Original Article

Spectrophotometric Determination Of Anti-Ulcer Drug (Cimetidine) By 2.4-Dinitrophenylhydrazine Reagent

Fatima Abdali Hassoni[1] and Muthana Saleh Mashkour*[2]

[1,2*]Department of Chemistry, Faculty of Science, Kufa University, E-mail:muthanahana74@gmail.com

***Corresponding Author:** - Muthana Saleh Mashkour
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Abstract

An innovative, simple, and sensitive spectrophotometric method was described for the determination of cimetidine in both its pure form and in pharmaceutical formulations. By oxidizing 2.4-dinitrophenylhydrazine (2.4-DNPH) and mixing it with cimetidine in an alkaline solution, a brightly colored chromogen with a maximum absorption wavelength of 586 nm is created. After that, spectrophotometry was used to quantify the amount of cimetidine. Other analytical criteria, such as the ideal reaction circumstances, were evaluated. These criteria included variables like the volumes of 2.4-DNPH and potassium iodate, the amount of base, the coupling reaction time, temperature, and the order in which the constituents of the final product are added. Beer's law is applied to concentrations of (25–250) µg/mL^{-1} with correlation coefficient (R^2) = 0.9964, molar absorptivity 353.276 (L.mol^{-1}.cm^{-1}), and Sandell's law. The recommended method for locating cimetidine in pharmaceutical formulations worked well.

KeyWords: Cimetidine, 2,4DNPH, azo coupling reaction, anti-ulcer drug

INTRODUCTION:

Cimetidine (N" -cyano-N -meth yl-N'-[2([(5-methyl-1 H-imidazol-4-yl) methyl] thio) ethyl] guanidine) has a distinctive antagonistic effect on histamine Hrreceptors and is a member of a new subclass of drugs in this category. Its primary mechanism of action on parietal cell histamine Hrreceptors is to prevent the formation of gastric acid that is triggered by histamine, pentagastrin, acetylcholine, insulin, food, and other secretagogues.[1, 2].Its chemical structure is depicted in figure (1).

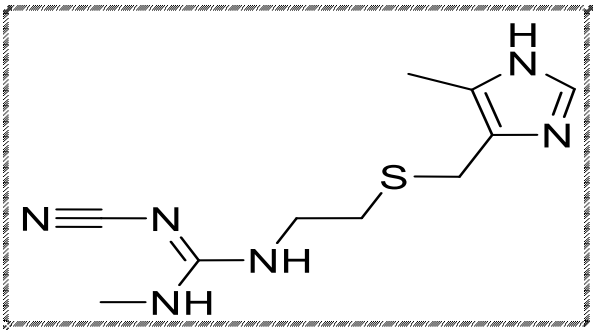


Figure (1): The Chemical Structure Of Cimetidine

Since assay method development is crucial for the pharmaceutical industries and pathological laboratories, it is always preferable to select and develop an easy-to-use, accurate, precise, and affordable method for the detection of pharmaceuticals in pharmaceutical dosage forms and pathological samples. [3,4]Numerous chemical mechanisms could cause the drug to break down. These include photochemical reactions, hydrolysis, dehydration, oxidation, polymerization, racemization, isomerization, and polymerization.[5- 7]Absorption Spectrophotometry is the measurement of the interaction between electromagnetic radiation and the molecules or atoms of a chemical substance. [8] Numerous techniques, including UV, visible, infrared, and atomic absorption, are utilized in pharmaceutical analysis. [9] Numerous elements in colorimetry need to be carefully and critically considered. To start, the colorant ought to be picky. Having the chemical formula [C6H3(NO2)2NHNH2], 2,4-Dinitrophenylhydrazine is one of the significant organic compounds. [10]. 2.4-Dnphh is a solid compound with an orange-red tint. Its chemical structure is shown in Figure 2, where it is substituted for hydrazine by two nitro groups in the ortho and para positions.[11]

