RESEARCH ARTICLE

Determination of Ofloxacin in Pure and Pharmaceutical Formulation using Reagent 2-amino-6-Nitrobenzothiazole by Visible Spectrophotometric Method

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Received: 19th March, 2021; Revised: 16th April, 2021; Accepted: 25th May, 2021; Available Online: 25th June, 2021

ABSTRACT

A Simple, accurate, and highly sensitive spectrophotometric method has been developed to rapidly determine ofloxacin (OFX) in pure form and pharmaceutical formulations. The visible spectrophotometric method was based on forming an Orange Yellow-colored complex between the OFX and 2-amino-6-nitrobenzothiazole (ANB) at pH 5.2 and 80 ± 5ºC in 5 minutes, and λ_{max} at 442 nm. The effects of analytical parameters on the reported system were investigated. Beer's law was obeyed in the concentration range of 1.80-86.72 µg/ mL, with a percentage relative standard deviation not exceed ± 2.54, and the molar absorptivity coefficients were 3.952×10^3 (L/mol.cm). Interferences of the other ingredients and excipients were not observed; the results showed that the developed method with the accuracy and sensitivity highs.

Keywords: Determination Ofloxacin, Complex formation, Spectrophotometry

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.2.20

How to cite this article: Mhemeed AH. Determination of Ofloxacin in Pure and Pharmaceutical Formulation using Reagent 2-amino-6-Nitrobenzothiazole by Visible Spectrophotometric Method. International Journal of Drug Delivery Technology. 2021;11(2):351-357.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Ofloxacin is a broad-spectrum antibiotic belonging to the second-generation fluoroquinolone group known by its brand name Floxin. It is used as an antibacterial and in the treatment of certain types of bacterial infections, and it is believed that it works to inhibit the enzyme deoxyribonucleic acid (DNA) and thus prevent its synthesis.[1-4] It is a white, crystalline, odorless powder that dissolves in water and Dimethylsulfoxide (DMSO) to a weak degree in methanol and methylene chloride. Its chemical formula $C_{18}H_{20}F_1N_3O_4$ and its molecular weight 361,368 g/mol.

Several studies have been published relating to the determination of OFX, both in its pure state and in its liquid pharmaceutical preparations, as determined by the capillary electrophoresis method.[5-7] Sriveda and others identified it with the high-performance liquid chromatography (HPLC) method,[8-10] when using the batal plate and colleagues, a spectroscopic method was used to determine ofloxacin based on the formation of a pink complex between OFX and citric acid with anhydrous acetic acid it has an absorption peak at the wavelength of 552nm.[11] Issa applied this method to determine OFX by using the reagents for bromophenol BPB, bromothymol BTB and purple bromocresol BCP,[12] Sastry also determined it using reagents Tropaeolin and Supracene Violet 3B and Ce(IV)-MBTH,[13,14] hosker, and colleagues also applied a spectroscopic method with a reagent N-bromosuccinimide[15] brachynth and colleagues determined it according to a spectroscopic method using reagents black and orange aerochrome and 3-methyl-2-penthosolanone hydrazine[16] a simultaneous spectroscopy method was used to determine afloxacin in pharmaceutical preparations.[17,22] In this study, ofloxacin was determined in its pure state and in its pharmaceutical preparations by a new easy and fast spectroscopic method by forming a complex between ofloxacin OFX and the reagent 2-amino-6-nitro–benzothiazole. An organic reagent is not previously used to determine ofloxacin and then to measure its color complex absorbance in the visible spectrum. This method is characterized by being easy and simple with good accuracy and sensitivity.

EXPERIMENTAL

Apparatus

The following devices were used in this research: UV Vis Spectrophotometer Korean – made model (SP3000 OpTMA), Sensitive balance from the German company Sartorius Model 2474, PH meter from company Sartorius model PB -11, Pipettes of variable size with capacities 10 µL and 1000 µL and 5000 µL from company Eppendorf, and Volumetric glass pipettes and flasks.

