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Effect of pH and Temperature on the degradation of ciprofloxacin and ofloxacin in aqueous solution.

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Abstract:

This study aims to evaluate the impact of physical and chemical factors specifically pH and temperature—on the stability and degradation of two fluoroquinolone antibiotics: Ofloxacin and Ciprofloxacin in aqueous solutions. Standard solutions of varying concentrations were prepared, and their absorbance and maximum wavelength (λ max) were measured using a UV-Vis spectrophotometer under different pH and temperature conditions.The results showed that an acidic medium (pH = 4) increased the absorbance of both antibiotics, with slight changes in λ max. Elevated temperatures led to a decrease in absorbance, indicating gradual thermal degradation of the compounds.The study highlights the importance of understanding environmental influences on antibiotic efficacy and recommends further research involving additional factors and longer exposure periods to enhance pharmaceutical stability assessments.

Keywords:

• Ofloxacin ,Ciprofloxacin, Fluoroquinolones, UV-Vis Spectrophotometer , pH Effect ,Absorbance, λ max (Maximum Wavelength)

1-Introduction:

Fluoroquinolones (FQs) are one type of synthesized antibiotics and have different antibacterial activities. Because of their continuous usage in both human and veterinary medicine beside environmental impacts on antibacterial agents are very essential and their potential alterations have decline in efficacy on human health. During storage or transfer of these antibiotic , various chemical and physical conditions, such as temperature, pH, light , chemical compounds, and other factors, have an effect on the chemical structure and efficiency of these antibiotics ***(Hartmut***

,et al .,1987).

1.1. Ofloxacin:

1. According to the IUPAC system, the ofloxacin name is −9−fluoro−2,3−dihydro3−methyl−10−(4−methyl−1−piperazinyl)−7−oxo−7H pyrido[1,2,3de] [1,4]benzoxacine−6−carboxylic acid, it is acarboxylic acid derivative of the quinolone that has a broad antibacterial range against gram−positive and gram−negative bacteria. This compound was discovered to be more active than norfloxacin and pipemidic acid, with antibacterial activity in vitro that was virtually comparable to gentamicin, tobramycin, and newer cephalosporin's. Ofloxacin could only be taken orally when it was first introduced, but a parenteral form was recently created***(National Committee for Clinical Laboratory Standards1997a)***

1.1.2. Chemical Structure of ofloxacin:

The chemical structure of ofloxacin is shown in Figure (1).

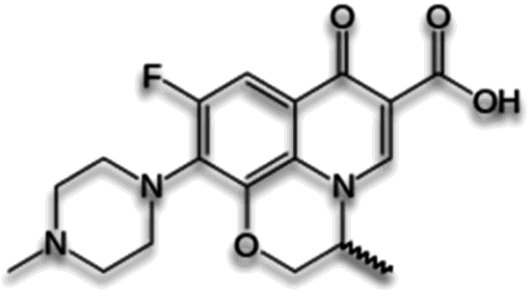


Figure (1): Chemical structure of ofloxacin.

2. Its molecular weight is 361.4 g/mole, the empirical formula is $C_{18}H_{20}FN_3O_4$. Ofloxacin is a crystalline powder that is white to pale yellow in color. The molecule occurs as a zwitterions at low pH values. Ofloxacin relative solubility properties at room temperature, as defined by USP nomenclature, indicate that it is soluble in aqueous solutions with a pH between 2 and 5, little soluble in aqueous solutions with a pH of 7 and freely soluble in aqueous solutions with a pH of 9 or above. It is react with several metal ion generate stable coordination complexes. The following is the formation order of chelating stabilities of complexes with some metals: Fe^{+3}> Al^{+3}> Cu^{+2}> Ni^{+2}> Pb^{+2}> Zn^{+2}> Mg^{+2}> Ca^{+2}> Ba^{+2}.***(Nationa Committee for Clinical Laboratory Standards1997b)***

1.1.3. Medical uses:

Ofloxacin tablets are indicated for the treatment of adults with mild to moderate infections (unless otherwise indicated) caused by susceptible strains of the designated microorganisms in the infections listed below:

3. Haemophilus influenza or *Streptococcus pneumonia*related acute bacterial exacerbations of chronic bronchitis.
4. Pneumonia caused by Haemophilus influenza or *Streptococcus pneumonia* acquired in the community.
5. Methicillin-susceptible: *Staphylococcus aureus, Streptococcus pyogenes*, or Proteus mirabilis infections of the skin and skin structures.
6. Neisseria gonorrhea urethral and cervical gonorrhea with no complications.***(National Committee for Clinical Laboratory Standards1997b)***

1.2. Ciprofloxacin:

Ciprofloxacin belongs to the Fluoroquinolones class of antibiotics. The types of fluorinated quinolone, norfloxacin and other members of these classes have been developed, including ciprofloxacin, which has a wide range of clinical applications, better safety profile and good in vitro effectiveness against resistant pathogenic organisms when compared to other antibiotic classes.(***Herrlin., et al 2000).***

Fluoroquinolones are bactericidal drugs that damage bacteria based on their AUC/MIC. They are efficient against Gram-negative bacteria and certain mycobacteria in general. ***(Johnson et all.,2006)***

1.2.1. Chemical Structure of ciprofloxacin:

The chemical structure of ciprofloxacin is shown in Figure (2).

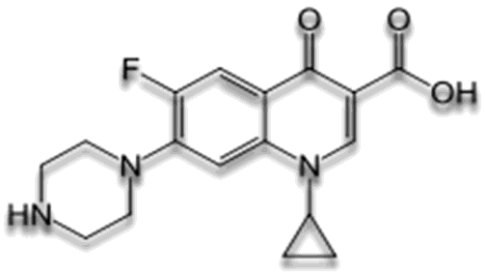


Figure (2): Chemical structure of ciprofloxacin.

