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New Spectrophotometric Method for The Determination of Nifedipine in Pharmaceutical Preparations by Formation of Azo Dye with Dopamine Reagent

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Dedication

بسمك اللهم

(يرفع الله الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ)

Content of the Research Includes:

New Spectrophotometric Method for The Determination of Nifedipine in Pharmaceutical Preparations by Formation of Azo Dye with Dopamine Reagent

Abstract

A new sensitive colorimetric determination of nifedipine has been developed following azo dye formation with dopamine reagent. This research was conceived as a means of developing an alternative cost effective and readily adaptable method for the assay of nifedipine in tablets. In this communication, new spectrophotometric methods for the determination of nifedipine have been discussed. The methods are based on the reduction of nitro group of nifedipine by Zn/HCl into primary amino derivative.

Nifedipine was reduced with Zn/HCl reduction system and then the diazo coupling reaction was carried out with the dopamine reagent to generate a new azo adduct with optimal wavelength at 320 nm. Optimal time for coupling were selected as 10 minutes. A linear response was observed over 5-30 µg/mL of nifedipine with a correlation coefficient of 0.9985 and the drug combined with dopamine at a stoichiometric ratio of 1:1. The method has limits of detection and quantitation of 0.1344 µg mL-1 and 0.4074 µg mL-1 respectively. The Sandell's sensitivity obtained is 4.673 ng/cm2

Key Words: **Nifedipine tablets, dopamine, diazonium ion, Azo dye , Colorimetric analyses**

Background

Nifedipine chemically dimethyl - 2 , 6 - dimethyl - 4- (2 nitrophenyl) -1 , 4 - dihydropyridine - 3 , 5 - dicarboxylate , has an empirical formula of C17H18N2O6 .

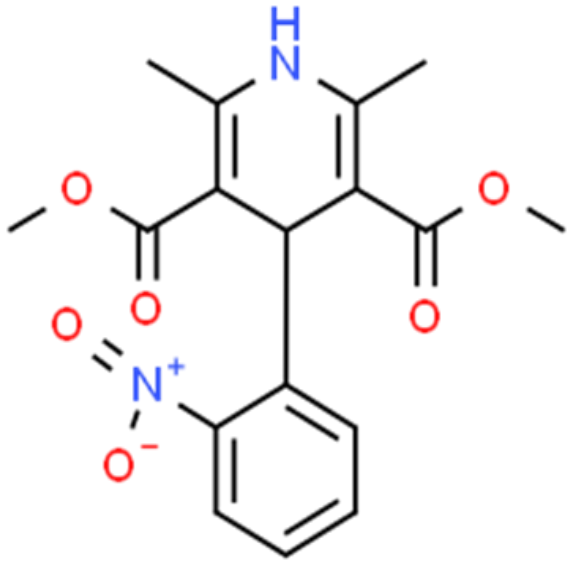


Figure 1structure of nifidipine

It is commonly available in capsule and extended-release tablet dosage forms [1-2]. Nifedipine, which occurs as a yellow crystalline powder, has a melting point of 172°C to 174°C and is practically insoluble in water, sparingly soluble in dehydrated alcohol and freely soluble in acetone [1–3].

When exposed to daylight or to certain wavelengths of artificial light, it is converted to a nitroso phenyl pyridine derivative, and exposure to ultraviolet light leads to the formation of the same derivative[1-3] The drug is official in the United States Pharmacopoeia (USP) [4] which recommends high performance liquid chromatography (HPLC) for its assay (both the pure drug and its dosage forms), and in the BP [3] which recommends redox titration using cerium sulphate and HPLC for the assay of the drug and its dosage forms, respectively.

