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Spectrophotometric Determination of Nitazoxinide Antiviral Drugs by Reaction of Diazonium salt

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Abstract— Spectrophotometric techniques can be used to accurately and rapidly determine the concentration of Nitazoxanide in body fluids (serum and urine) through react with diazonium salt ,which result from hydrolyze amino group of 2-Aminobenzothiazole by hydrochloric acid , to formation a precipitate a yellow-orange azo dye. Showed obeyed beers law between 50- 450 $\mu g.mL^{-1}$, Molar absorptivity of 0.4916 $x10^3$ L mol^{-1}/cm^{-1} was measured at 478 nm, . for Sandal's sensitivity 0.0132 has a limit of detection (LOD) of 2.96 $\mu g.mL^{-1}$ and a limit of quantification (LOQ) of 9.875 $\mu g.mL^{-1}$. This technique has been successfully used to quantify various concentrations of Nitazoxanide in body fluids.

Keywords: Nitazoxanide Antiviral (NTZ), Diazotization, Spectrophotometry, 2-aminobenzothyazol

1. Introduction

Drugs with new modes of action that may be employed alone or in conjunction with neuraminidase inhibitors are needed in order to offer more effective medical treatment for seasonal and pandemic influenza. As a result, new influenza strains are always on the rise, and the present therapeutic options are unable to combat them effectively. A significant contribution to this field might be made by the redistribution of Nitazoxanide as an influenza therapy. It has been discovered via research into The mechanism of action of Nitazoxanide against influenza viruses is that the medication interferes with the maturation of the viral hemagglutinin during the post-translational stage of the virus's replication cycle [1].as a method of providing stronger protection against a broader range of viruses while avoiding the dangers of resistance and reducing the costs associated with developing treatments that are tailored to each specific virus type [2,3].

Use to treat and prevent a broad range of protozoa, parasites, and gram-negative organisms, Nitazoxanide is the most often used antiprotozoal and antihelmintic agent. Intestinal protozoal diseases as well as helminthiasis are both treated . All patients with impaired immune systems, including those with HIV or AIDS, Giardia lamblia-caused diarrhea .Antiprotozoal effectiveness may be based on NTZ's capacity to disrupt the PFOR enzyme-dependent electron transfer mechanism, which is essential to anaerobic energy metabolism, which NTZ inhibits.

Nitazoxanide (NTZ) is a novel antiparasitic and antiprotozoal compound with a wide range of action against a variety of parasites and protozoa. It is a nitrothiazole derivative with the chemical name 2-acetyloxyl-N-(5-nitro-2-thiazolyl) benzamide as its chemical name. It is a nitrothiazole derivative (Fig. 1) [4].

In its pure form, NTZ is a white crystalline powder with a light yellow or pink tint that is insoluble in water and only mildly soluble in alcohol. It has a molecular mass of 307.283 g/mole and a chemical formula of $C_{12}H_9N_3O_5S$, indicating that it is a carboxylic acid, according to the IUPAC classification. A class IV medication, Nitazoxanide is classified as such by the Biopharmaceutical Classification System (BCS) (low solubility and low permeability), Acetone, chloroform, and methanol are all mildly soluble in it, while water is completely insoluble. Nitazoxanide has a melting point of 202 degrees Celsius. There is a wide range of actions for this compound ,the active metabolite after treatment when taken orally, is detected within 1-4 hours. It is eliminated in urine, bile, feces, and other places in the body.

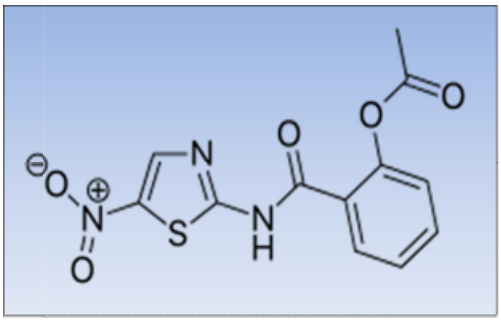


Figure (1): The Chemical Structure of Nitazoxanide.

2. Experimental :

Instrument: Double – beam Uv-Vis spectrophotometer (Biochrom England origin) was utilized with quartz 1cm length.

3. Material and reagent:

Nitazoxanide were obtained from China (Company Name: Nanjing Duly Biotech Co., Ltd) the purity of which was 99.3% and 2-Aminobenzothiazole from China purity of which was 98%

3.1 Solutions:

2-Aminobenzothiazole was prepared solution by dissolving 0.024g in hydraulic acid (BDH) 1M and complete to the mark by distilled water in cold water bath(0-5)C^{o} with continuous shake , then addition Sodium nitrate (BDH) 0.01M .