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MATERIALS AND SOLVENTS USED

Preparation of the Main Standard Solutions

Regulating Solutions

The following structured solutions were prepared according to sources,[23-25]

Breton regulator: It was prepared at a concentration of 0.2 M by dissolving 12.37 gm. boric acid in dipole distillation water, in addition to 13.72 mL concentrated phosphorous acid and (11.44) mL from acetic acid and completing the volume with distilled water in a standard flask of 1 L capacity. From the previous solution, a series of Breton buffer solutions was prepared in which the medium pH value ranges between 12–2 by taking 50 mL of a Briton buffer solution and adding the sodium hydroxide solution until the required PH value was reached in a volumetric flask with a capacity of 100 mL and complete the volume to the mark with distilled water.

Stearate Organizer: It is prepared at a concentration of 0.2 M by dissolving 42.03 gm of citric acid with water in a 1 L capacity flask. Take 50 mL of this solution in a 100 mL flask and adjust the specific PH value with sodium hydroxide (5) M and complete the volume to the mark with distilled water.

Borat Regulator: Several solutions of borate regulator were prepared by adding the appropriate amount of sodium hydroxide solution of 0.1 M to 50 mL of a mixture containing boric acid and potassium chloride, each concentration of 0.1 M, then complete the volume to the mark with distilled water.

Ammonia Regulator: Several solutions of ammonia regulator in flask 100 mL By adding the necessary amount of ammonium hydroxide solution to 25 mL of 0.1 M from 0.1 M ammonium chloride solution, then complete the volume to the mark with distilled water.

Ofloxacin Solution: The solution was prepared at a concentration of 1.10^{-3} M by dissolving (36.13) gm of pure ofloxacin in a quantity of DMSO and then supplementing the volume with the solvent used to the limit of the mark in a 100 mL volumetric vial.

Reagent Solution 2-amino-6-nitrobenzothiazole: The solution was prepared at a concentration of (1.10^{-3}) M by dissolving a suitable weight of the reagent in DMSO and then completing the volume with the solvent used to the limit of the mark in a 100 mL volumetric vial.

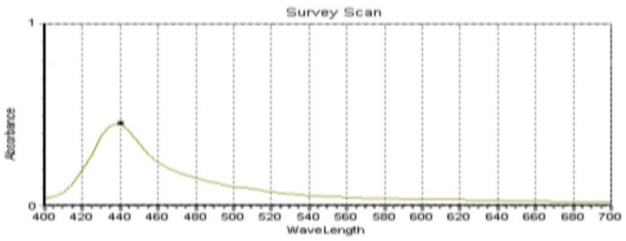

Figure 1: Absorption spectrum for a complex (Ofloxacin with Reagent)

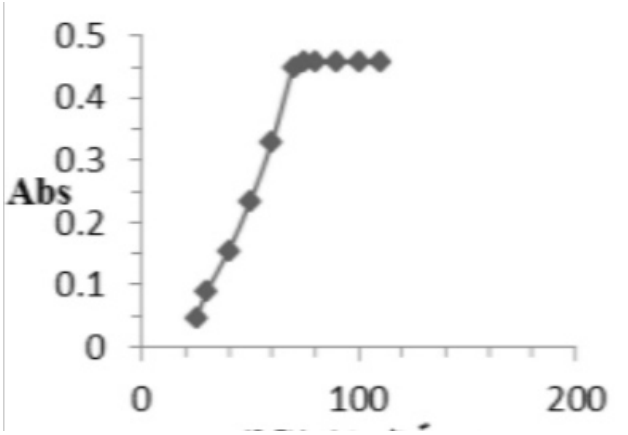

Figure 2: The effect of temperature on absorbance complex at wavelength 442nm

RESULTS AND DISCUSSION

Ofloxacin forms with the reagent a fixed complex that has an absorption peak at the wavelength of 442 nm compared to the reagent solution, which has a negligible absorbency, as shown in Figure 1.