Ciprofloxacin has the chemical formula of $C_{17}H_{18}FN_3O_3$ and a molar mass of 331.4g/mol. According to IUPAC system its chemical name is :

1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo7-(1-piperazinyl)-3- quinoline carboxylic acid . At room temperature, the substance occurs in crystalline form with a bright yellow color. ***(Hossen, et all.,2012).***

After oral dosing, Ciprofloxacin is efficiently absorbed from the gastrointestinal system, with a serum protein binding of 20 to 40%. The drug's absolute bioavailability is over 70%, and it is unaffected by first- pass metabolism.***(Lister, P. D.& Sanders,1999).***

1.2.2. Medical uses:

Ciprofloxacin is also used to treat infections such as typhoid fever (enteric fever), urinary tract infections, chronic bacterial prostatitis, acute exacerbations of chronic bronchitis, skin and skin structure infections, complicated intra-abdominal infections, acute uncomplicated cystitis in females, acute sinusitis, bone and joint infections, infectious diarrhea, and lower respiratory tract infections . Ciprofloxacin is frequently prescribed in veterinary medicine for respiratory tract infections, gastrointestinal tract infections, and urinary tract infections in poultry ***.(Halling et all.,2000).***

1.3-Literature reviews:

The study of some factors which affecting on drug stability were taken place in many studies. Some of previous works and their results can be summarizing as following:

Within the consider recorded that the photodegradation responses of FQ followed first arrange energy which in freshwater and saline water. The ponder too ponder the photo debasement and energy of FQ at diverse pH values. The comes about recorded that at pH values of(5 - 11) , the rate of debasement increased.***(peres.,etall;2012).***

Smith (2021) conducted inquire about on the steadiness of ibuprofen

at changing pH levels, utilizing mass spectrometry to identify debasement items and analyze the soundness profile.

The consider included planning ibuprofen arrangements at pH levels from 3 to 9 and measuring the debasement over time. Smith found that ibuprofen was most steady at pH 4 to 6, with corruption rates of 5% at pH 4.5 and 6% at pH 6. Noteworthy corruption happened at pH levels underneath 3 and above7, with rates surpassing 15%. The consider prescribed buffering ibuprofen details to preserve a somewhat acidic environment to guarantee medicate soundness, especially for fluid details and suspensions. This inquire about is basic for the pharmaceutical industry to move forward the steadiness and rack life of ibuprofen items, guaranteeing steady helpful impacts. Encourage ponders were reco mmended to investigate the combined impacts of pH and other detailing variables, such as excipients and additive on ibuprofen solidness.

Brown (2020) explored the soundness of vitamin C (ascorbic corrosive)beneath diverse pH conditions utilizing HPLC examination, centering on the corruption energy and potential misfortune of viability. The ponder included planning vitamin C arrangements at pH levels extending from 2 to 8 and putting away them beneath controlled conditions to screen debasement. The comes about demonstrated that vitamin C corrupted quickly in impartial to soluble conditions, with a corruption rate of 25% at pH 7.5 and 30% at pH 8.5. The most noteworthy steadiness was watched at a pH of 2, where the debasement rate was as it were 8%. Brown prompted that vitamin C supplements ought to be defined with acidic excipients to improve their rack life and adequacy. This finding is vital for the dietary supplement industry, as keeping up the steadiness of vitamin C can anticipate the misfortune of its dietary benefits.

The ponder too suggested investigating the affect of pH on other vitamins and cancer prevention agents to create comprehensive soundness profiles for wholesome items.

Wang (2021) inspected the affect of pH on the solidness of ibuprofen in watery solutions ,employing a comprehensive test plan that included UV-Vis spectroscopy to track the hydrolysis of headache medicine into salicylic corrosive.

The think about efficiently shifted the pH of ibuprofen arrangements from 3 to 11and observed the debasement rates over time. Comes about appeared that headache medicine debasement rates were altogether higher at pH levels over 7, with a corruption rate of 20% at pH 9.
The ideal soundness was watched at acidic pH values around 3, where the corruption rate was as it were 5%.

2- Material and Methods

Chemicals and studied anti –biotic :

The chemical used in this study was chemical grad including: .Two types of anti-biotics (Ofloxacin and Ciprofloxacin)were selected in this study and purchased from local Libya pharmacies.

2.1-Apparatus:

Different instruments and equipment's were used and summarized as following:

1. Computerized spectrophotometer type DU 800.
2. pH meter.
3. Digital Heater

2.2- Solutions preparation:

30mg/L of every antibiotic was prepared by dissolved 30mg in 1L distilled water. The solutions were filtered before the measurements.

Ofloxacin (30mg/l) and working solutions:

30mg/l of ofloxacin was prepared by dissolved 30 mg of it in distilled water then completed to 1L volumetric flask. Working (standard) solutions were freshly

prepared by diluting the stock solution with distilled water to obtain the appropriate concentrations of (3, 6, 9, 12, 15, 18, 21 and 24mg/l).

Ciprofloxacin (30mg/l) and working solutions:

30mg/l was prepared by dissolved 30mg of CIP in distilled water then completed to 1L volumetric flask. Working standard solutions were freshly prepared by diluting the stock solution with distilled water

to obtain the appropriate concentrations of (3, 6 ,9, 12, 15, 18, 21 and 24mg/l).

2.3- The procedure:

Two different parameters were applied on the standard solutions to estimate and measurement their effects on the absorbance and λ max values of each one of the studied solutions. The parameters including (Effect of pH , Effect of temperature).

2.4-Effects of Temperature.

The stability of ofloxacin and ciprofloxacin in aqueous solution was studied by applied different values of Temperature (30, 35, 40 and 45^0C) on the standard solutions, then the absorbance values were recorded during different durations of (5,10,15,20 and 25 min).

2.5-Effect of pH:

The Effect of PH values on the working solutions was carried out by used different pH vales (4, 7, 9, and 10 pH). All prepared solutions of the studied antibiotic were mixed with 1ml of different type of pH . The effecting of every pH on the absorbance and (λ **max**)was observed from the

U.V curves. The values of absorbance were compared before and after addition the pH solutions, then the absorbance values were recorded at different times of (5, 10, 15, 20, 25, 30,and 35 minutes).

Software programs:

Origin 0.7 was used for draw the Figures and calculate the kinetic

parameters.

3-Results and Discussion:

The obtained results of this study were divided into different parts according to the selected parameters in this study the results can described as following:

3.1-Ofloxacin:

The standard curve of ofloxacin solutions was shown in Figures of (3&4). The results showed the maximum wave length (λmax) was obtained at (283nm) and the high absorbance value was 1.76 for the concentration of (24 mg/l) before used or addition any parameter.

