitrophenyl)-dimethyl ester, Formula: $C_{17}H_{18}N_2O_6$: figure (1) and molar mass : 346. 335 $g{\cdot}mol^{-1}$(1,2).

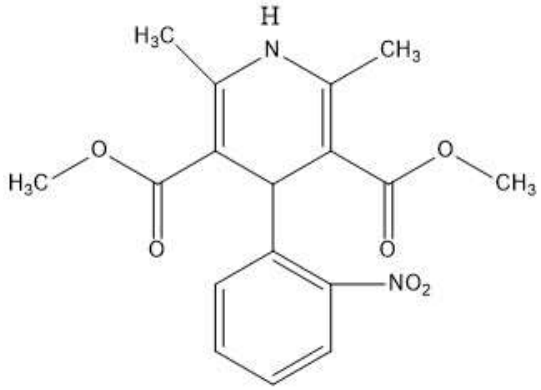


Figure 1: Chemical structure of Nifedipine

Nifedipine is a yellow crystalline material, nearly insoluble in water but soluble in ethanol and have low bioavailability (**3) .**Nifedipine, is a nonpolar compound, Nifedipine is similarly metabolized within the minor intestine and liver to extra polar mixes that are more often than not eliminated with the aid of the kidney The literature has stated a few approaches for Nifedipine in organic fluids, which comprised gas chromatography, performance liquid chromatography with either UV discovery (4)or electrochemical discovery, fluorescence tactics, first spinoff spectroscopy, volta metric approach and LC–MS uniting a humble liquid–liquid removal and cloud-Point extraction. (5).In this study has been studied Sulfamethoxazole reagent Figure (2) as a reagent short word (SMX or (SMZ) is an isoxazole (1,2-oxazole) compound having a methyl substituent at the 5-position and a 4-aminobenzenesulfonamido group at the 3-position . It works by eliminating the bacteria that cause many kinds of infections. This medicine will not work for colds, flu, or other virus infections. Sulfamethaxazole is chemically known as [4-amino -N-(5-methylisoxazole-3-yl) benzenesulfonamide], Is white crystallized powder. It does not dissolve in ether and chloroform solvents. It has low solubility in water, it dissolves in acetone , in ethanol. And in alkaline hydroxide solutions .Is a highly effective chemotherapeutic agent (6,7,8,9). The drug nifedipine was reduced in this research to obtain the amine group and then its association with the diazotized SMZ reagent .Most of techniques are time consuming and expensive, the most nFIA method in this work used Sulfamethoxazole drug as safe and green diazotization agent in medicinal preparations examples via oxidative reaction .

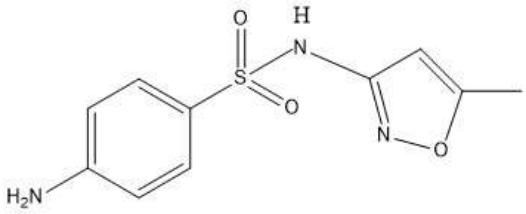


Figure 2: Structure of Sulfamethoxazole chromogen reagent

Spectrophotometric methods are the most commonly used techniques in chemical analysis due to the availability of instruments, simplicity of procedures, precision, and wide applicability. Based on the laws governing absorption and emission phenomena, it is possible to determine the concentrations of compounds in solutions, notably those of biological, chemical, or pharmaceutical interest . In this research chosen new idea for Drug analysis, involving the pharmaceutical preparations or the raw materials used for their production using Normal Flow Injection analysis (nFIA)) (10,11, 12) has been very fruitful in abridging chemical assays., based on chromogenic reactions or light absorption by the analyte. Chromogenic reactions for drugs include redox reactions, and FIA publication always derived the analytical readout from the peak maximum. Homogeneously mixing sample with reagent ,the injected sample zone within the reagent stream — corresponding to after establishment of chemical equilibrium, , minimizing the amount of waste generated and containing different concentration ratios of sample and reagent than exploit a single one of them , continuous-flow system where procedure are measured peak areas (13,14) .The aim of the study was to develop a simple method for precise routine determination of important drug Nifedipine (NIF) in our medical community in Iraq . In this work described to prepared new compounds via diazotization- coupling reactions , which have gained considerable popularity because of their variety of

applications in green chemistry ,analytical clinical laboratories, , agricultural, and industrial and environmental fields . By alkaline medium in the procedure of reaction between reduced Nifedipine (NIF) drug with Sulfamethaxazole to obtained color product with high chemical resistance and stable state by normal flow injection with UV-Vis spectrophotometer detector .