The optimal factors and conditions required in the formation of a complex between ofloxacin and the reagent were studied by changing one indication each time and confirming the other. To achieve this, the following studies were conducted:

1. Study the effect of both temperature and heating time on complex formation

The optimum temperature was determined for the complex to be formed as it was observed that the complex formed by a degree 80 ± 5°C (Figure 2). A series of 10 mL volumetric flasks was prepared and transferred to each of the 1.2 mL of ofloxacin at a concentration of 10^{-3} M and 2.4 mL of 2-amino-6-nitrobenzothiazole reagent at a concentration of 10^{-3}M and in the presence of 1 mL of the peritoneal buffer solution at PH= 5.2. Complete the volume to the mark with the solvent used and then heat each of the complex solutions formed in a water bath at different temperatures. The heated solutions absorbance was measured after being cooled to the estimated laboratory temperature 25 ± 2°C; compared with the reagent solution prepared by the same method, except for the medicinal compound ofloxacin at wavelength 442 nm. The relationship between the absorbance values of the formed complex at the wavelength 442 nm was drawn in terms of temperature. The curve is shown in Figure 2 was obtained. It is noticed from this figure that heating to a temperature of 80 ± 5°C gives the best absorbency and stability of the formed complex. The study effect of the heating time at temperature 80°C on the absorbance of the complex formed, prepared a series of standardized solutions of the complex according to the same previous method. Then its temperature was fixed at the temperature of 80°C by a water bath. It was shown different heating times at the mentioned temperature. The heated solutions absorbance was measured after being cooled to the estimated laboratory temperature 25 ± 2°C. In comparison with the reagent solution prepared by the same method, except for the medicinal compound ofloxacin at wavelength 442 nm, the relationship between the compound's absorption values

formed at the wavelength of 442 nm was plotted in terms of heating time. The curve shown in Figure 3 was obtained. It is noted from this figure that heating for 5 minutes gives the best absorbency and stability of the complex formed at wavelength 442 nm.

2. Study the Effect of Time on the Stability of the Complex Formed

The effect of time on the stability of the complex was studied, where 10 mL volumetric flask was taken to transfer 1.2 mL of ofloxacin solution at a concentration of 10^{-3}M, and 2.4 mL of a reagent solution with a concentration of 10^{-3} M and a fixed volume of 1 mL of the peritoneal buffer solution of a concentration of 0.2 M at PH = 5.2. A complete volume to the mark with the solvent was used. Then the solution was heated for 5 minutes at 80°C until the complexity formed then measure the absorbance of the complex solution at different times after cooling it to laboratory temperature 25 ± 2°C at the wavelength 442 nm, the relationship between the absorbance values of the formed complex was drawn in terms of time and the curve shown in Figure 4, was obtained which shows that the time of stability of the complex is 6 hours at the laboratory temperature 25 ± 2°C.

3. Study the Effect of Reagent Concentration

Study effect of changing the reagent concentration on the absorbance of the complex formed by the constant volume and PH value of the peritoneal regulator solution and in the presence of a fixed (3.9–126.75 µg/mL) Where a series of 10 mL flasks were taken, each containing 43.36 µg/mL of ofloxacin and 1 mL of a peritoneal buffer solution with a value of PH = 5.2 and an increasing number of different concentrations from the reagent, then the volume was completed with the solvent used up to the mark, the solutions were heated to a temperature of 80 °C for a period of five minutes after which the absorbance of the solutions was measured after cooling them to the laboratory temperature 25 ± 2°C at the wavelength of 442 nm and then the relationship between the absorption changes of the complex formed in terms of the change of the reagent concentration was noticed from Figure 5 that the concentration of 46.80 µg/mL of the detector concentration is the optimal concentration for the highest value absorption.

4. Study the Effect of Drug Concentration:

Study effect of drug concentration on the absorbance of the complex formed by the constant volume and PH value of the peritoneal regulator solution and in the presence of a fixed (1.8-151.77µg/ mL)**,** Where a series of 10 mL flasks were taken, each containing 46.8 µg/mL of reagent solution and 1 mL of a peritoneal buffer solution with a value of PH = 5.2 and an increasing number of different concentrations from the drug substance then the volume was completed with the solvent used up to the mark, the solutions were heated to a temperature of 80°C for a period of five minutes after which the absorbance of the solutions was measured after cooling them to the laboratory temperature 25 ± 2°C at the wavelength of 442nm and then the relationship between the absorption changes of the complex formed in terms of the change of the drug concentration was noticed from Figure 6 that the concentration of 86.72 µg/mL of the drug concentration is the optimal concentration for the highest value absorption.