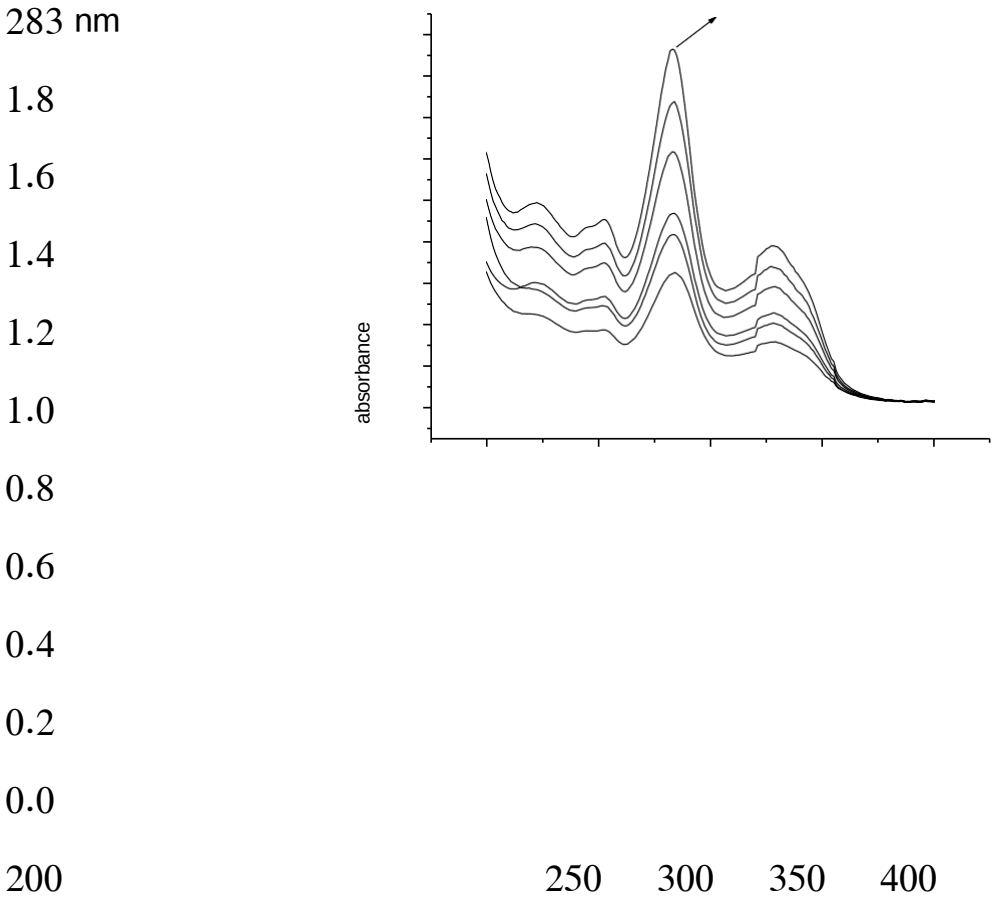
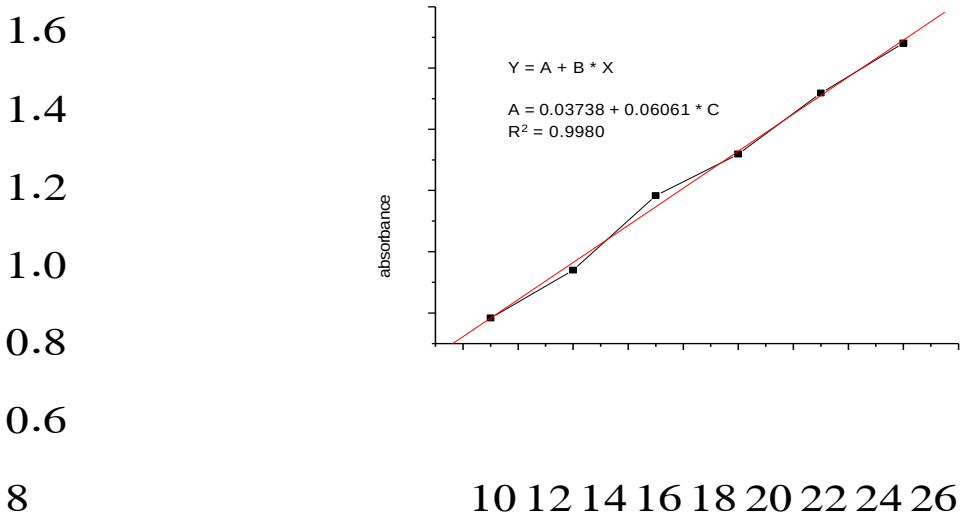


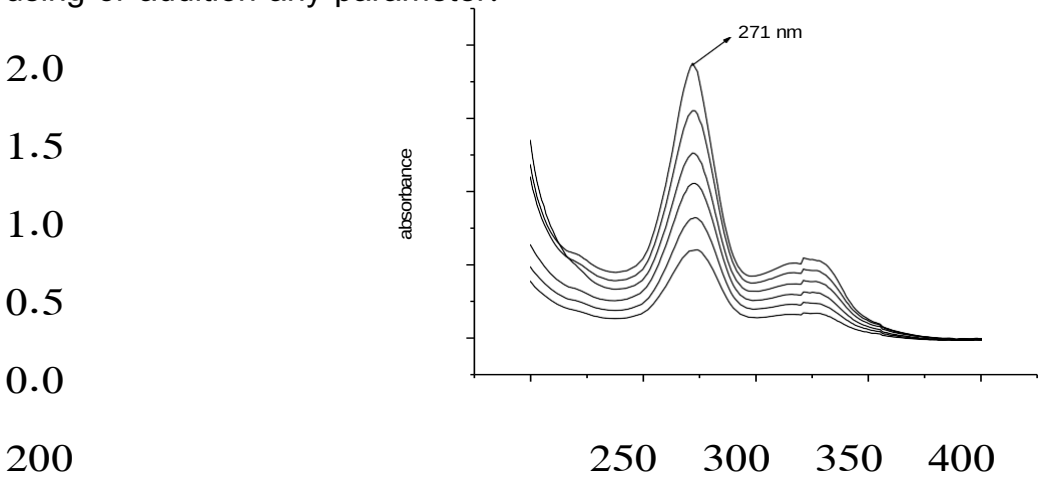
Figure (3): The standard curve of ofloxacin.



wave length (nm) Figure (4): The fit linear of ofloxacin solutions.