MATERIALS AND METHODS

Shimadzu UV mini -1240 digital single beam (UV –Vis spectrophotometer) has been used for absorbance and spectral measurements containing movement cell of 50 µl internal volume with 1 cm path length . A normal flow injection for determination of Nifedipine was achieved with a peristaltic pump (shenchen , LabM1 model, China). The injection of samples volume and standard solutions was accomplished by Injection valve (Knauer (6-Port/3-channel). The reaction coil (RC) was made of Teflon with 0.5 mm of an internal diameter. Manifolds of two channel were utilized for nFIA spectrophotometric determination of Nifedipine.

Chemicals and Drugs: All the reagents, solvents and chemicals were of analytical grade. NIF as Pharmaceutical grade was supplied from sigma chemical co. used two applications from Pharmaceutical dosage as tablets : EPILAT Retard 20 mg Nifedipine S.R. flim coated tablets manufactured Egyptian ,int ,pharmaceutical industries co . E.I.R.I.CO . and bayer : Adalat LA 30 tablets /comprimes (30 mg of Nifedipine manufactured Bayer AG,D-51368Leverkusen , Germany / Allemagne) made by /Fabr1que .par , made in Germany .

Preparation of Solutions

Nifedipine (NIF) stock solution (500 µg/ml) : This solution is prepared via reducing process including dissolving 0.05 g of pure Nifedipine in 50 ml Ethanol ,transferor to beaker contain 20 ml of distilled water , add to it 20 ml of conc HCL , 3g of Zn metal powder followed by completed the volumetric flask with distilled water to the mark. Then it is left to settle for 15 minutes, then filtered and the volume is finished to 100 ml in a volumetric bottle after the precipitate was washed with a little distilled water.

Hydrochloric acid solution (1M): Diluting 8.4mL of 11.97M concentrated hydrochloric acid (New Delhi 37%, M. Wt.=36.46 $g.mol^{-1}$) with distilled water in 100 mL volumetric flask.

Diazotized of Sulfamethoxazole (SMZ): Prepared 0.003 M in 100 ml from reagent by weighted 0.1266 g and dissolved in 20 ml ethanol ,put beaker for solution in ice bath , than add to it 3ml of diluted HCL slowly and sodium nitrite solid about 0.0345 g than transfer solution quantitively in volumetric flask 100 ml and complete volume with distilled water to the mark .

Sodium hydroxide solution (0.5M) as Alkali medium : dissolving 2.00 g of sodium hydroxide (BDH, England, M. Wt.=39.997g.mol-1) using distilled water and complete the volume to 100mL with the same solvent.

Experimental

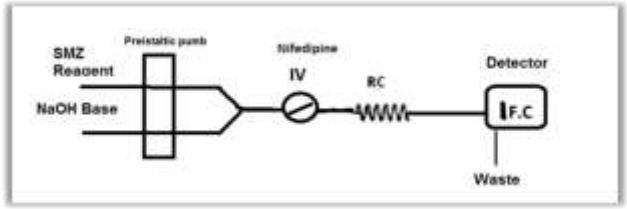
Reduction nitro group to amino group in NIF: Reduced NIF solution was performed by weighing pure NIF 50mg and dissolved in 50mL of ethanol, transferred the solution into 150mL beaker; 20mL of distilled water was added then 20mL of conc. HCl (11.97M) and 3g of zinc powder. The solution was stood for 15 min at 25°C then filtered in 100mL volumetric flask and completed with distilled water to get 500$\mu g.mL^{-1}$ stock solution and was ready for next experiments.(15)

Preparation of pharmaceutical form samples: Two type of Commercial Nifedipine (NIF): EPILAT Retard 20 mg Nifedipine S.R. film coated tablets manufactured egyptian int,pharmaceutical industries co . E.I.R.I. CO . and bayer : Adalat LA 30 tablets /comp rimes (30 mg of Nifedipine manufactured Bayer AG,D-51368Leverkusen , Germany / Allemagne) made by /Fabr1que .par , made in Germany .Weighted 10 pellets for each form and grinded. The amount of (NIF) equivalent to 500 mg / L was taken, melted in 50mL of ethanol. The solution fill to a 100 mL in volumetric flask, washed and completed with distilled water 100mL beaker and reduced as previously described. Working solutions of pharmaceutical drugs were complete usage distilled water.