Nifedipine, the prototypical 1,4-dihydropyridine, is a calcium channel blocker with peripheral and coronary vasodilator activity. It is used in the management of hypertension, angina and some other cardiovascular disorders. Its uses result primarily in vasodilatation, with reduced peripheral resistance, blood pressure, and afterload; increased coronary blood flow; and a reflex increase in heart rate. This in turn results in an increase in myocardial oxygen supply and cardiac output. It acts

by inhibiting the transmembrane influx of calcium into cardiac and vascular smooth muscle cells. Nifedipine is rapidly and almost completely absorbed from the gastrointestinal tract but undergoes extensive hepatic first-pass metabolism. Bioavailability of oral capsules is between 45% and 75% but is lower for longer-acting formulations. Peak blood concentrations were reported to occur 30 min after oral doses of capsules. It is about 92% to 98% bound to plasma proteins and is distributed into breast milk. It is extensively metabolized in the liver, and 70% to 80% of a dose is excreted in the urine almost entirely as inactive metabolites. The half-life is about 2 h after intravenous doses or oral capsules [1]

Used in the management of hypertension, angina and some other cardiovascular disorders Its uses result primarily in vasodilatation, with reduced peripheral resistance , blood pressure , and afterload ; increased coronary blood flow ; and a reflex increase in heart rate . This in turn results in an increase in myocardial oxygen supply and cardiac output [10,11]

Side effect of nifedipine include headache, nausea, dizziness or lightheadedness.[12]

Detailed survey of literature for nifedipine revealed several methods that have been reported for the assay of nifedipine either alone or in combined form in drug formulations. These analytical techniques include UV-Visible (Vis)

spectrophotometry[5-13] HPLC[14-15] high performance thin layer chromatography[16] micellar electrokinetic chromatography electroanalytical methods flow injection analysis and mass spectrometry

Dopamine:

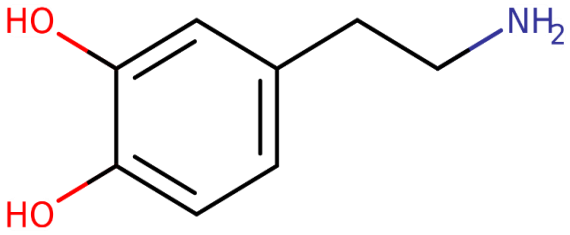


Figure 2 structure of dopamine

(chemically 4-(2-Aminoethyl) benzene -1,2-diol) having the formula (C8H11NO2) and the molecular weight 153.17844g/mol [17].

molecule consists of a catechol structure (a benzene ring with two hydroxyl side groups) with one amine group attached via an ethyl chain.[18] As such, dopamine is the simplest possible catecholamine,

a family that also includes the neurotransmitters norepinephrine and epinephrine.[19] The presence of a benzene ring with this amine attachment makes it a substituted phenethylamine, a family that includes numerous psychoactive drugs.

Like most amines, dopamine is an organic base, As a base, it is generally protonated in acidic environments (in an acid-base reaction). The protonated form is highly water-soluble and relatively stable, but can become oxidized if exposed to oxygen or other oxidants. In basic environments, dopamine is not protonated [20]

Freely soluble in water; sol in methanol, in hot 95% ethanol, in aqueous solution of alkali hydroxides; practically insoluble in petroleum ether, ether, benzene, chloroform, toluene and so, Hydrochloride it is highly sensitive to oxygen and discolors quickly.

Dopamine is primarily a dopamine receptor agonist; however, at higher doses, dopamine activates α- and β-adrenergic receptors, too. Dopamine is administered as a continuous intravenous infusion. At low doses, dopamine preferentially stimulates D1 and D2 receptors in the renal vasculature, which leads to vasodilation and promotes renal blood flow to preserve glomerular filtration, at intermediate doses, dopamine also stimulates β1-receptors on the heart. At high doses, dopamine stimulates α-adrenergic receptors in the vasculature, which exacerbates HF by increasing afterload Dopamine is rapidly absorbed from the small intestine [21].

Biotransformation of dopamine proceeds rapidly to yield the principal excretion products, 3-4-dihydroxy-phenylacetic acid (DOPAC) and 3-methoxy-4-hydroxy-phenylacetic acid (homovanillic acid, HVA). It has been reported that about 80% of the drug is excreted in the urine within 24 hours, primarily as HVA and its sulfate and glucuronide conjugates and as 3,4-dihydroxyphenylacetic acid. A very small portion is excreted unchanged.