The coupling reagent was prepared by dissolving 0.05 g from Nitazoxanide in 0.1 M sodium hydroxide.

3.2 Procedure:

To detect Nitazoxanide take 1ml of serum or urine diluted with distil water were taken then addition to coupling Nitazoxanide reagent solution.

The coupling method formation by gradually mixed of Nitazoxanide coupling solution with 2-aminobenzothiozole solution and continues shake up to appeared the precipitate color of reaction.

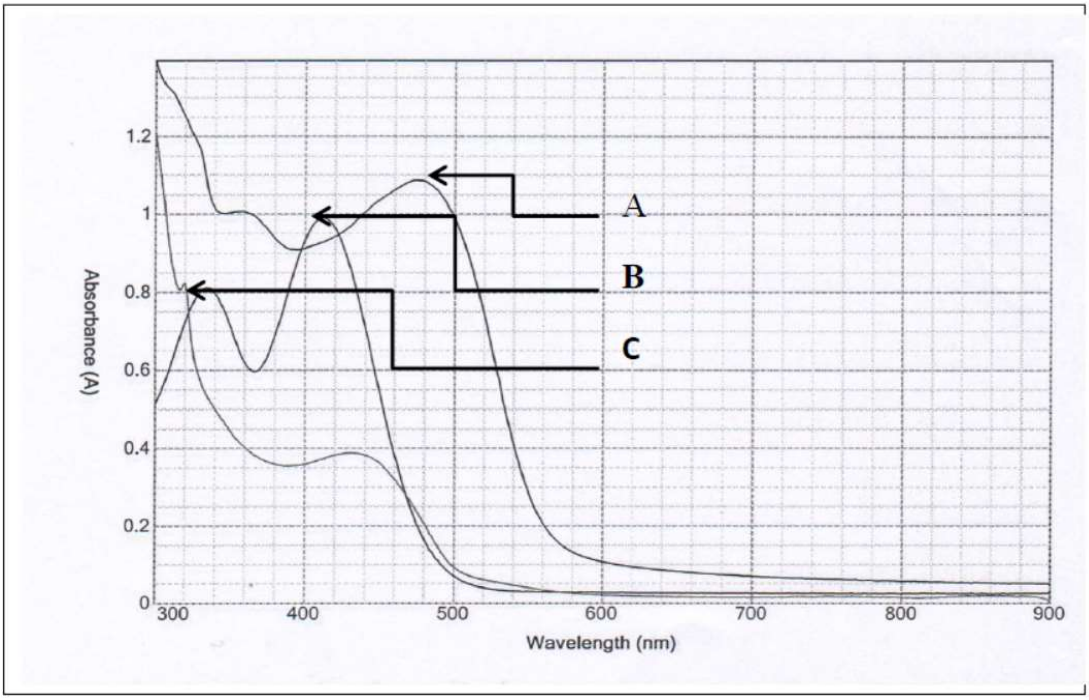
4. Results and discussion

4.1 choosing of coupling reagent:

Several coupling reagent such as 2-Aminobenzothiazole ,Resorcinol and Dapsone were used in this study. The useful analytical results were obtained with Nitazoxanide with 2-aminobenzothiozole to give a stable azo dye.

4.2 Analytical Spectral trails:

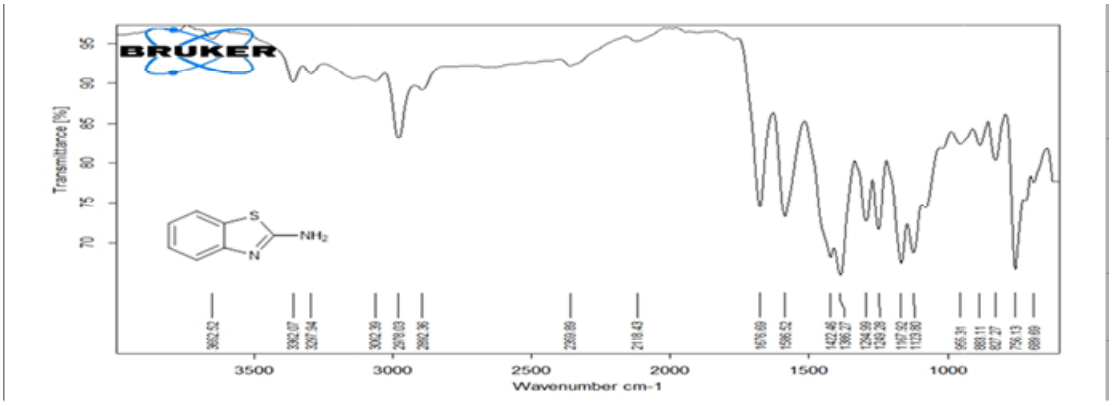
The reaction takes place by addition Nitazoxanide dissolved in sodium hydroxide to 2-aminobenzothiazole solution the cold and colorless solution dissolved in concentrated hydraulic acid which contains sodium nitrite, where we get a colored precipitate that dissolved in ethanol. Which leads to the formation of a yellow-orange solution using ultraviolet light Scan, the final product as well as the reactants in order to confirm the reaction. Figure (2) shows the spectra of the colored product. As shown in these data, the maximum absorbance of the enclosed product is at 478 nm. To identify antiviral Nitazoxanide in a range of applications, this red-shift assay approach can be used in conjunction with a variety of other screening techniques.