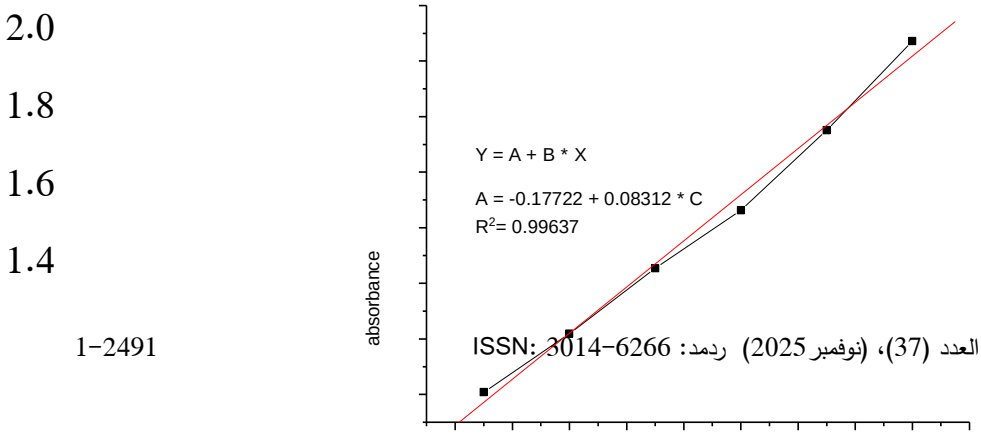
3.2-Ciprofloxacin:

The standard curve of ciprofloxacin solutions was shown in Figures (5&6). The results showed the maximum wave length (λmax) was obtained at (271nm)and the high absorbance value was 1.90 for the concentration of (24 mg/l) before using or addition any parameter.



wave length (nm)

Figure (5): The standard curve of ciprofloxacin.



1.2

1.0

0.8

0.6

8 10 12 14 16 18 20 22 24 26

wave length (nm)

Figure (6): The fit linear of ciprofloxacin solutions.

3.3-Effect of pH:

3.3.1-Ofloxacin:

The effect of PH on the ofloxacin solutions was studied b added different buffer solutions of (4, 7 ,9 and 10) of pH, the wave length and absorbance values were recorded after each addition of buffer solutions to the standard solutions of ofloxacin, the results were shown in the Figures of (**7 & 8)** , where the absorbance value was highly increased in the acidic media of pH (4) , also there is an increase of absorbance at the other buffer solutions, on the other side the wave recorded slight changes to 284 nm.

5

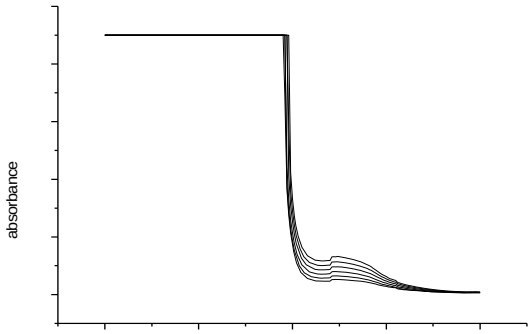
4

3

2

1

0

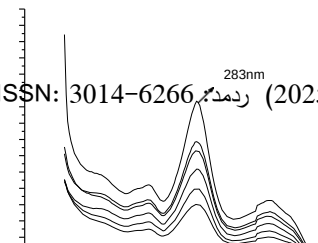


200 250 300 350 400

wave lengt (nm)

Figure (7): Effect of pH= 4 on the absorbance of ofloxacin.

3.2



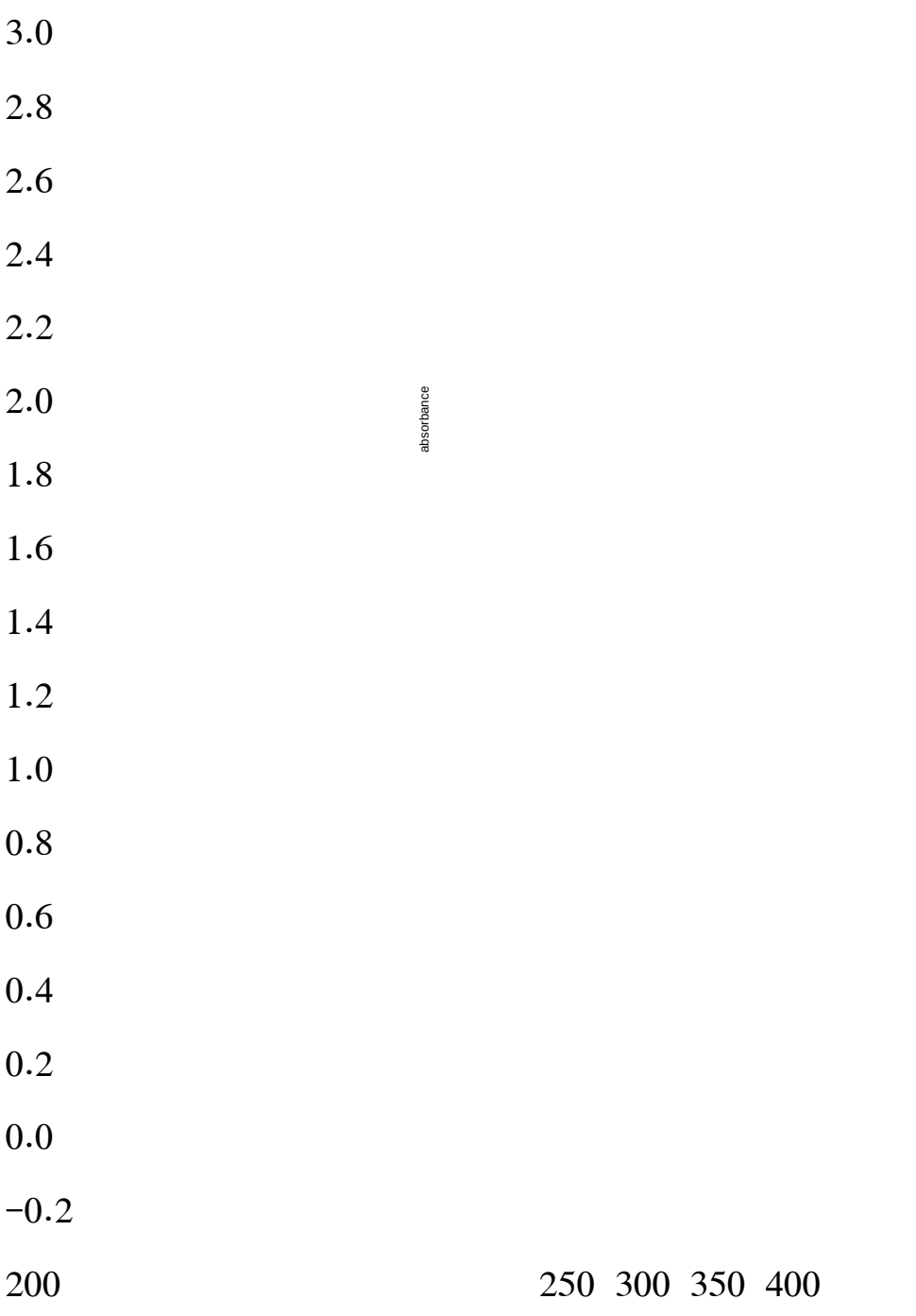
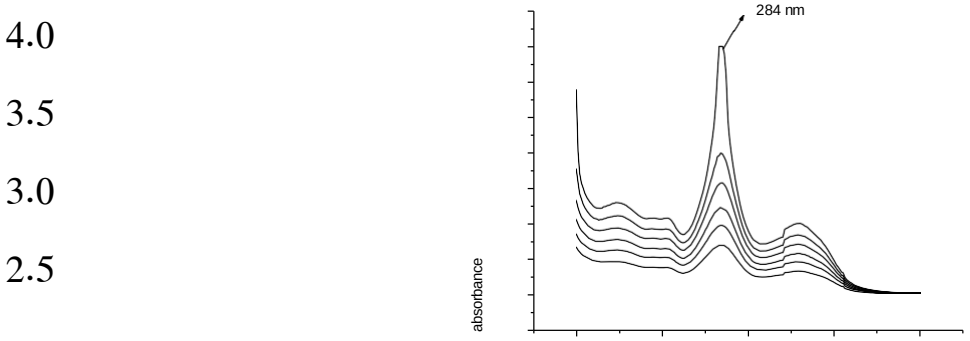