Dopamine is a prescription medicine used to treat the symptoms of low blood pressure, and low cardiac output and improves blood flow to the kidneys. Dopamine may be used alone or with other medications and treat condition such as Parkinson's disease

Side effects include irregular heartbeats, Nausea, Vomiting, Anxiety, Headache, Chills, and Shortness of breath [22].

A bright red azo dye will be formed between the reduced nifedipine and dopamine, azo dyes are synthetic compounds containing an azo bond –N=N-, obtained mainly from the aromatic amine substrate, nitro and nitroso. The synthesis processes rely on the use of an appropriate oxidizing/reducing reaction or a diazotization/coupling reaction. This is considered one of the most important reaction in the development

of industrial organic chemistry. The synthesis requires a diazonium salt and a coupling component. [23,24].

Most diazonium salts are unstable and can be explosive when they are dry. They are always prepared in an acid medium with good stirring at 0 °C to minimize contact with water to produce phenol and are used immediately in the coupling reaction.

To complete the synthesis of the azo dye, the diazonium salt reacts as an electrophile with a coupling component is rich in electrons (a phenol or an aniline). This reaction was carried out by an electrophilic aromatic substitution mechanism. The hydroxyl or amine group directs the aryl diazonium ion to the para site in the event that this site is not occupied, otherwise it will be attached to the ortho position. There are other methods for the synthesis of azo dyes among which we found the reduction of the nitroaromatic derivatives in alkaline medium, the reduction of the nitrosated compounds by AlLiH4, the oxidation of the primary amines by the potassium permanganate or lead tetraacetate, condensation of hydrazines and quinones and condensation of primary amines of nitrosated derivatives.

The azo dyes and pigments are manufactured on an industrial scale by the same reaction sequence in two stages, diazotization and azo coupling. Overall, there are five strategies for the synthesis of azo dyes based on the diazotization/coupling reaction. Each strategy has certain restrictions associated with the availability of substrates. The two reactions 1 and 2 below are intended for the preparation of symmetrical azo compounds

The color of the azo dyes is determined by the azo bonds and their associated chromophores and auxochromes.

Owing to the presence of an azo group, these compounds are mainly used as dyes and pigments. Azo dyes are primarily used in textiles, leather, paint, food, solvents,

and printing (fat and oil-soluble dyes). Moreover, azo compounds have also demonstrated biomedical applications involving their use as antitumor, antidiabetic, and antiseptic agents [25–27]. Antitumor agents, antioxidants, drug derivatives, and drug polymers are among the medical uses of azo compounds, which exhibit antibacterial, antifungal, pesticidal, antiviral, and anti-inflammatory properties [28–30]. owing to their use in dyes, pigments, functional materials, and optical computing, metal complexes of azo compounds have garnered significant attention [31-33]. Azo compounds produce a wider range of colors throughout the ultra-violet-visible spectrum, compared to those produced by conventional aromatic compounds. Several researchers have demonstrated interest in preparing these compounds and studying their properties and effectiveness, as well as the distribution of compensated groups at different sites in the aromatic ring relative to the azo group, such as hydrazine.

As well as their harmful effects of azo dyes on humans and aquatic life, have aroused urgent calls for the treatment of effluents containing azo dyes to eliminate them or convert them into useful and safe products, [8, 9].

In addition, the distribution of dyes in water increased with the increase in the molecular weight of the azo dyes, probably caused by the increase in the molecular weight of the azo dyes in the form of increased azo bonds, resulting in a decrease in the rate degradation of azo dyes.

Materials and Method

Apparatus

Shimadzu UV–visible double beam spectrophotometer (model 2450) with 1 cm matched quartz cells was used for all the spectral measurements.

Materials

All chemicals and reagents were of analytical grade and water was always double distilled water.

Reduction of nitro group in Nifedipine [34]

100 mg of nifedipine pure or equivalent tablet powder was accurately weighed and dissolved in 20 ml of ethanol. This solution was treated with 10 ml of 5 N HCl and 1.0 g of Zinc powder was added in the portions, while shaking at 80 C^0 for 30 min. The solution was filtered using a Whattman filter paper 41 to remove the insoluble matter and the volume was made up to 100 ml with ethanol to get the concentration 1 mg/ml

Preparation working standard solution The resulting amine from the above solution 10 ml was taken into 100 ml volumetric flask and made up to the mark with ethanol to get the concentration 100 mg/ml and dilution was carried out to the further working standards.