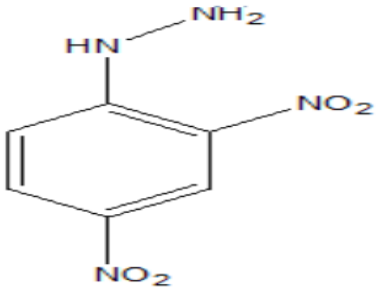


Figure (2): The Chemical Structure Of 2.4-DNPH

Brady's reagent or Borche's reagent, which contains 2.4-dinitro phenyl hydrazine and is dissolved in methanol with a small amount of strong sulfuric acid, was developed by other researchers. [12] An essential reagent, 2,4-Dinitrophenylhydrazine is utilized in numerous sorts of processes, including oxidative coupling, to quantitatively measure the presence of diverse molecules. [13] For the pharmaceutical industry, pharmaceutical preparation analysis is essential because it defines the standard specifications of these preparations and assesses how well they correspond to the pharmacopoeia. The discipline of analytical chemistry in pharmaceuticals.[14] Given that the method of analysis employed determines how well a medicine is judged for quality,[15], For the analysis of cimetidine in pharmaceutical formulations, various types of analytical techniques have been documented. Among them is high performance liquid chromatography.[16-,18] flow injection analysis, [19-20] potentiometry[21] and spectrophotometric methods [22-23] . Some of these procedures need an unreasonable amount of time and money for routine analysis. Since it provides sensitivity, precision, and accuracy of analysis, the current study offers practical and financial improvements over earlier techniques.

EXPERIMENTAL PART:

Instruments:

UV-1650PC UV-Visible Spectrophotometer, SHIMADZU, Japan (Double beam (, 303 PD UV-Visible Spectrophotometer, Apel, Japan (Single beam) and pH meter, Spinbot, thephaw

Materials and Reagents

All of the chemicals and reagents employed were of analytical grade, and China was the source of the cimetidine powder in its purest form.

Preparation of solutions:

1-Standard Cimetidine solution (1000 µg.mL^{-1}):

By combining 0.1 grams of powdered medication with the necessary amount of deionized water, transferring the mixture to a 100 ml volumetric flask, and topping off the volume to the mark with deionized water, you can create a solution of cimetidine with a concentration of (1000 µg.mL^{-1}).

2- 2, 4-Dinitrophenylhydrazine reagent solution (2.4-DNPH) 5×10^{-3} M:

The detector solution (2.4-DNPH) is made by dissolving 0.01 g of 2,4-DNPH in 0.15 mL of concentrated sulphuric acid (H_2SO_4) at a concentration of (5×10^{-3}M). This mixture is then transferred to a 10 ml volume flask, filled to the mark with deionized water, kept out of the light, and used right away.

3-Potassium iodate oxidized solution (KIO_3) 4.6×10^{-3} M:

By dissolving 0.01g of KIO_3 in the necessary volume of deionized water, and then pouring the resulting solution into a 10 mL volumetric flask and topping it off with deionized water, the oxidizing agent solution (KIO_3) is created with a concentration of (4.6×10^{-3} M).

4- Sodium hydroxide solution (NaOH) 10 M:

By dissolving 4 g of sodium hydroxide in the necessary amount of deionized water, the basic solution sodium hydroxide (NaOH) is created with a concentration (10 M). And then poured into a 10 ml volumetric flask before adding deionized water to fill the flask to the mark.

5-Preparation of Solutions for the analysis of Cimetidine in pharmaceutical preparations

i. In Tablet

One tablet's(200mg/tablet CIM) worth of material was precisely weighed, ground into a fine powder, dissolved in a quantity of deionized water, and agitated for 10 minutes to ensure that the medication was completely dissolved. The resulting solution was then poured into a 200 mL volumetric flask and diluted with deionized water to the proper concentration to make 1000 µg. mL^{-1}. The solutions were diluted properly, filtered via filter paper, and then evaluated utilizing the research procedure.

ii. In Ampoule

The injection ampoule contains 2 ml/200 mg of cimetidine; 1 ml was taken, transferred to a volumetric flask measuring 100 mL, and diluted with deionized water to the proper concentration to provide 1000 $\mu g.mL^{-1}$. Working solutions created and evaluated using the suggested methodology.

Analytical procedure:

After adding 0.25 ml of 10 M sodium hydroxide and waiting 10 minutes to stabilize the color, 2 ml of 2.4-DNPH reagent was added to 2.25 mL of standard Cimetidine solution in the presence of 2.25 mLof potassium iodate solution in basic medium to create a dark green color product. At 586 nm, the absorbance was measured in comparison to the identically prepared reagent blank that was devoid of any medication.