Figure (8): Effect of pH = 7 on the absorbance of ofloxacin.



2.0

1.5

1.0

0.5

0.0

−0.5

200 250 300 350 400

wave length (nm)

Figure (9): Effect of pH= 9 on the absorbance of ofloxacin.

5

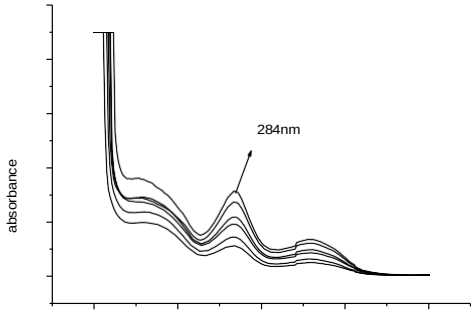
4

3

2

1

0



200 250 300 350 400

wave length (nm)

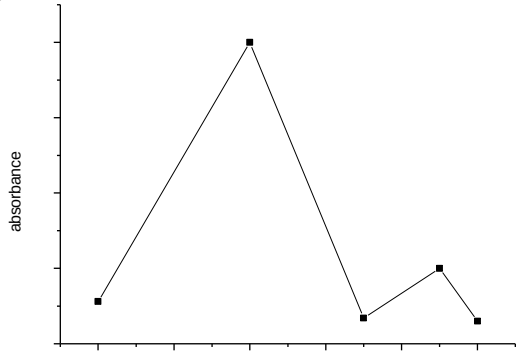
Figure (10): Effect of pH =10 on the absorbance of ofloxacin.

3.5

3.0

2.5

2.0



1.5

0 2 4 6 8 10

pH

Figure (11): Effect of different pH values on the absorbance of ofloxacin (24mg/l).

3.3.2-Ciprofloxacin:

The effect of PH on the ofloxacin solutions was studied by added different buffer solutions of (4, 7 ,9 and 10) of pH, the wave length and absorbance values were recorded after each addition of buffer solutions to the standard solutions of Ciprofloxacin, the results were shown in the Figures of (12 -16) ,where the absorbance value was highly increased in the acidic media of pH (4) , also there is an increase of absorbance at the other buffer solutions, on the other side the wave length recorded slight changes to 267 nm.

5

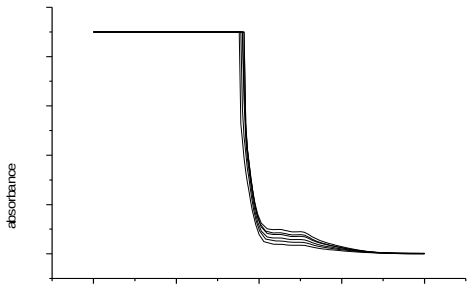
4

3

2

1

0



200 250 300 350 400

wave length (nm)