5. Study the Volume of the Reagent

A study was conducted on the effect of the volume of the added reagent on the absorbance of the complex formed. A series of 10 mL volumetric flasks were taken, each containing a fixed volume of ofloxacin solution of 1.2 mL at a concentration of 10^{-3}M and 1 mL of a buffer solution with a value of PH = 5.2 and different and increasing quantities of the solution. The reagent at a 10^{-3}M concentration within the range of 0.2-7.5 mL and then complete the volume with the applied solvent to the mark. The solutions were heated to a temperature of 80°C for

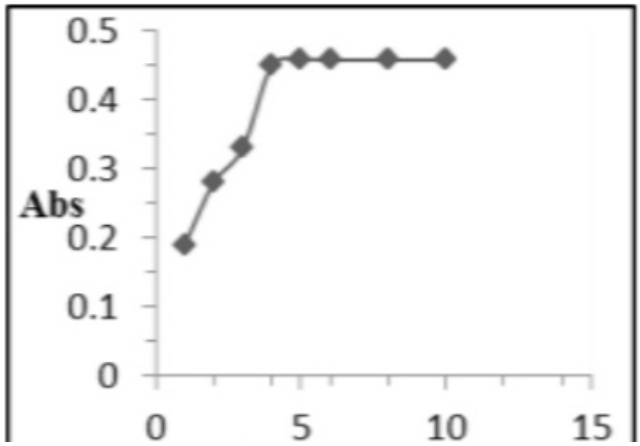

Figure 3: The effect of heating time at 80°C on the absorbance of a complex at wavelength 442 nm

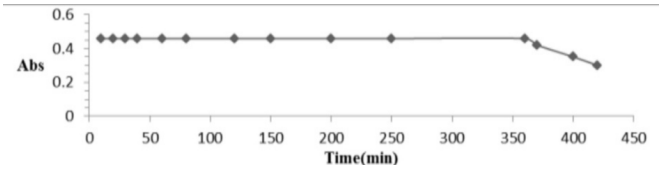

Figure 4: The effect of time on its complex stability

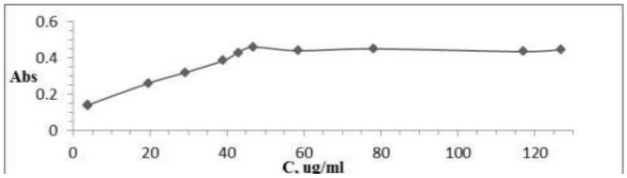

Figure 5: The effect of reagent concentration on the absorbance of the complex formed

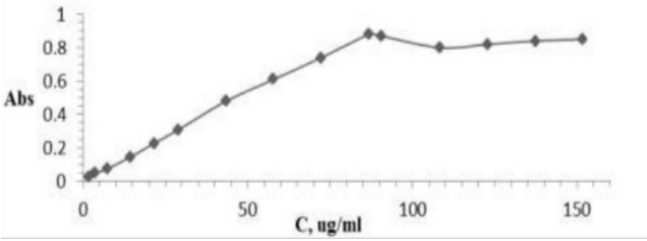

Figure 6: The effect of ofloxacin concentration on the absorbability of the formed complex

5 minutes, after which the absorbance of the solutions was measured after being cooled to the laboratory temperature of 25 ± 2°C at the wavelength of 442 nm, and then the relationship between the absorbance changes of the complex formed in terms of the change in the volume of the added reagent. It is noted from Figure 7 that the volume of 2.4 mL of the reagent solution is the optimal volume for the highest absorption value.