General nFIA procedure: Two channel manifold was used for the spectrophotometric willpower of NIF**.** Online Sample concentration in FIA/UV-Vis Hyphenated System Working solutions of reduced NIF ranged from (2 to 180) $\mu g.mL^{-1}$ were ready. The solutions were injected in 150µL sample loop through solution of Sulfamethoxazole (0.005M) then mixed with a coupling reagent solution of NaOH (0.5M). The flow rate was 25 mL.min- 1, the stream solutions mixed together in 50 cm reaction coil .The red orange dye absorbance was measured at 496 nm. Optimization of circumstances was approved out using 50$\mu g.mL^{-1}$ of NIF .

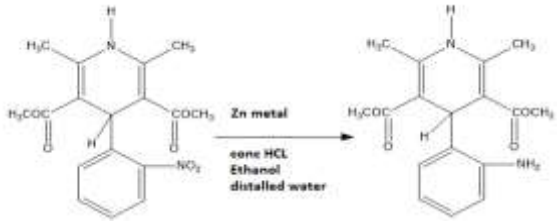
RESULTS AND DISCUSSION

First step : tested manifold of nFIA in four ways to work procedure , the result gives first one manifold the higher absorbance so the it chosen (Scheme 1) below shows Manifold of nFIA :



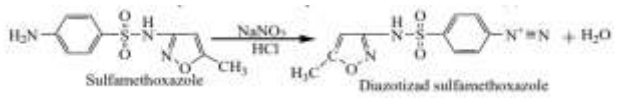
Scheme 1 :Manifold of nFIA method for determination of NIF via diazotization coupling reaction with diazotized Sulfamethoxazole and NaOH: Injection valve (IV); Reaction coil (RC): Peristaltic pump ; :Flow cell F.C ; UV-Vis detector; waste

Second step : must reduce step for Nifedipine Analyte in pure form using Zn metal .Scheme 2 :



Scheme 2 :Reducing process for Nifedipine drug

Third step : Sulfamethoxazole SMZ was diazotized with sodium nitrite solution 0.005 M in the presence of acid solution of HCl to give the diazonium salt (16) . Scheme 3.



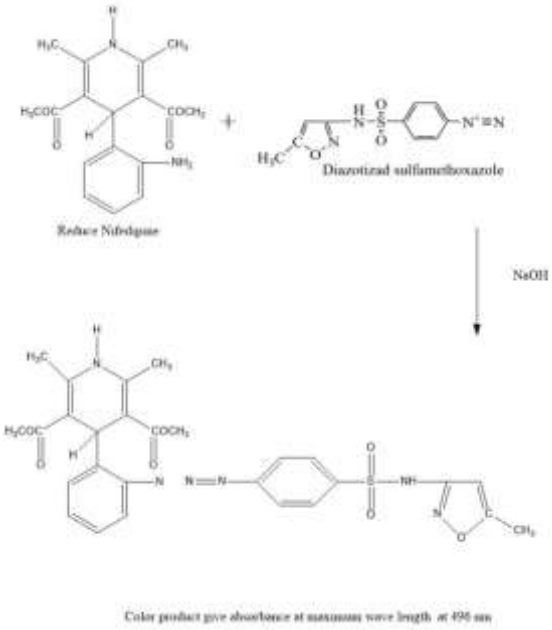
Scheme 3: Diazotized Sulfamethoxazole

After reduced step in pure form and prepered diazotized Sulfamethoxazole reagent .The next step including reaction between diazotized Sulfamethoxazole SMZ reagent with reduced Nifidipine drug to form to form colored product (orange liquid compound)The colored chromogenic shows absorption maximum at 496nm. Figure 3:

The use of experimental conditions in this research was the beginning of setting the correct path for optimal conditions for interaction between Nifedipine and the reagent drug in the attendance of a base medium to crop a colored product reaching the maximum wave length at 496 nm. The absorption spectrum of the tinted species

(scheme 4) color product compound after coupling .