Figure (12): Effect of pH =4 on the absorbance of ciprofloxacin

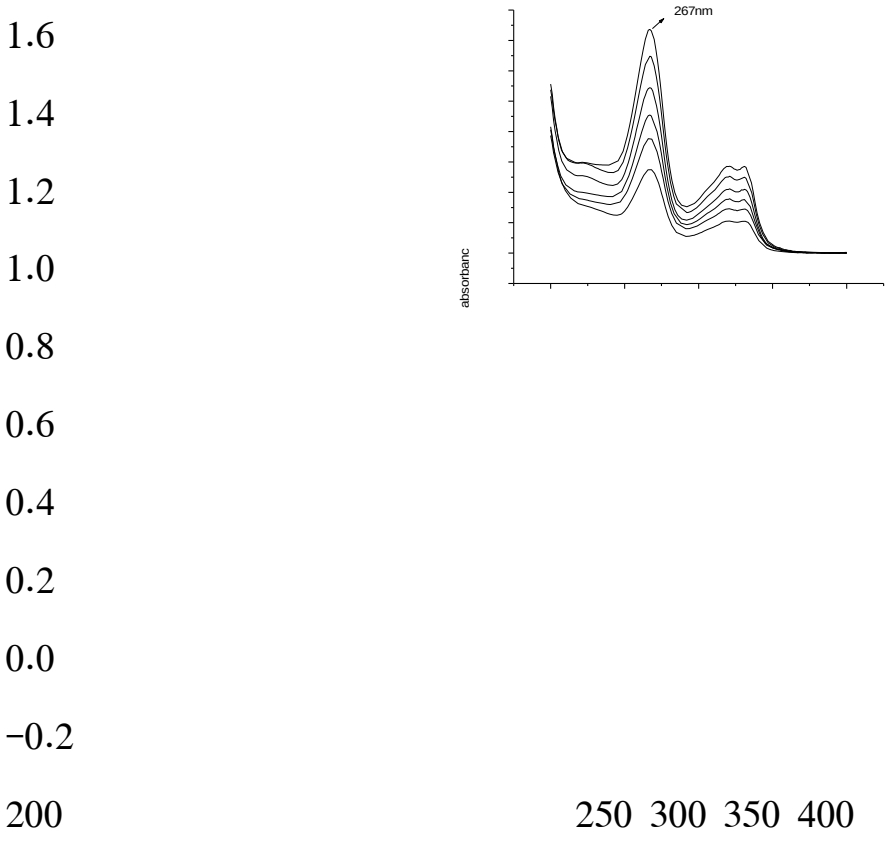
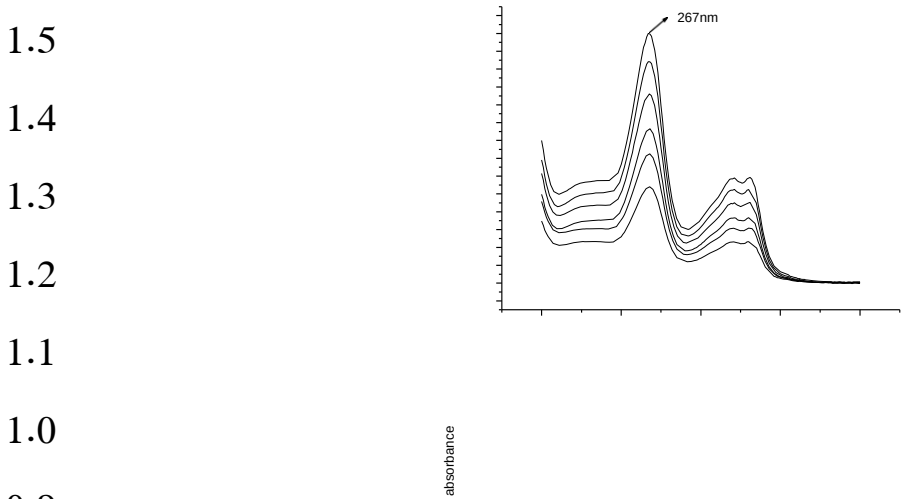


Figure (13): Effect of pH = 7 on the absorbance of ciprofloxacin.



0.6

0.5

0.4

0.3

0.2

0.1

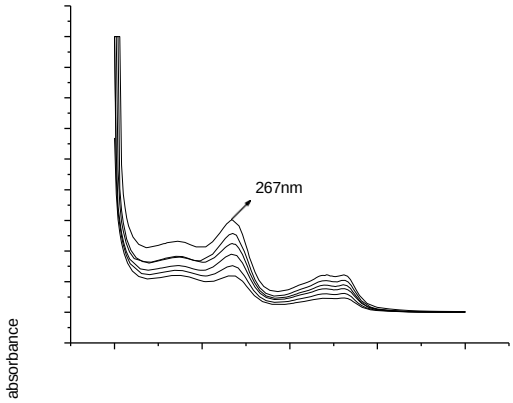
0.0

−0.1

200 250 300 350 400

wave length (nm)

Figure (14): Effect of pH = 9 on the absorbance of ciprofloxacin



5.0

4.5

4.0

3.5

3.0

2.5

2.0

1.5

1.0

0.5

0.0

−0.5

200 250 300 350 400

wave length (nm)

Figure (15): Effect of pH= 10 on the absorbance of ciprofloxacin.

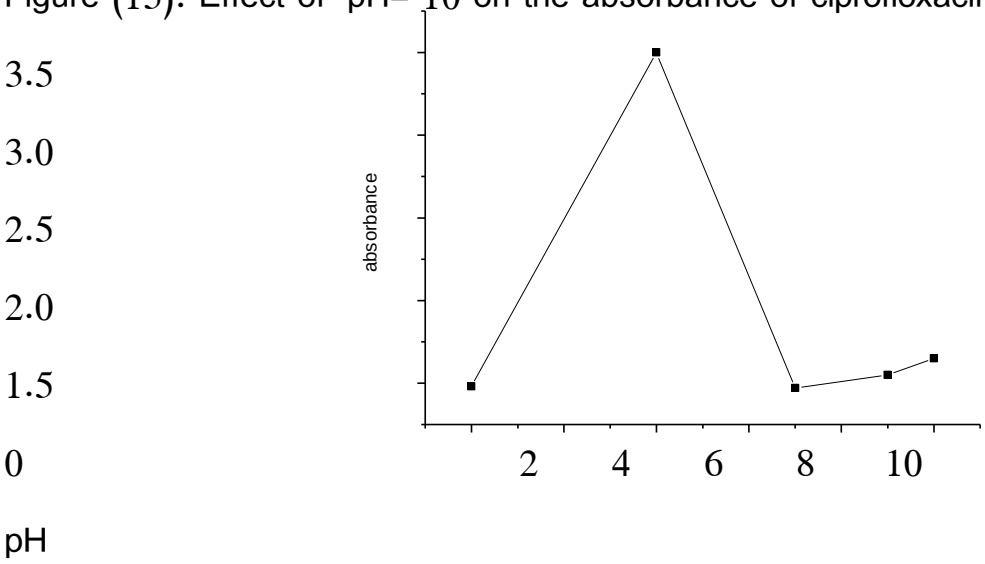


Figure (16): Effect of pH values on the absorbance of ciprofloxacin.

The pH is an important parameter that can affect on photocatalytic reactions. In this view, the photo catalytic degradation of ofloxacin and as amphoteric Fluoroquinolones, CIP has zwitterions ion functional group, namely, piperazine moiety with positive charge and carboxyl group with negative charge, which can influence the physicochemical properties. CIP exists as protonated or deprotonated form, and only at neutral pH could CIP reach the new potential points.