6. Effect of PH Buffer Solution

The effect of some buffer solutions (peritoneal regulator, citrate regulator, borate regulator and ammonia regulator) was studied in the complex formation as it was observed that these buffer solutions gave close results in terms of the length of the absorption wave its absorbance value and the stability of the complex, but the peritoneal regulator solution was the best among those buffer solutions used because it gave the best absorbency at the wavelength of 442 nm for the complex therefore a series of buffer solution were prepared from a Breton regulator within a range of values ranging from PH = (4.6–7) and a diagram of the visible absorption spectrum of the complexes formed between ofloxacin and the reagent in the presence of a constant concentration from ofloxacin 43.36 µg/mL, By taking 1.2 mL of the drug solution at a concentration of 10^{-3}M, adding a fixed volume of reagent solution of 2.4 mL at a concentration of 10^{-3}M and adding a fixed volume of 1 mL of a 0.1 M peritoneal buffer solution, then completing the volume with the used solvent to the mark and heating it to a temperature of 80°C for 5 minutes It was observed to form a yellow-orange complex at the wavelength of 442 nm, so its absorbency was measured for the colored complex formed in each solution after cooling it at the laboratory temperature of 25 ± °C. The relationship between the absorbance changes of the complex formed at the wavelength 442nm for each complex was drawn in terms of the PH value of the buffer solution, and it was found that the ideal value was at PH = 5.2, as shown in Figure 8.

7. Effect of Buffer Solution Volume

The effect of the added volume of the peritoneal regulator solution at PH = 5.2 within the range of (0.2–6.0 mL) on the absorbance of the complex formed where a series of solutions were prepared in volumetric flasks with a capacity of 10 mL and placed in each of them a fixed volume of ofloxacin solution of 1.2 mL in concentration 10^{-3} M and volume constant of reagent solution in concentration 10^{-3}M and variable volumes of the peritoneal regulator solution at PH = 5.2 then complete the volume up to the mark with the used solvent and heat it to a temperature of 80°C for 5 minutes the absorbance of the complex solutions formed after cooling them was measured to a laboratory temperature 25 ± 2°C at the wavelength of 442 nm then the relationship between the absorbance values of the complex formed in terms of the volume of the buffer solution was measured the curve was obtained shown in Figure 9 and it was found that when adding a volume of buffer solution 1 mL the highest absorbance was obtained.

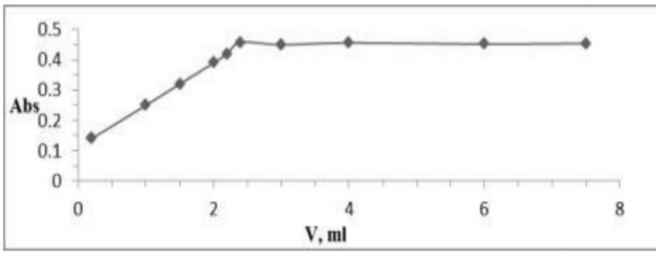

Figure 7: the effect of the volume of the reagent solution on its absorbance of the complex formed

Determining the correlation rate is complex (ofloxacin and the 2-amino-6-nitrobenzothiazole):

The optimal correlation ratio between ofloxacin and the reagent in the staining complex was determined using the continuous change method.[26]

The Continuous Change Method

This method is based on preparing a series of complex solutions formed between the ofloxacin drug and the reagent with changing the concentration of ofloxacin and the reagent concentration. Their total concentration in all solutions remains constant and equals 300 µM. The absorbance values of each solution were determined at the wavelength 442 nm, and the