After oxidation, the procedure involved an oxidative coupling reaction between 2.4-DNPH, which was followed by a coupling step using cimetidine in an alkaline medium.

To determine the ideal circumstances for the detection of cimetidine in pharmaceutical preparations, the impact of different variables on the color development was examined.

RESULT AND DISCUSSION

Spectrum of absorption and proposed reaction The colorful product created from the reaction of oxidized 2.4-DNPH with Cimetidine in an alkaline solution during the primary test has a maximum absorption (max) at 586 nm in the absorption spectrum. The hardly noticeable absorbance of the slightly yellowish blank solution at the maximum concentration at which the medication was tested. Figure (3) displays the maximum absorption of 2,4-DNPH (354 nm), CIM (257 nm), and the color result of the reaction between CIM and 2.4-DNPH.

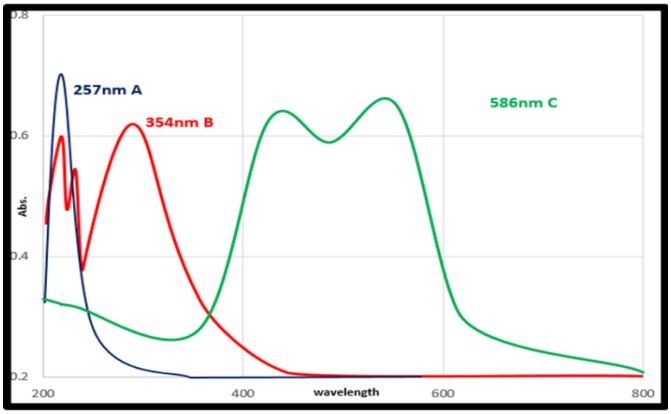


Figure (3): Absorption spectra A :(λmax) of 2.4-DNPH (354nm) , B: (λmax) of CIM (257nm) and C: (λmax) of Color product for reaction CIM and 2,4-DNPH.

OPTIMIZATION OF THE EXPERIMENTAL CONDITIONS

Univariate method

The impact of various parameters on the production of color products were methodically examined by altering each parameter separately while keeping the others constant. These factors include the amounts of 2.4-DNPH and potassium iodate solution used, the amount of base used, the coupling reaction duration, temperature, and the order in which the constituent parts of the finished product are added.

1. Effect Volume Reagent of the 2,4-DNPH on the Reaction with CIM drug:

Different volumes of reagent solution ranging between (0.25 - 2.50) mL to the volumetric flasks with installations of the other components as a drug, oxidizing agent KIO_3, and sodium hydroxide were used to determine the effect amount of 2.4-DNPH(5×10^{-3} M) on the measured absorbance of the colored product that was formed. Deionized water is used to bring the volume up to 10 mL. The amount of 2.4-DNPH used determines how intensely colored the reaction product will be. In order to use the recommended amount of 2.4-DNPH for all subsequent measurements, 2 mL of (5×10^{-3} M) was chosen. The findings are displayed in figure (4).

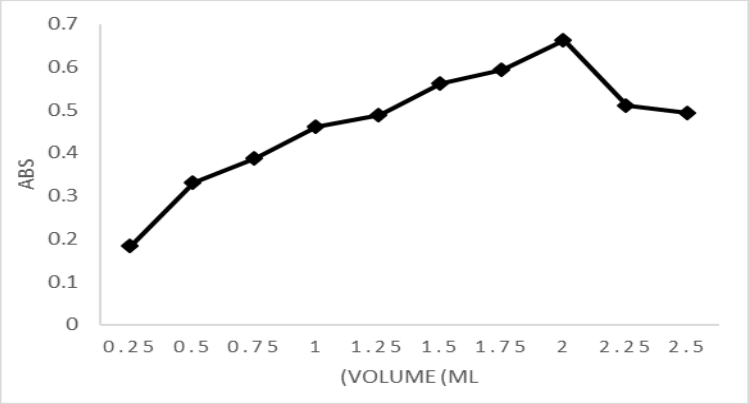


Figure (4): Effect Volume Reagent Of (2.4-DNPH) On The Reaction With CIM Drug

2. Effect Volume of the Potassium Iodate on the Reaction with CIM drug:

In Figure (5), the measured absorbance values are shown against various oxidant volumes (0.25 – 2.5) mL, A 2.25 mL of KIO_3 solution producing the highest absorption. Therefore, 2.25 mL of solutions were sufficient for the work that followed.