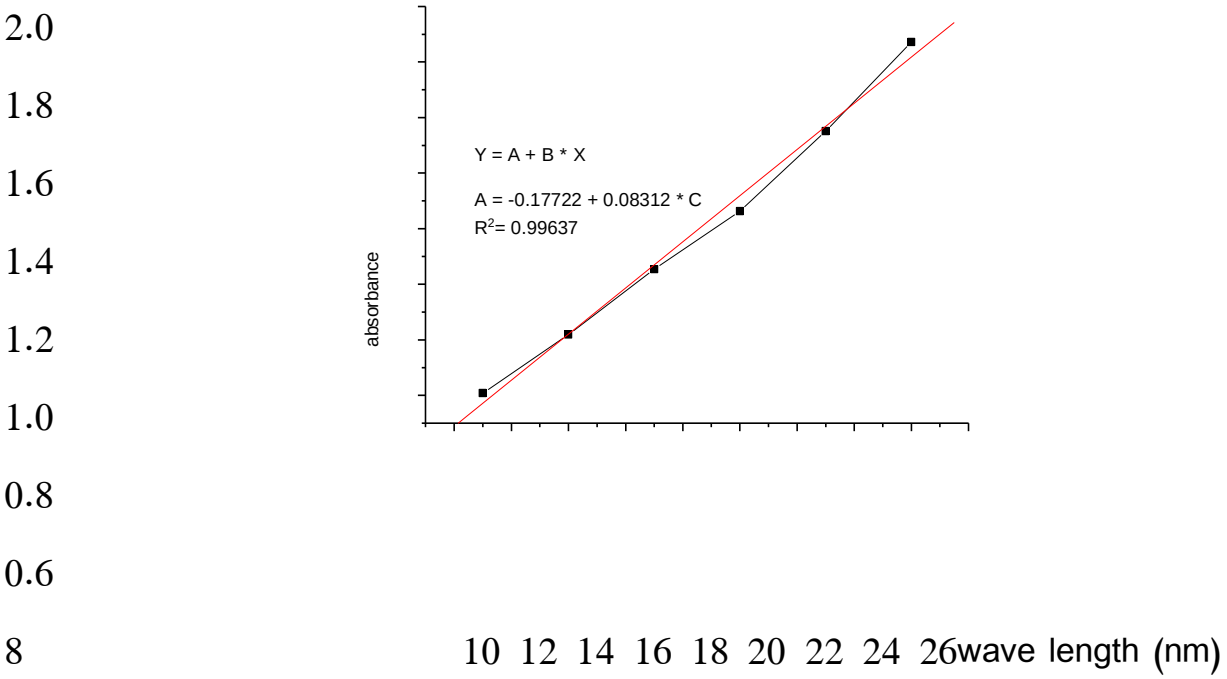


Figure (17): The fit linear of ciprofloxacin solutions.

2-Effect of Temperature on the studied solutions:

2.1-Ofloxacin:

Different values of temperature were applied on the standard solutions of the selected anti –biotic to study the effect of it on the (λ max) and absorbance values. The results were shown in Figures of (18 – 22), and recorded that, there is effect of the temperature on the absorbance values at each of temperature degree which used. The absorbance values of standard solutions were ranged between (0.6 – 1.76) before effect of temperature. The values of absorbance were decreased after heating the solutions at ($30–45^0$C) were the high absorbance values decreased to (1.35) for the highest concentration of Ofloxacinof (24mg/dl). Also the results showed small changes in (λ max) values, where the (λ max) values were (283, 283, 284 and 284 nm) after heating the solutions at temperature degrees of (30, 35 , 40 and 45 ^{0}C).A slight increase of (λ max) values with the temperature of heating increasing.

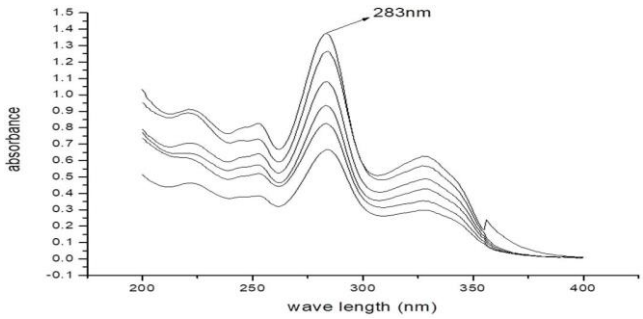


Figure (18): Effect of temperature at (30^0C) on (λ max) and the absorbance of ofloxacin.

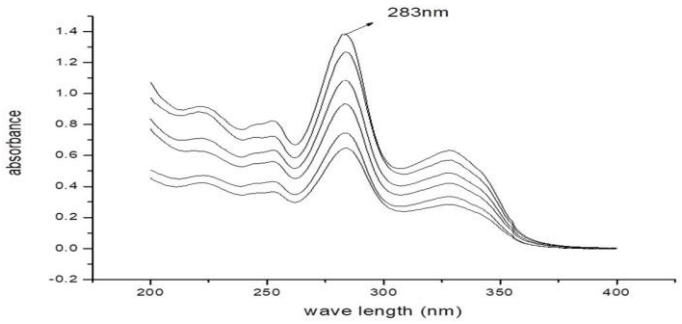
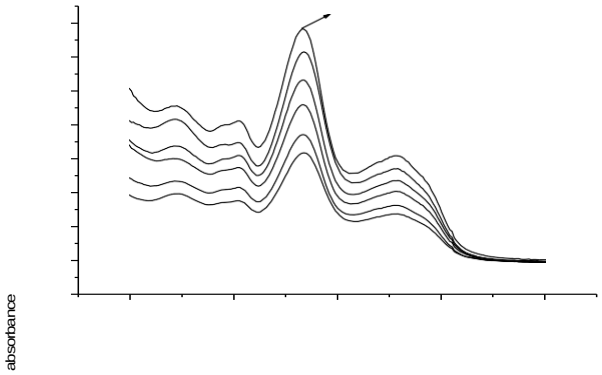


Figure (19): Effect of temperature at (35^0C) on (λ max) and the absorbance of ofloxacin.

284 nm



1.4

1.2

1.0

0.8

0.6

0.4

0.2

0.0

−0.2

200 250 300 350 400

wave length (nm)

of temperature at (40^0C) on (λ max) and the absorbance of ofloxacin.