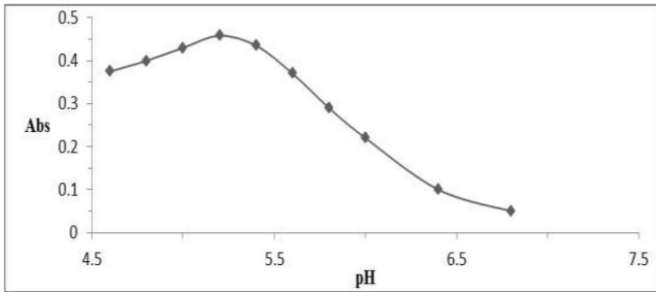

Figure 8: The effect of medium PH on the absorbance of the complex formed

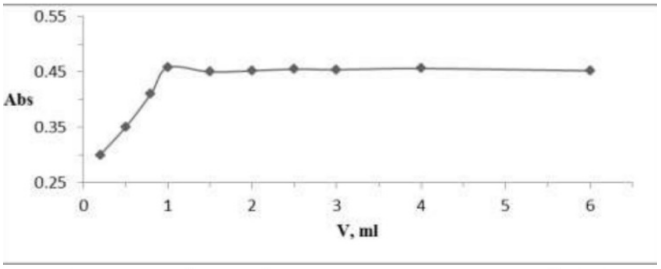

Figure 9: The effect of the volume of the peritoneal buffer solution (PH=5.2) on its absorbance of the complex formed

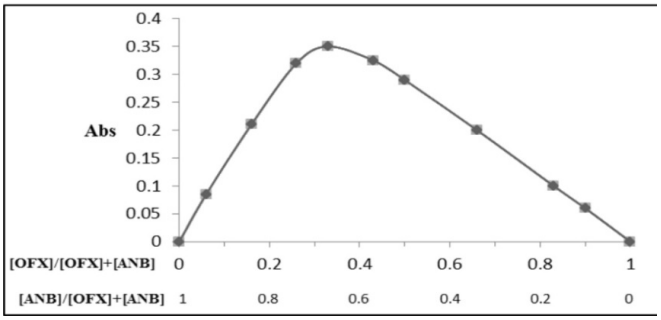

Figure 10: Calculate the correlation ratio of the formed complex by the method of continuous change

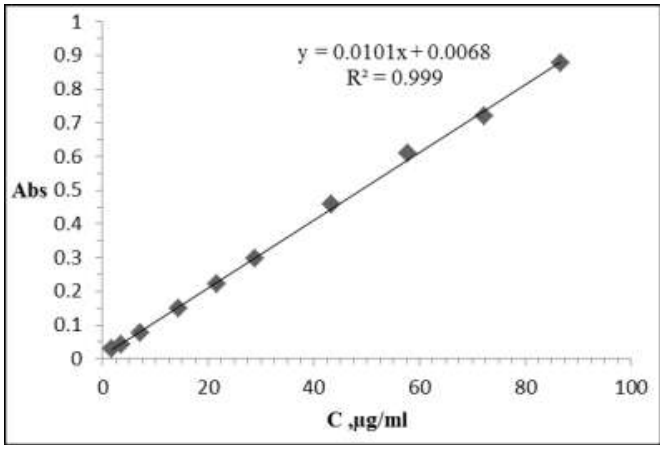

Figure 11: Standard curve of ofloxacin complex with the reagent at wavelength 442nm

Table 1: Quantitative factors for complex (Ofloxacin-reagent)

1.80-86.72	Beers law field µg/ mL
3.952×10^3	The molecular absorption coefficient at the greatest wave length $mol^{-1}cm^{-1}$ L
0.091	Sandals allergy (A= 0.001), $\mu g.cm^{-2}$
440	Great wave length nm
2.54	Percentile relative standard deviation (RSD %)
5.2	PH peritoneal regulator solution
1.71	The logarithm of the conformation constant by continuous change
6	Complex constancy time hours
1:2	Correlation rate between ofloxacin drug and reagent
0.95	Detection limit (LoD) µg. mL^{-1}
3.17	Limit to quantify (LoQ) µg. mL^{-1}
A =mC + b equation of the standard curve	
0.0101	Inclination
0.0068	The intersection (b)
0.999	Correlation coefficient (R^2)

absorbance changes of the complex solutions formed were plotted in terms of the molar fraction [ANB]/[OFX]+[ANB] and [OFX]/[OFX]+[ANB] as shown in Figure 10.

Quantification of Ofloxacin with Reagent the 2-amino-6-nitrobenzothiazole)

Ofloxacin was quantified by spectrophotometric color method within a linear range of concentrations to achieve the beer-lambert law where a series of solutions of the complex was prepared in which the concentration of ofloxacin ranged within the field of 1.80-86.72 µg/mL with a fixed concentration of the reagent 2-amino-6-nitrobenzothiazole 46.8 µg/mL, the absorbance values of the studied complex were recorded at the wavelength 442 nm. The relationship between the absorbance of the formed complex and the concentration of ofloxacin was plotted in Figure 11.