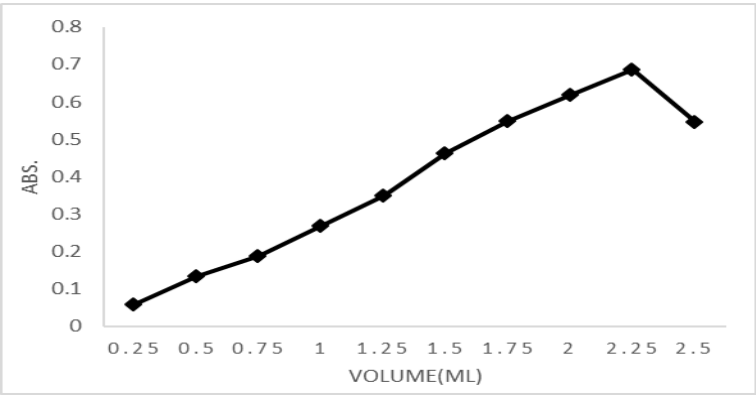


Figure (5): Effect Volume Of KIO_3 On The Reaction (2.4-DNPH) With CIM Drug

3. Effect Volume of Sodium Hydroxide on the Reaction with CIM drug:

The range of sodium hydroxide (10 M) study volumes was (0.25-2.50) mL. Figure (6), which displays the data, shows that the addition of 0.25 mL of NaOH produced the maximum absorbance. Therefore, 0.25 mL of NaOH(10M) was utilized in each experiment that followed.

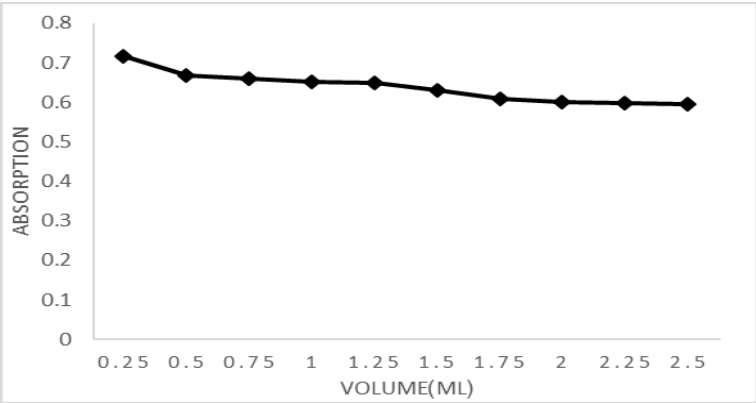


Figure (6): Effect Volume Of Base (Naoh) On The Reaction (2.4-DNPH) With CIM Drug

4. Effect temperature on the Reaction with CIM drug:

It was investigated how temperature affected the intensity of the colors. According to Figure (7), the ideal temperature is 20C° because in practice the color developed with the highest absorption at this temperature. Therefore, it is applied in later experiments.

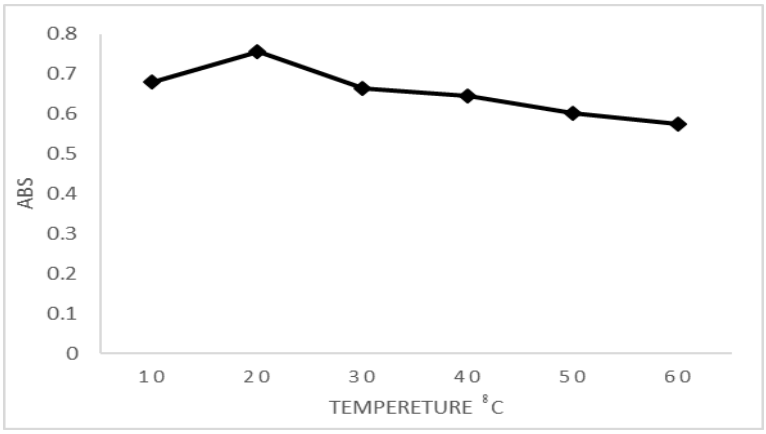


Figure (7): Effect Temperature On The Reaction (2,4-DNPH) With CIM Drug

5. Effect Time on Coupling Reaction with CIM drug:

Absorbance measurements were taken at various intervals, from right away to after 60 minutes of waiting. According to Figure, the oxidative coupling reaction was finished in 10 minutes (8).