Figure(2):(A) The reaction product of Nitazoxanide with 2-Aminobenzothiazole , (B) Absorption spectrum of the 2-Aminobenzothiazole (C) Absorption spectrum of the Nitazoxanide.

4.3 FTIR spectra:

The FTIR spectra of the product and Nitazoxanide shown in Figures (3,4,5) reveal 3325 (OH, carboxyl), 3085 (C-H, aromatic), 1723 (C=O, carboxyl), 1624-1533 (C=C, aromatic), and 1481 (N=N) in the case of the product.



Figure(3):Shows FT-IR for 2-Aminobenzothiazole.

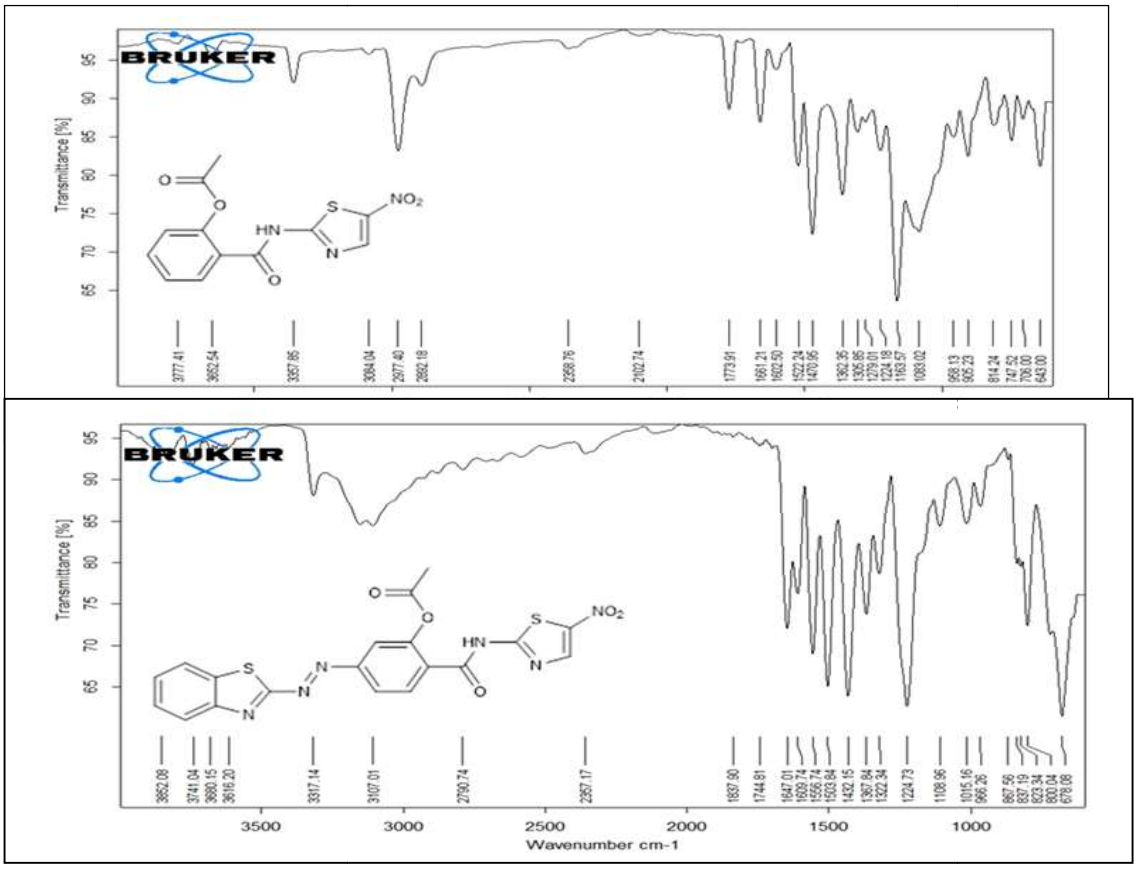


Figure (5):shows FT-IR Product (BTNCPA)*.