Figure 3: Orange colored product for Nifedipine product and yellow color for blank



Scheme 4: Assumed reaction mechanics after coupling at 496 nm .

Diazonium ion was produced through the reaction of SMZ with sodium nitrite in the presence of acidic medium followed by the coupling of NIF with diazonium ion in a basic medium to form the colored products than measure color compound in UV-VIS spectroscopy to show figure 4 .

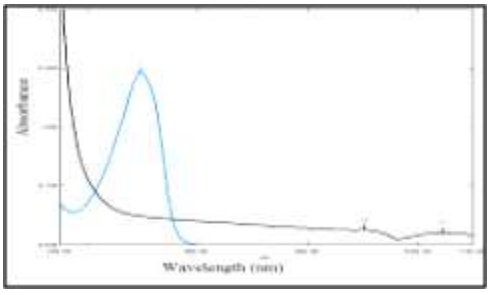


Figure 4: Absorption spectra of the suggested reactions solutions when,absorption spectrum of 50 µg/mL of reduced NIF drug coupled with SMZ reagent against blank, and absorption spectrum of reagent blank against distilled water.

Chemical optimization: The chemical variables that impact in the performance of the proposed methods have been studied. The optimum conditions of method were selected based on reproducibility, throughput and sensitivity. For the optimization of circumstances in very following trials, 50µgmL^{-1} of NIF was chosen.

Effect of reagent concentration: Different concentrations of Sulfamethoxazole in the range between (0.001 to 0.007) M were examined . It was give the maximum absorbance at 0.003 M to coupling method were selected for further used as given in Figure 5.

Figure 5: The effect of changing the concentration of the reagent on the absorbance of the colored product

Effect of alkaline medium: Experiments indicated that the orange products were showed more efficiently in alkaline medium, different types of basic solutions were examined including (Sodium hydroxide , Ammonium hydroxide, potassium hydroxide and Sodium carbonate).After testing found that NaOH (0.5M) base for method was gave the maximum absorbance 0.416 among tested bases (KOH :Na_2CO_3: NH_4OH : Na OH) figure 6, stability and sensitivity Various concentrations (0.1-1.5) M of NaOH was studied to method Figure 7 .

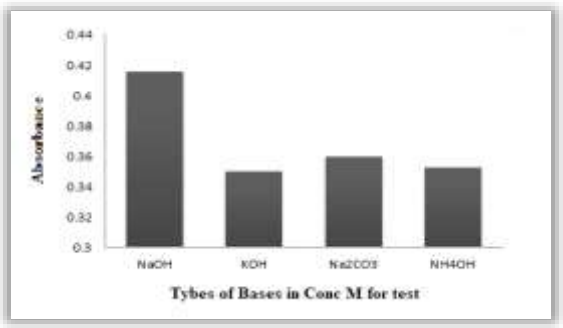


Figure 6: Concentration of base versus the versus the absorbance

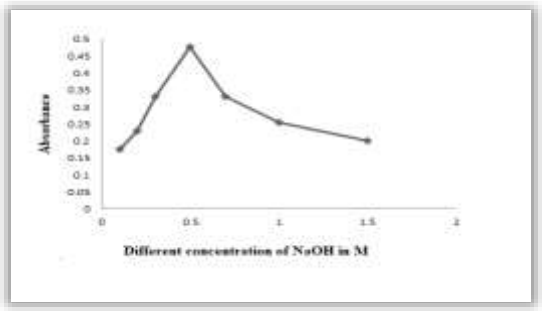


Figure 7: Different concentrations tested for NaOH chosen

Physical optimization: The physical parameters studied were reaction coil, injection volume, and total flow rate. The reaction coil length was studied in the range from (0 with out reaction coil - 250 cm), the results indicated that reaction coil length of 50 cm for method gave maximum absorbance and therefore was selected. Higher lengths perhaps increase the dispersion of reactant zone. The influence of injection volume for Nif drug was investigated with varying sample loops in the range of (60- 250 µL), and the volumes 150 µL was selected for method. the flow rates that accomplished sampling rate and sensitivity was chosen, which was 2 mL/min method Figure 8 (A , B,C)shows the results obtained from the physical conditions studies , shows all parameters.