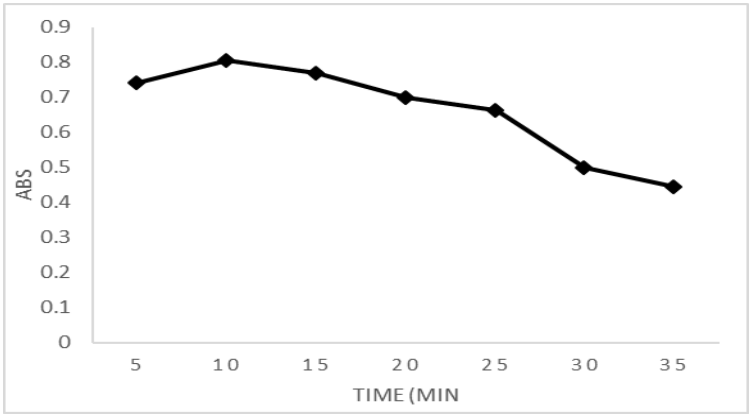


Figure (8): Effect Time On The Reaction (2.4-DNPH) With CIM Drug

6. Order of Additions for Reaction 2.4-DNPH with CIM drug:

We looked at four processes with various component adding orders. The sequences (1) and (2) and (3) produced absorbance values that were higher, while the sequences (1) and (4) produced absorbance values that were lower because the blank value was more than the absorbance value of the solution. The movements

Table (1) The following is an explanation for this behavior: 2.4-DNPH's hydrazine group is oxidized by KIO_3 to diazonium cation, which interacts with the drug in an alkaline medium to produce a stable derivative. So, it was decided to use sequence 1 in the procedure under study.

Table (1): Order Of Additions For Reaction 2.4-Dnph With Cim Drug.

NO.	Addition	Absorption
1	2,4-DNPH + KIO_3+ Drug + NaOH	0.817
2	Drug + 2,4-DNPH + KIO_3 + NaOH	0.502
3	2,4-DNPH + Drug + KIO_3 + NaOH	0.411
4	Drug + NaOH + 2,4-DNPH + KIO_3	0

Calibration Curve and Analytical Data

In a series of volumetric flasks (10mL), (0.25-2.50 mL) of (1000 $\mu g.mL^{-1}$) of Cimetidine were transferred, 2.25 mL of KIO_3 (4.6×10^{-3}) M. and 2 mL of 2.4-DNPH reagent (5×10^{-3} M), 0.25 mLof sodium hydroxide solution, equal to approximately (pH 13) were added at 20C°. After, that the solutions, were left for 10 min to complete the reaction, then the volumes were, completed to the mark with deionized water. The absorbance was measured at 586 nm against the blank reagent Linear relationship was observed between the absorbance and concentration of Cimetidine ranged from (25-250 $\mu g.mL^{-1}$) as shown in Figure (9). The correlation coefficient (R^2) = 0.9964, molar absorptivity =353.276 $L.mol^{-1}.cm^{-1}$, Sandell's sensitivity = 0.7142µg. Cm-2 limit of detection (LOD) = 0.012$\mu g.mL^{-1}$and limit of quantification (LOQ) = 0.037 $\mu g.mL^{-1}$were calculated.

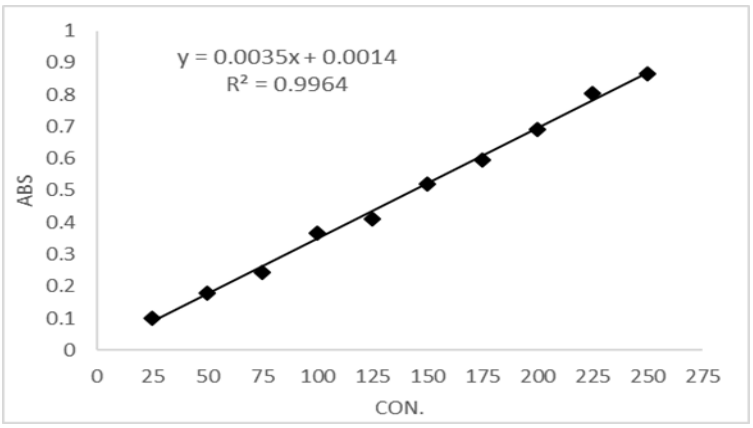


Figure (9): The Calibration Curve Of (CIM) Drug With (2,4-DNPH)