The Absorption Spectra of Azo Dye Solution

Into a 25mL standard volumetric flasks, 5ml of working standard solution nifedipine (20ppm) was added,0.75 mL of 0.1% NaNO2, 1.0 mL of 2N HCl were pipetted and shake well, 1.5mL of 0.1% M dopamine reagent were pipetted kept for 5.0 min to complete diazotization reaction. followed by 2.0 mL of 2 M sodium hydroxide and then diluted to volume with distilled water at room temperature. The absorbance was

measured against the reagent blank prepared similarly except for the drug. The color was stable up to 3 h. Figure (1) show the Absorption spectra of azo dye solution.

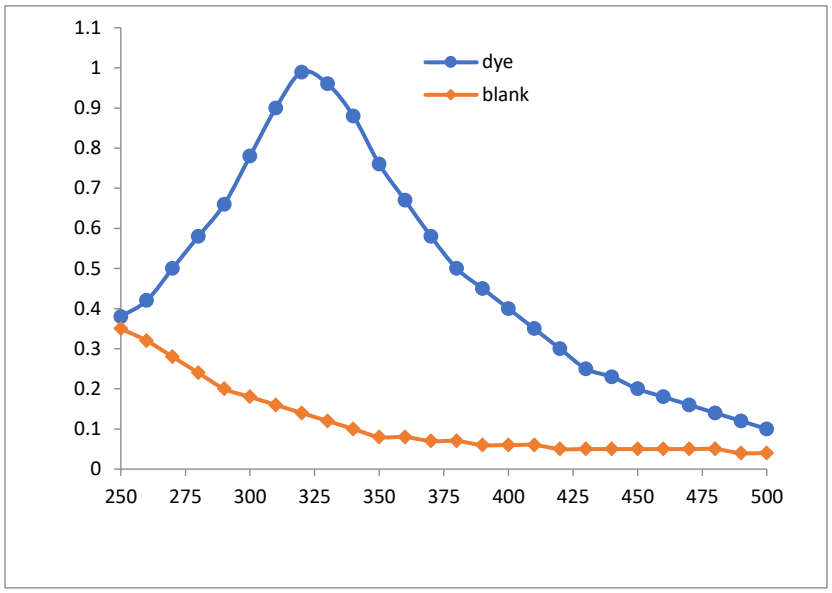


Figure (1): The Absorption spectra of azo dye solution

Optimization Studies

The optimizations of the method was carefully studied to achieve complete reaction formation, highest sensitivity and a maximum absorbance. Reaction conditions of e coupling complex were found by studying with preliminary experiments.

Effect of NaNO2 concentration

The effect of the concentration of $NaNO_2$ on the color development was investigated in the range of (0.25-1.25 ml)of 0.1% NaNO2. The absorbance of 0.75 ml was chosen as an optimum value for the determination process (Fig. 2).