According to the standard data, the median value of the molecular absorption coefficient of the complexes, formed at the wavelength 442 nm, was calculated based on the beer-lambert law, sandals sensitivity S and the detection limit, the correlation coefficient R^2 and the intersection point of the titration curve with the absorbance axis b and the slope m for standard data were also determined as shown in Table 1.

A comparison was made between this spectrophotometric method and the reference methods used worldwide, as shown in Table 3, where it was noticed that this method converges with the results of the reference methods.

Determination of Ofloxacin in Pharmaceuticals using Reagent 2-amino-6-nitrobenzothiazole

The spectroscopic method developed in this research was used to determine ofloxacin in its pharmaceutical preparations (tablets) using a reagent 2-amino-6-nitrobenzothiazole; the results indicated that the determination of ofloxacin in its pharmaceutical preparations is not affected by the presence of the adjuvants as shown in Table 4.

Table 2: Estimating the accuracy and validity of the proposed spectral method for quantifying ofloxacin using a reagent

Percentage Return R % (Retrospective)	*Limit of confidence* $x \pm [txSD/(n)^{1/2}]$	*Analytical measurement error* $SD\backslash(n)^{1/2}$	*RSD %*	*standard deviation SD*	*Specific focus * (µg. mL^{-1})*	*Focus taken (µg. mL^{-1})*
98.33	1.77 ± 0.055	0.020	2.54	0.045	1.77	1.80
99.16	3.58 ± 0.063	0.023	1.54	0.052	3.58	3.61
99.03	7.15 ± 0.127	0.046	1.44	0.103	7.15	7.22
98.13	14.18 ± 0.610	0.220	3.47	0.493	14.18	14.45
100.59	21.81 ± 0.810	0.292	2.99	0.653	21.81	21.68
98.71	28.53 ± 0.904	0.326	2.56	0.731	28.53	28.90
98.50	42.71 ± 1.104	0.398	2.08	0.892	42.71	43.36
99.61	57.59 ± 1.199	0.432	1.67	0.967	57.59	57.81
99.14	71.65 ± 1.712	0.617	1.92	1.38	71.65	72.27
99.44	86.24 ± 2.184	0.787	2.04	1.76	86.24	86.72

* average of five experiments (repeat the experiment five times for each concentration, repeat the preparation of each solution from the standard series five times and measure the absorbance for them at the wavelength 442 nm then calculate the concentration from beers law – lambert or by projecting the absorbance on the standard curve and calculating the specific concentration by taking the average for them).

Table 3: A comparison between the studied method and the reference methods

Reagent name	*Great wave-length λmax (nm)*	*Linear µg/ mL ε = L/mol.cm*	*Reference*
Citric acid-acetic anhydride	552	5 – 55 ε= 6.04 x 10^3	[11]
a) Bromophenol blue	410	5 – 25 ε= 1.04 x 103	[12]
b) Bromothymol blue	415	2 – 15 ε= 2.01 x 103	
c) Bromocresol purple	410	2-20 ε= 1.04 x 103	
a) Tropaeolin 000 (TP 000)	485	2.5-30 ε= 8.24 x 103	[13,14]
b) Supracene Violet 3B (SV3B	575	2.5-25 ε= 1.09 x 104	
2-amino-6-nitrobenzothiazole	442	1.80 – 86.72 ε= 3.952×103	The studied method

Table 4: Determine the amount of ofloxacin in the tablets

Retrospective R %	*RSD%*	*SD*	*The specified quantity mg*	*Specific focus µg. mL^{-1}*	*Focus taken µg. mL^{-1}*	*Amount of ofloxacin mg*	*The name of the preparation*
99.6	2.710	1.35	398.40	49.8	50	400	Azoflox
99.64	0.843	0.84	398.56	99.64	100		

a: average of five measurements (average of five experiments repeat the preparation of the solution five times and measure the absorbance of the solutions at the wavelength of 442 nm and calculate the specific concentration by taking the average for them).

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