Accuracy and precision

In order to confirm the precision and accuracy of the offered procedure. First, we determined precision by doing five times of each experiment at the concentration (225 $\mu g.mL^{-1}$) of the cimetidine solution. The R.S.D. percent computation and values for this medicine, which were equivalent to (1.839%), show the method's high level of precision. The results are shown in Table (2).

Table (2): Accuracy And Precision For The Studied Method To Determine Drug (CIM)

X	$X - X^{-}$	$(X - X^{-})^2$
0.801	7.19×10^{-3}	5.16961×10^{-5}
0.807	0.01319	1.73976×10^{-4}
0.805	0.01119	1.252161×10^{-4}
0.777	0.01681-	2.825761×10^{-4}
0.779	0.01481-	2.193361×10^{-4}
$\sum_{X} = 3.969$		$\sum_{(X-X^{-})^2} = 8.52 \times 10^{-4}$

$$X^{-} = \frac{\sum X}{n} = \frac{3.969}{5} = 0.79381$$

$$S.D = \sqrt{\frac{\sum (X-X^{-})^2}{n-1}} = \sqrt{\frac{8.52\times 10^{-4}}{5-1}} = 0.01460$$

$$R.S.D\% = \frac{S.D}{X^{-}} \times 100 = \frac{0.01460}{0.79381} \times 100 = 1.839\%$$

Recovery % = 98.7%

The Stoichiometry of the formed product

Both the Job's approach and the molar ratio method were used to determine the generated dark green color product (stoichiometry of medication to reagent). In both approaches (the concentration of each of the standard Cimetidine solution and 2.4-DNPH reagent solution was equal) In a succession of volumetric flasks, Job's method (10 mL), Different volumes of the reagent solution (0.9-0.1) mL were combined with various volumes (0.1-0.9) mLof the drug solution. A 2.25 mLof potassium Iodate (4.6×10^{-3}M) and 0.25 mLof sodium hydroxide solution were added .and volumes were completed, to the mark with deionized water. The absorbance was measured, at 586 nm against the blank reagent. The results as it Figure (10) show that the ratio is 1:1.

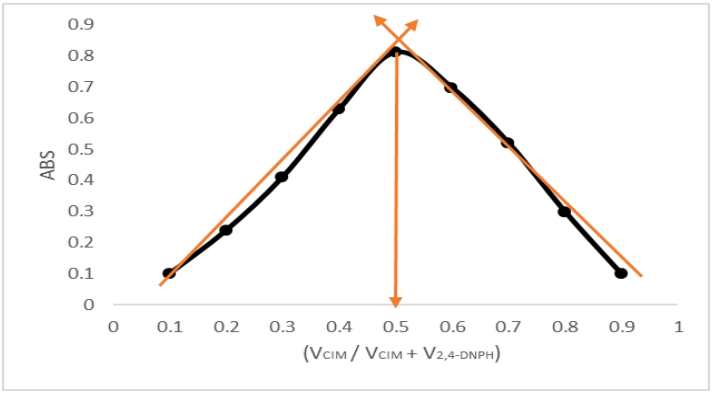


Figure (10): The Continuous Variation (Job's Method) Cim Drug With 2,4-Dnph

In the molar ratio approach, 2.25 mL of the standard drug solution in a series of volumetric flasks (10 ml) was transferred, and various volumes of (0.25–0.25) mL of 2.4-DNPH reagent solution, 2.25 mL of potassium Iodate (4.6×10^{-3} M) and 0.25 mL of sodium hydroxide solution were added. Deionized water was used to fill the volumes exactly to the mark, and the absorbance was measured at 586 nm in comparison to the blank reagent. Molar ratio was found to be 1:1. The results are shown in Figure (11) which is in agreement with the Job's method results and the reaction can be represented as in Scheme (1) as below.
the degree of the disintegration for CIM drug was equal to (0.059) the value of the stability constant was equal to (2.364×10^{5}) L. mol^{-1}