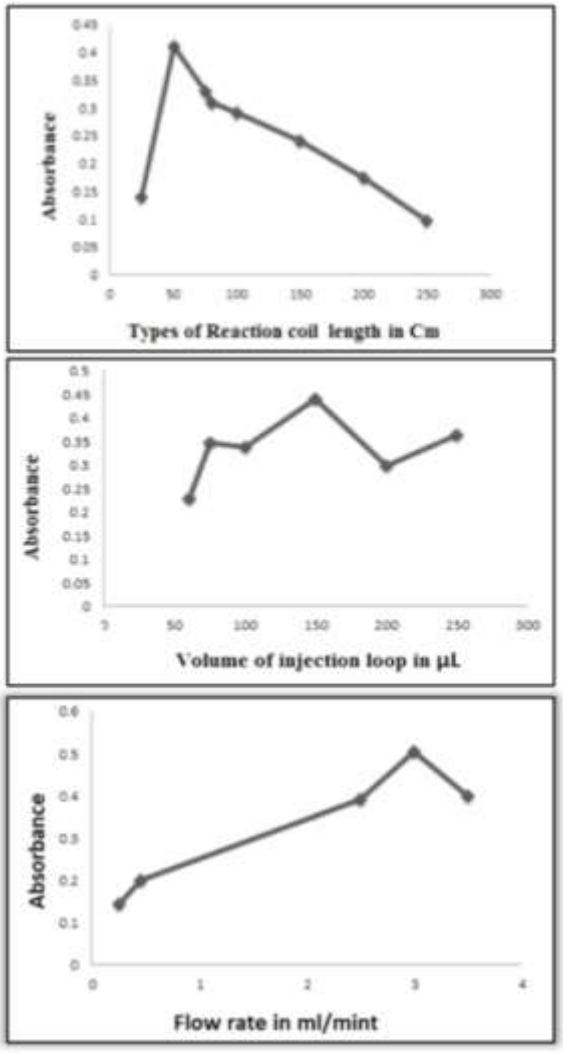


Figure 8: Effect of physical parameters : A :Effect of Reaction coil in Cm , B : Effect of total injection volume, C : Effect of total flow rate in mL/min,

Validation of the current method: Analytical characteristics; linearity, sampling rate, detection limit, correlation constant and relative standard deviation(RSD) of technique were calculated (Table 1) ,more sensitive and wider linear range furthermore it was rapid ,using Analytical statically laws to calculate results [17] .Accuracy and exactness of the future Method were estimated, six dissimilar attentions of NIF were applied for nFIA with five replicate. The values of RE%, Rec%, and RSD% summarized in (Table2) and indicated a good accuracy, and high precision for the current Method. Figure 9 shows lireanty relashionship of the different concentration for NIF drug from (2-180 ppm) taken through current nFIA manifold against absorbance .

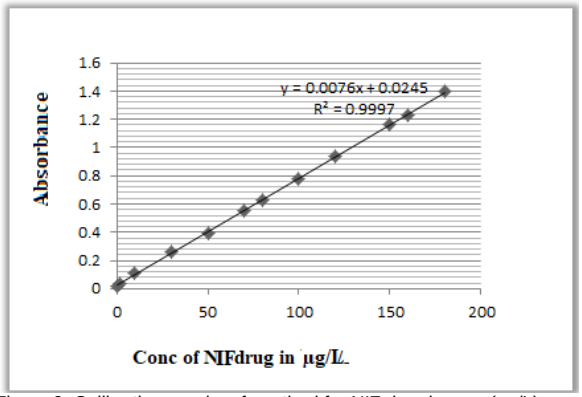


Figure 9: Calibration graphs of method for NIF drug in ppm (µg/L)