(BTNCPA)* (E)-5-(benzo[d]thiazol-2-yldiazenyl)-2-((5-nitrothiazol-2-yl)carbamoyl)phenyl acetate.

4.4Optimization of reaction conditions:

4.4.1 Effect of acid:

were used differ acids to investigation the effect on the color product development table (1) revealed A comparison between the acidity and the absorption of the colored product .the HCL acid achieved best absorbance.

Table 1: Effect type of acid on absorbance of the product.

Acids	HCl	HNO3	H_2SO4	H_3PO4
Abs.	**1.086**	**0.778**	**0.328**	**0.641**

After selected the suitable acid , the volume is modified depend of suitable acid. In order to get the best results, the experiment was carried out in the range of (1 – 5) ml of 1M HCl, as indicated in Figure (6). The absorbance reaches its maximum when 1 mL of the solution is added.

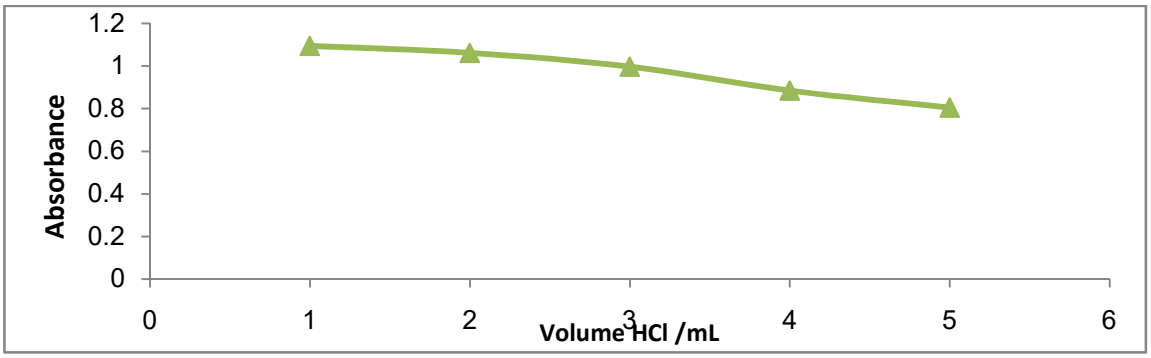


Figure (6): Acid volume has an effect on color product absorption.

4.4.2 Effect of Bases:

An investigation is conducted into the influence of various bases on the product's color intensity. The results are summarized in Table (2). The alkalis KOH and NH4OH are ruled out in favor of the alkaline solution NaOH because of their low absorbance.

Table 2: Effect type of bases on absorbance of the product.

Base	NaOH	KOH	NH4OH
Abs.	**1.115**	**0.734**	**0.701**

Following the selection of the most appropriate base, the volume is adjusted accordingly. In order to get the results shown in Figure (7), the experiment was conducted in a range of (1 – 5) ml of 0.1M NaOH. The finding shows that 2.5 ml of Sodium hydroxide is required for this process.

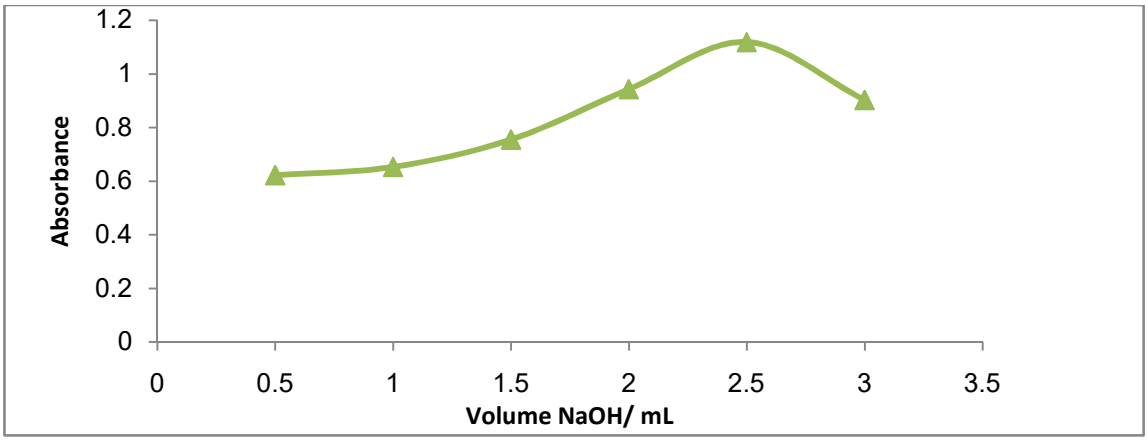


Figure (7): Base volume has an effect on color product absorption.