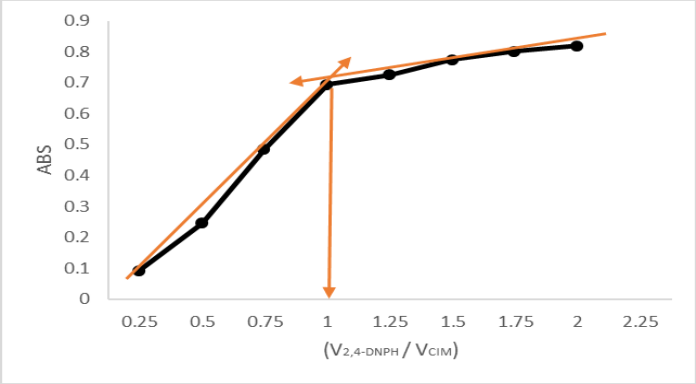
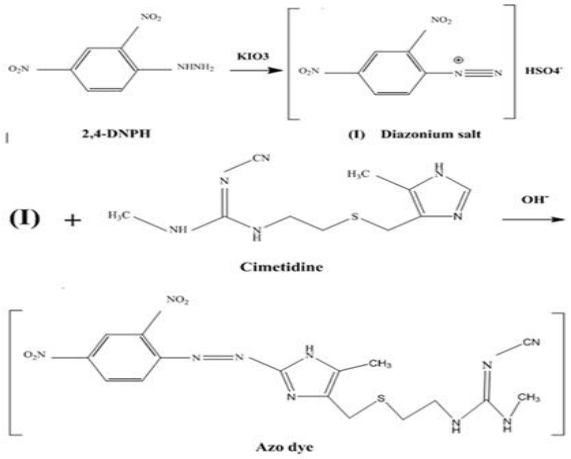


Figure (11): Mole Ratio Of Cim Drug With 2.4-Dnph

Suggest steps for reactions 2.4-DNPH with CIM drug in presence of oxidizing agent and alkaline medium:

The steps for reaction of 2,4 DNPH reagent with oxidizing agent KIO_3 to form azo coupling to react with CIM drug in alkaline medium to form color azo compound as shown in scheme 1..



Scheme (1): Steps For The Reactions 2.4-Dnph With Cim Drug.

Effect of Interferences on the Reaction (2,4-DNPH) with CIM drug

The influence of certain common excipients, starch, sodium lauryl sulfate, and microcrystalline cellulose were evaluated by performing the determination of cimetidine in order to evaluate the analytical potential of the suggested approach. Excipients did not interfere with the experimental procedure, according to experimental data, which are shown in Table (3).

Table (3): Effect Of Interferences On The Reaction (2.4-Dnph) With Cim Drug

Interference	Absorbance
Starch	0
Sodium lauryl Sulfate	0
Micro Crystalline Cellulose	0
Corn Starch	0
Magnesium sterate	0
Povidone	0
Sodium Starch Glycolate	0

Application in Pharmaceutical Preparation of CIM drug

Two real samples with known Cimetidine contents were used to test the effectiveness of the approach under study: an ampoule (2mL/200mg Cimetidine) and a tablet (200 mg Cimetidine/tablet). The adoption of the research method yielded good results, as shown in Table (4).

Table (4): Analytical Applications Of Pharmaceutical Formulation For CIM Drug

Sample	Concentration($\mu g.mL^{-1}$)		Error (%)	Recovery (%)	RSD (%)
	Taken	Found			
200 mg/tablet (Jordan)	200	198	1	99	0.27
200 mg/2ml Ampoule (uk)	25	25.04	0.16	100.16	1.27
	50	49.82	-0.36	99.64	1.73

CONCLUSION

For the quantitative determination of (CIM) in pure form and pharmaceutical preparations, the oxidative coupling reaction between 2.4-DNPH after oxidation was followed by coupling with CIM in alkaline medium was found to be a straightforward, sensitive, exact, and affordable spectrophotometric method. The investigated approach has good linearity and accuracy.

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