Table 1: Analytical values of the suggested methods for determinate of Nif

Parameter	Value for method
Regression equation	Y=0.0076X+0.0245
Correlation coefficient, r	0.9997
Linearity percentage ,r^2	99.97
Dynamic range (µg /mL^{-1})	2-180
Slope, (mL / µg)	0.007583
Molar absorptivity (L $mol^{-1}cm^{-1}$)	3428.714
Specific absorptivity (ml /gm.Cm)	989.988
Intercept, a	0.024521
Throughput (Sample/h)	50
Sandell 's sensitivity S (Mg / Cm^{-2})	1.01×10^{-5}
Limit of detection (µg.mL-1)	4.606
Limit of quantification (µg.mL-1)	15.355
standard deviation of the residuals , Sy/x	0.5041
Standard deviation of slope, Sb	0.3162
Standard deviation of intercept, Sa	0.6858

Table 2: Accuracy and precision for suggested method.

Conc. of Nif µg /mL		E %	Rec. %	RSD %
Present	Found			
30	31.12	3.74	103.74	0.16
50	51.43	2.85	102.86	0.62
70	70.99	1.416	101.42	3.66

Pharmaceutical applications: nFIA Method were adopted to estimate NIF in pharmaceutical forms Type one : Adalat LA 30 tablets /comp rimes 30 mg NIF drug and type two : (EPILAT Retard 20 mg NIF drug) by taken six different concentrations of pharmaceutical dosages with five replicate for each type , The results are shown in the table 3 :

Table 3: direct applications of proposed methods for Dosage form of NIF drug by using flow injection analysis.

Dosage form	Taken conc. (µg/mL)	Found conc. (µg/mL)	Recovery (%)	RSD (%)
Adalat Tablet (30 mg NIF) Germany	30	30.73	102.43	0.62
	50	51.34	102.68	0.243
	70	70.38	100.54	0.284
Tablet (20 mg NIF Egyptian	20	20.51	102.54	1.19
	50	51.87	103.74	1.44
	70	71.56	102.23	0.306

Staticall Analytical calculation for NIF drug t- Calculate (theoretical= 5.212)

Give for Pharmaceutical Preparation 0.8087 while t- Calculate theoretical give 1.2004 in classical method and F- Calculate (theoretical =101.4) equil to 0.465 in pharmaceutical preparation while F- Calculate (theoretical =101.4) give 6.230 in classical method .

Stoichiometry of the formed product using Job's method: The suggested method flow injection analysis was applied successfully to determination Nifedepine with Sulfamethoxazole reagent in presences of strong base sodium hydroxide . Color product was characterized using continuous variation method , often known as Job's method, is a method for determining the stoichiometry of a binding event in analytical chemistry. which is named for Paul Job. Paul method is interested in the interactions of ions in solution. (18,19,20).

The job's method was applied by placing 0 to 5 ml of 4×10^{-4}M NIFsolutions in series of 10 ml volumetric flasks , mixing with 5 to 0 ml of 4×10^{-4}M SMZ reagent which flow at reagent solution . It's found that the ratio was 1:1 (NIF : SMZ) seen in figure 10 .

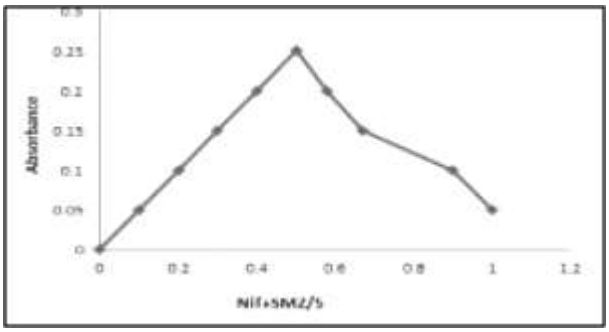


Figure 10: Job's method shows perfect addition drug with reagent in maxium absorbance appeared at 2/5 ratio

CONCLUSION

This study submitted new simple and sensitive Method including hyphenated technique normal Flow injection analysis for evaluation NIF drug in pure and pharmaceutical with diazotized reagent SMZ which is reacts and coupling with NIF drug to yield red orange product. Suggested Method was simple, rapid and effective without requiring any complicated steps like heating and difficult preparation. As compared with other expensive techniques, the proposed Method are economic and inexpensive with an excellent accuracy and precision. FIA method more convenient for determination drugs , the sensitivity was better and linear range wider . The proposed Method could be employed for routine analysis.

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