4.4.3 Effect of Sodium Nitrite.

The influence sodium nitrite volume was study (0.1M) Figure (8) showed the suitable volume was 3ml because high absorbance .

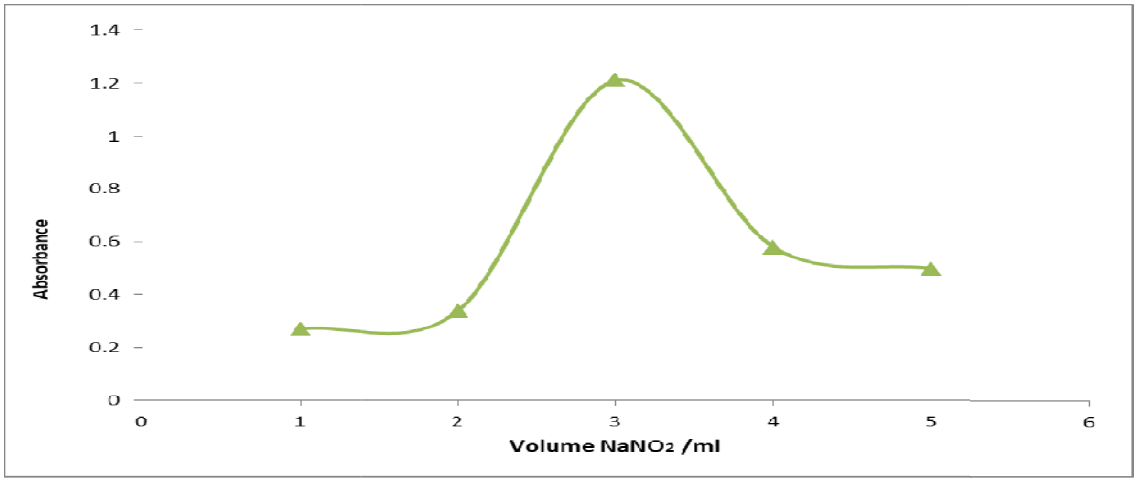


Figure (8): The effect of NaNO2 volume on color product absorbance.

4.4.4 Sequence of addition:

Table(3):Shows that 2-Aminobenzothiazole, diazotized Nitazoxanide, and sodium hydroxide (NIT+2-A+B) had the highest absorbance. You will get the finest results if you follow this method.

Sequence	NIT+2-A+B	2-A+B+NIT	B+NIT+2-A
Abs.	1.147	0.734	0.677

Nit: Nitazoxanide. 2-A: 2-Aminobenzothiazole. B:base.

4.4.5 The time required for the diazotization process:

The time intervals employed to explore the development of the azo product are shown in Figure (9). with the highest absorption observed after 5 minutes of experimentation.

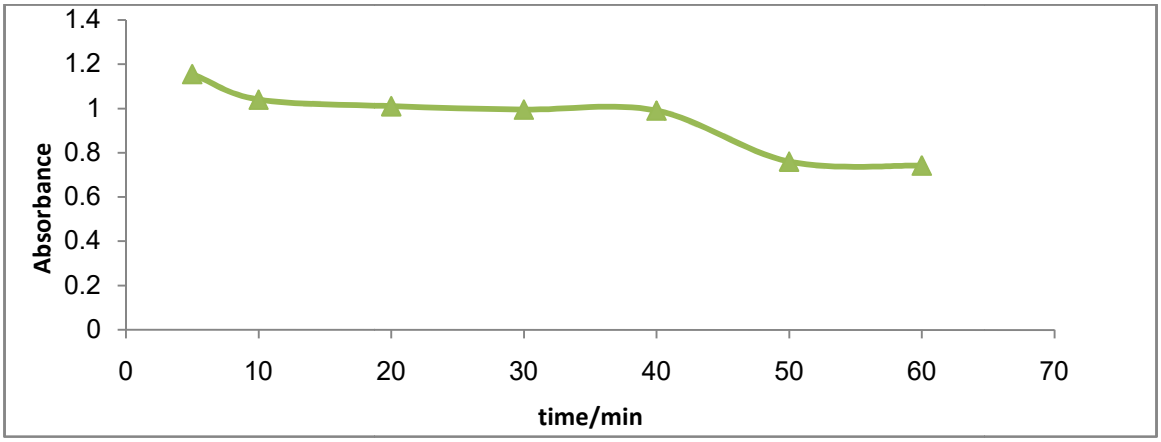


Figure (9):The Effect time on product.