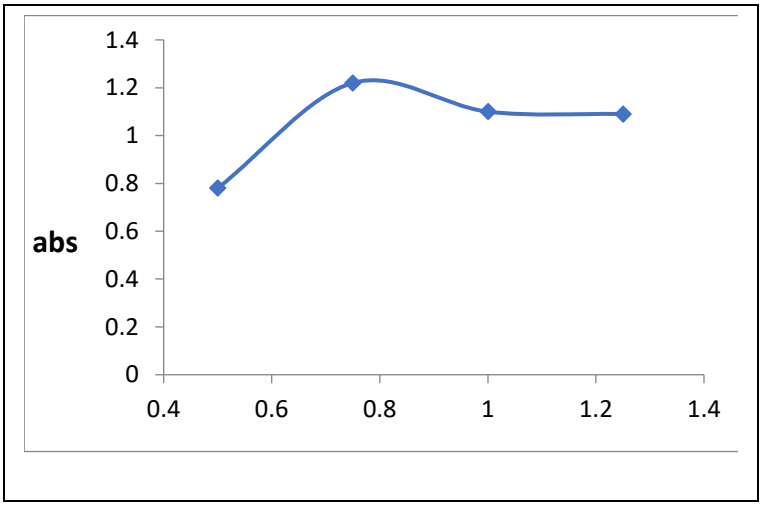


Figure (2) **Effect of NaNO2 concentration**

Effect of reaction time and stability of colored azo dye

the optimum reaction time was investigated by following the color development at ambient temperature (25 ± 2 C). Complete color intensity was attained after 5.0 min of mixing for complex. Rising the temperature up to 30 C has no effect on the absorbance of the formed complex. The absorbance remains stable for at least 3 h

Effect of HCl concentration

The influence of the concentration of HCl on color development has been investigated in the range of (0.25-1.5ml)and the results are shown in Fig. 3. The highest absorbance was obtained with 1ml HCl absorbance Therefore, the 1ml of 2 M HCl was used in all measurements

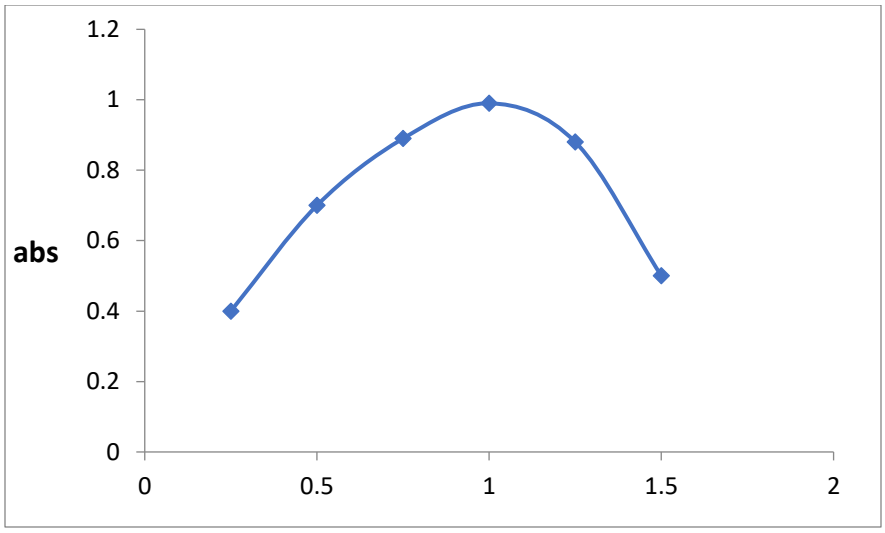


Figure (3) Effect of HCl concentration

Effect of NaOH concentration

The effect of the concentration of sodium hydroxide was studied in the range of (0.5-3ml) of 2M sodium hydroxide solution. As can be observed from Fig. 4, the absorbance of the colored product increased with increasing concentration of NaOH. 2ml was used as an optimum value for the determination of nifedipine drug.