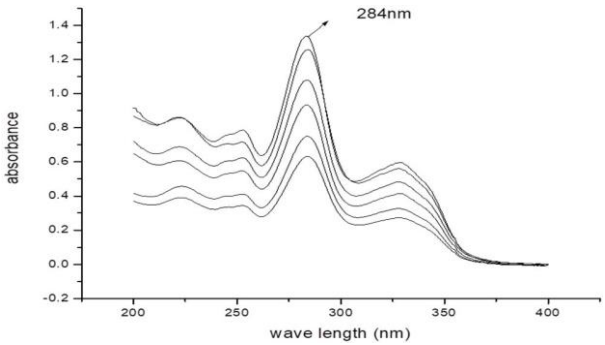
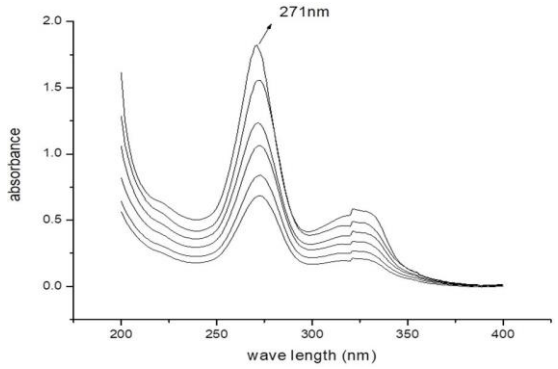


Figure (21): Effect of temperature at (45^0C) on (λ max) and the absorbance of ofloxacin.

2.2- Ciprofloxacin:

The values of absorbanceand (λmax)at each heating temperature values of (24mg/dl) of ciprofloxacin solutions where recorded ,the results showeddecreasing of the absorbance value of (1.90) to less different values at the used temperature values, the results were given in the Figures of (22 -26).Also the results showed not changes in (λ max) values, where the(λ max) values were (271, 271, 271 and 272 nm) after heating the solutions at temperature degrees of (30, 35 , 40 and 45 ^{0}C).A slight increase of (λ max) values with the temperature of 45 ^{0}C.



e (22): Effect of temperature of (30^0C) on (λ max) and the absorbance of Ciprofloxacin.

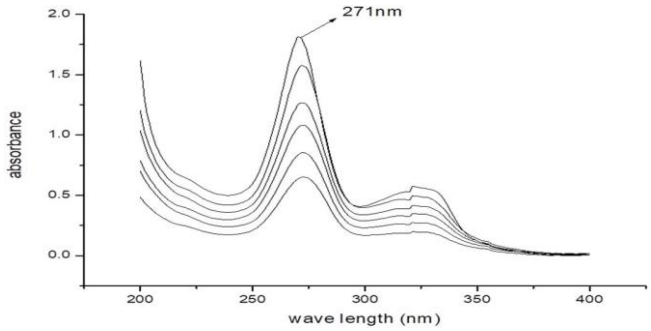


Figure (23): Effect of temperature at (35^0C) on (λ max) and the absorbance of ciprofloxacin.

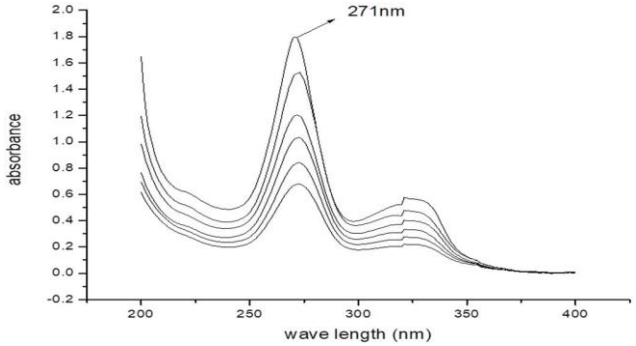


Figure (24): Effect of temperature at (40^0C) on (λ max) and the absorbance of ciprofloxacin.

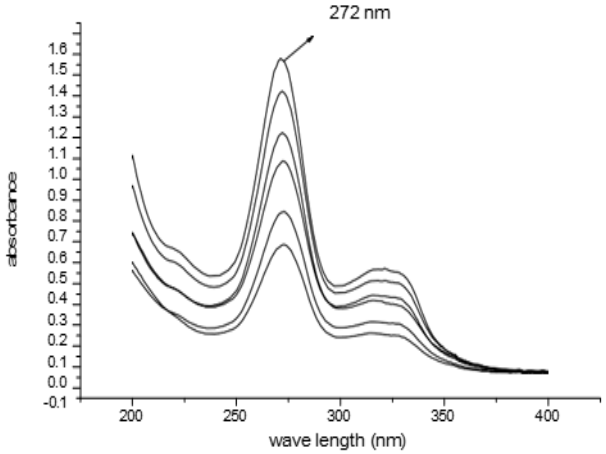


Figure (25): Effect of temperature at (45^0C) on (λ max) and the absorbance of ciprofloxacin.

Conclusion:

This study demonstrated that both pH and temperature significantly influence the stability and degradation behavior of Ofloxacin and Ciprofloxacin in aqueous solutions.

Acidic conditions (pH = 4) enhanced the absorbance of both antibiotics, suggesting increased solubility or stability, while elevated temperatures led to a decline in absorbance, indicating thermal degradation.

These findings underscore the importance of controlling environmental conditions during the storage and application of fluoroquinolone antibiotics to preserve their efficacy.

Further research is recommended to explore additional environmental factors, longer exposure durations, and real-world conditions to better understand the degradation pathways and improve pharmaceutical stability strategies.

Recommendation:

Storage Conditions: Antibiotic formulations containing Ofloxacin and Ciprofloxacin should be stored in cool, temperature-controlled environments to minimize thermal degradation and preserve efficacy.

1. pH Optimization: Pharmaceutical preparations should consider maintaining an acidic pH (around pH = 4) to enhance the stability and solubility of these antibiotics in solution.
2. Packaging Design: Use UV-protective packaging to reduce photodegradation caused by exposure to light, especially for medications stored in transparent containers.
3. Extended Studies: Future research should investigate the impact of other environmental factors such as humidity, light intensity, and ionic strength on antibiotic stability.
4. Clinical Implications: Healthcare providers should be informed about the influence of environmental conditions on antibiotic potency to ensure proper handling and administration.

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