4.4.6 Effect of Temperature :

Figure (10) depicts the various temperatures (0–40 C°) that were utilized in the investigation; the temperature (15C°) provides the greatest absorbance. The absorbance value decreased as the temperature increased, which is most likely owing to the product's dissipation throughout the process.

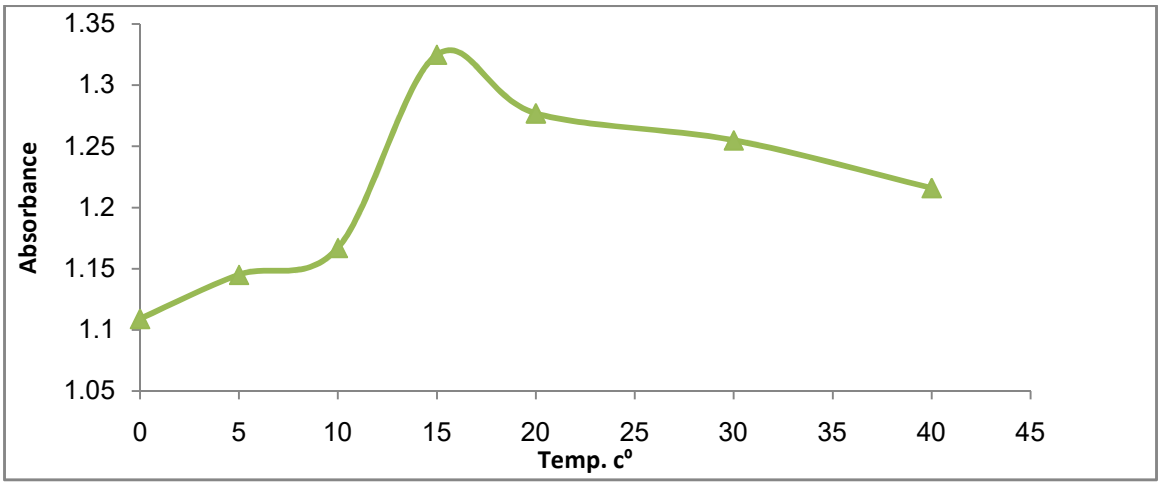


Figure (10):The Effect temperature on product.

4.5 Calibration curve:

When working under the ideal circumstances described above, it is possible to construct and store a standard calibration curve for the colored product. Using the chemical compound Nitazoxanide as an example, Figure (11) illustrates a calibration curve at 478 nm that follows Beer's law over a concentration range of (50 - 450 µg.mL^{-1}) and has a correlation value of 0.9969.

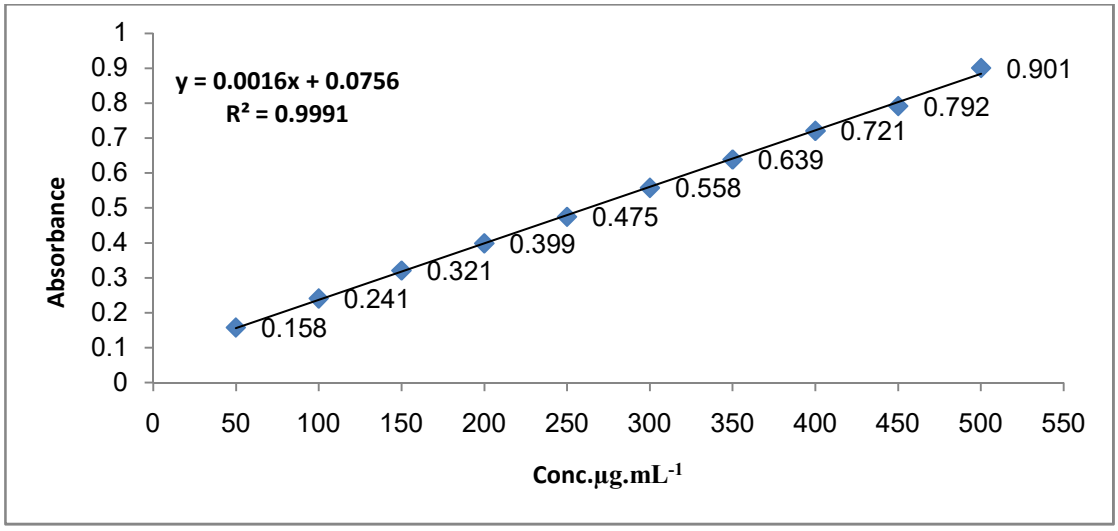


Figure (11):Calibration curve of Nitazoxanide.