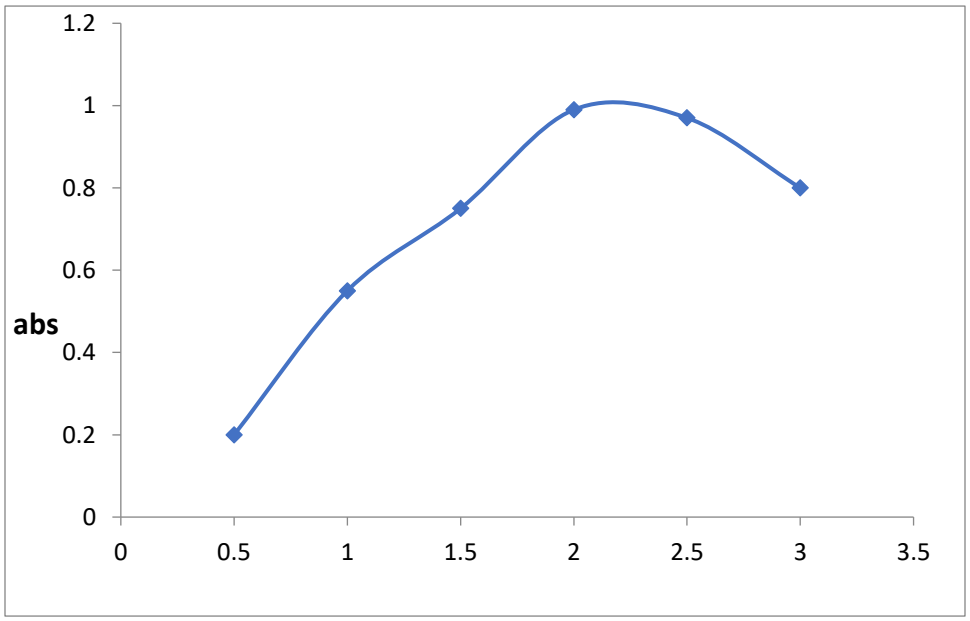


Figure (4) Effect of NaOH concentration

Effect of concentration of dopamine reagent

The effect of dopamine concentration on the intensity of the color developed at the selected wavelength and constant nifedipine concentration was critically examined using different millilitres of the reagent (0.1%). The results indicated (Fig. 5.) that the maximum absorbance obtained with 1ml, Therefore, the 1ml of 0.1% dopamine was used in all measurements.

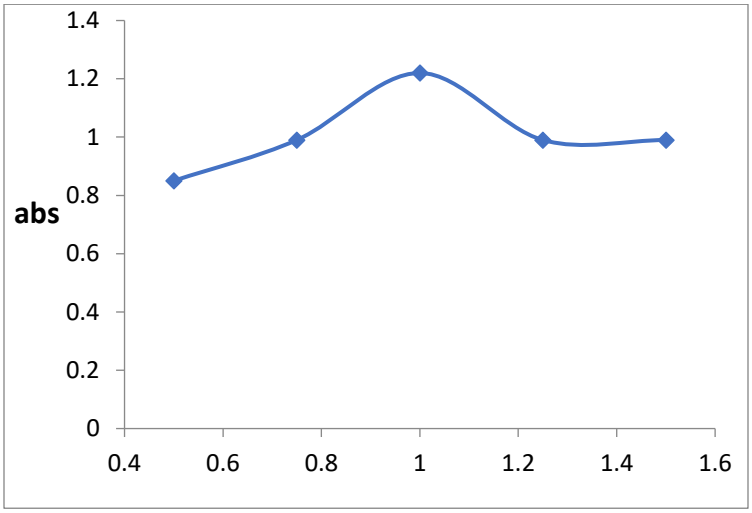


Figure (5) Effect of reagent concentration

Analytical Data and Calibration Curve

Aliquots of nifedipine solution 5-30 mg/ml were transferred into 25 ml volumetric flasks. To, this 1 ml of hydrochlpric acid (2N), 0.75ml sodium nitrite 0.1% solution and 1 ml of (0.1% N) dopamine reagent were added to each flask. The flasks were shaken thoroughly and placed for 5 min. The reaction mixture and total volume was adjusted to 25 ml with distilled water at room temperature. The absorbance of each solution was measured at 320 nm against a reagent blank. The amount of nifedipine present in the sample was computed from calibration curve (Fig. 6).

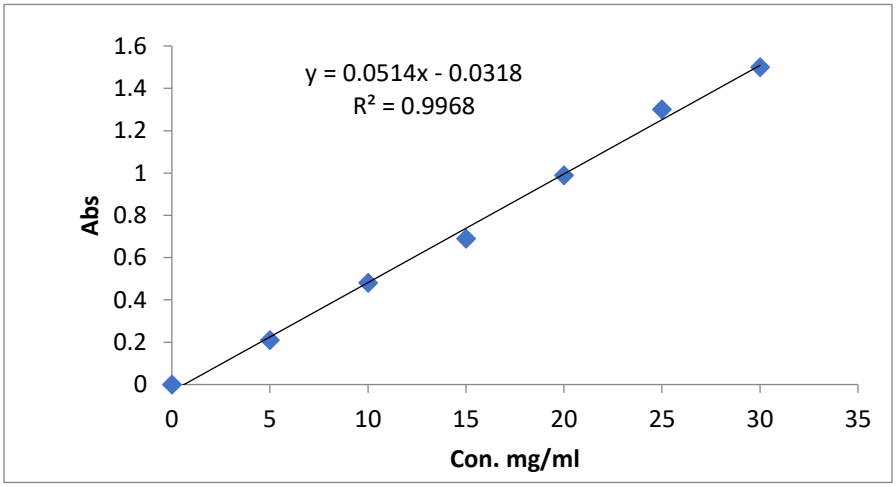
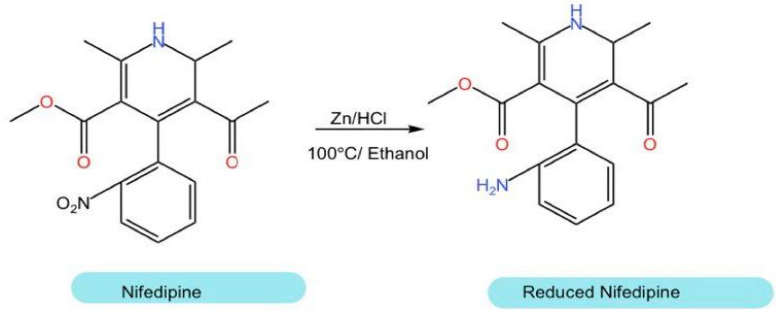


Figure (6) Calibration curve of Nifedipine

Table 1. Optical performance and regression characteristics of the proposed method

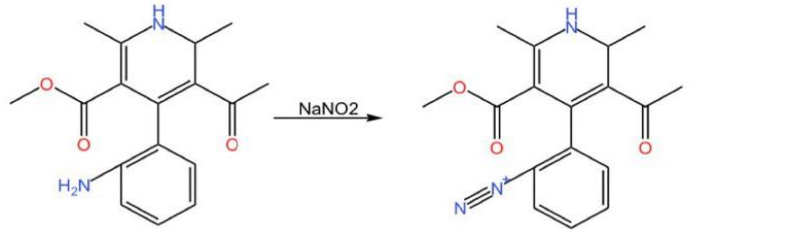
Parameters Proposed Method

Wavelength max (nm)	320
Stability (h)	3
Beer's law limit (_g mL-1)	5.0-30
Molar absorptivity (L mol-1 cm-1)	61×10^4
Linear regression equation	Y= 0.0514x - 0.0318
Detection limit (mg mL-1)	0.08
Correlation coefficient (r)	0.9968

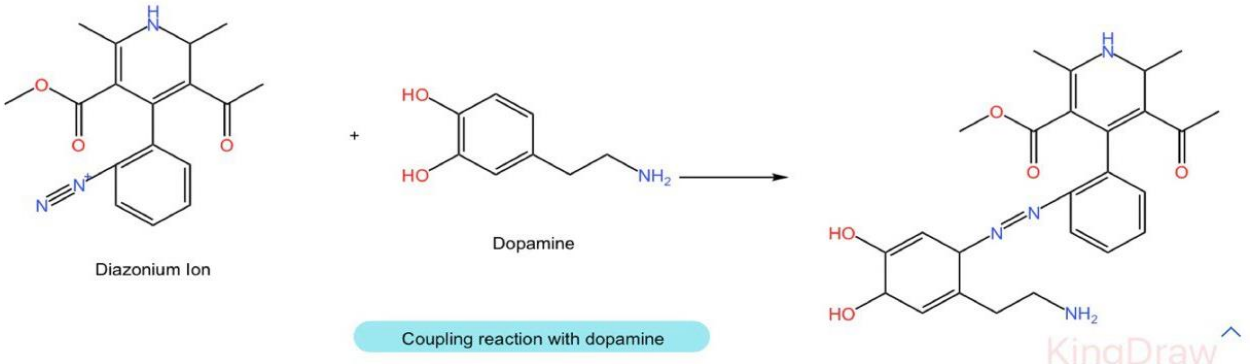


Nifedipine

Reduced Nifedipine



Diazonium Ion



Suggested mechanism of formation of dye

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