Table(4): Nitazoxanide analytical parameter Estimate the product's composition.

Parameter	Value
Measurement wavelength (nm)	**478**
Linear range (µg/mL)	**50-450**
Molar absorptivity(L.mol-1.cm-1)[6]	**$0.4916x10^3$**
Detection limit (µg/mL)[7]	**2.96**
Sandells sensitivity, S (µg.cm-2) (µg/cm)[8]	**0.0132**
LOQ (µg/mL)[9]	**9.875**
Correlation coefficient (R)	**0.9995**
Determination coefficient (R^2)	**0.9991**
Slope (b)	**0.0016**
Intercept (a)	**0.0756**

4.6 Stability and Nature The Product's Constant:

Stoichiometric ratios were calculated using the mole ratio[10,11,12] Jobs method [13], as illustrated in Figure (12). The findings revealed a Nitazoxanide and 2-Aminobenzothiazole ratio of 1:1. The production of the product occurs in the same manner as in Scheme 1. This finding refers to the formation of stable compounds between Nitazoxanide and 2-aminobenzothiazole.

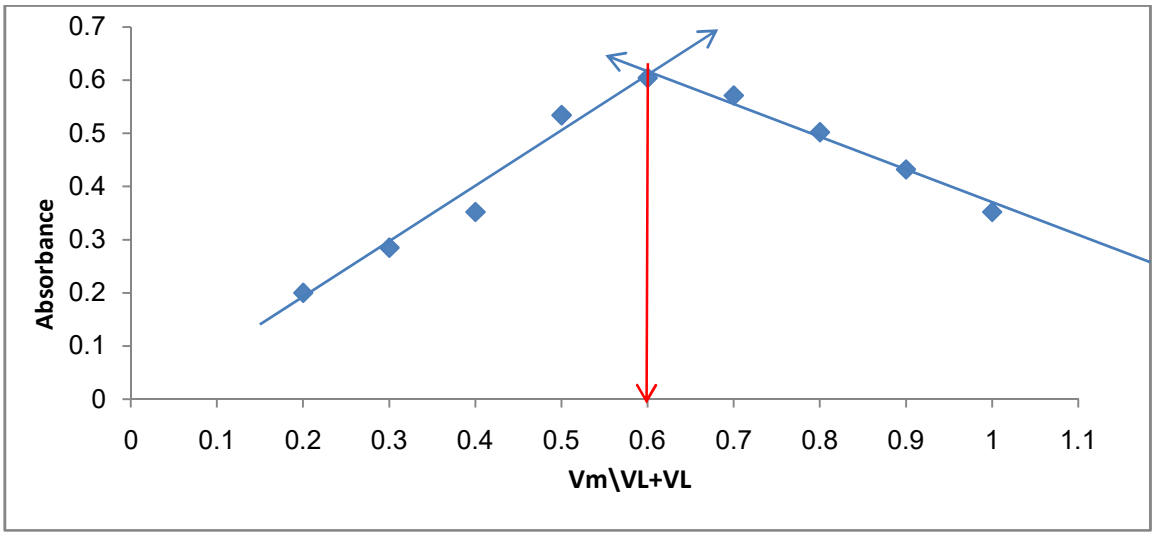


Figure (12):Jobs method.

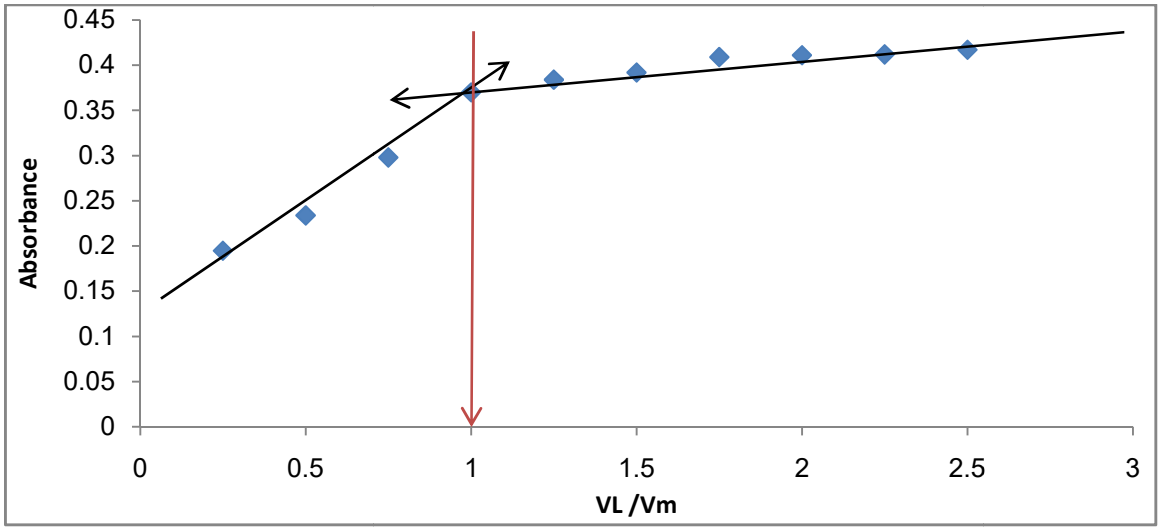
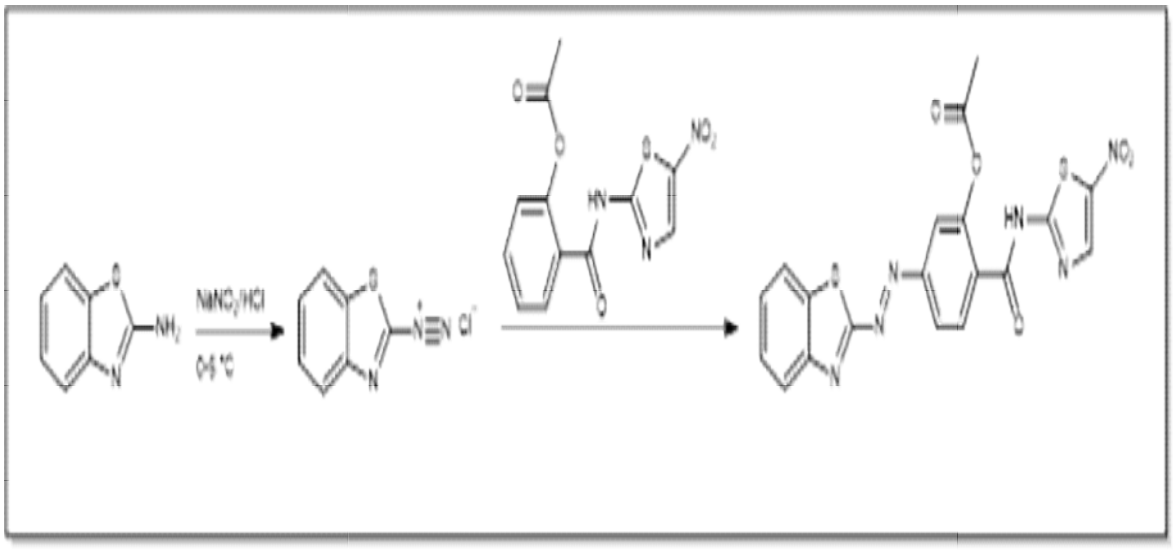


Figure (13) Mole-ratio.



Scheme (1) : The reaction between Nitazoxanide and 2Aminobenzothiazole has been proposed as having the following mechanism.

4.7 Applications:

4.7.1 Procedure

A mixture of serum [14] or urine (1 mL + 4 mL DW) containing Nitazoxanide at different concentrations (100,150 and 200 µg/mL) with serum, while the concentrations of Nitazoxanide in urine were (300,350 and 400 µg/mL) . 1) After dissolving in a basic solution (0.1 M), which is added to 2-aminobenzothiazole dissolved in acid (1M HCl) and a volume (1ml) with the addition of (0.1 M $NaNO_2$) and a volume (3 ml). By applying the optimal reaction conditions we to get the azo salt and after conducting a process Filtration and drying the colored precipitate, dissolved in ethanol measure the absorbance at λ_{max} 478 nm.

Table (5):Application of Nitazoxanide in Serum and Urine.

Sample	Present µg.mL^{-1}	Found µg.mL^{-1}	Error %	Recovery % [15]
Nitazoxanide In Serum	100	101.89	+1. 89	101.89
	150	150.87	+ 0.58	100.58
	200	199.02	- 0.49	99.51
Nitazoxanide In Urine	300	297.5	-0.83	99.17
	350	352.2	+ 0.628	100.628
	400	400.7	+ 0.175	100.175

5. Conclusion:

In comparison to other spectrophotometric techniques described in the literature, the suggested approach has been shown to be more quick, simple, selective, and extremely sensitive, and the results of a recovery study support this conclusion. This is an excellent spectrophotometric approach that may be used.

6. References

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