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Two Techniques (Spectrophotometric and Turbidimetric) for Determination of Ciprofloxacin HCl in Pharmaceutical Drugs with Comparison between the Techniques

Mohammad K. Hammood*, Jalal N. Jeber, Yasmeen H. Muhamad
Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

A new, effective, simple and inexpensive method was developed for determination the amounts of Ciprofloxacin HCl in solutions and in tablets by spectrophotometry and turbidity using sodium tungstate as reagent. Also, in this paper comparison between two techniques was conducted. The reaction between the Ciprofloxacin HCl and reagent in acidic media formed a yellowish white complex. Chemical and physical conditions have been investigated. The Linear range for spectroscopic and turbidimetric techniques were (**0.05-1.25**) and (**0.05-3.0**) mmol.L^{-1} with correlation coefficients (**0.9956**) and (**0.9941**) respectively. The limits of detection were 7×10^{-5} and 5×10^{-5} mol.L^{-1}. The proposed method is good alternative for determination of Ciprofloxacin HCl in tablets.

Keywords: Ciprofloxacin HCl, Spectrophotometric, Turbidimetric, Cipropharm

تقنيتان لتقدير الدواء السايبروفلوكسسين هيدروكلورايد (طيفية وتعكرية) في المستحضرات الصيدلانية واجراء مقارنة بين التقنيتين

محمد كاظم حمود*، جلال ناصر جبر، ياسمين حكمت محمد

قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد ، العراق

الخلاصة

طورت طريقة جديدة لتقدير دواء السايبروفلوكسسن هيدروكلورايد في المحاليل القياسية وفي المستحضرات الصيدلانية من خلال استخدام تنكستات الصوديوم كعامل مرسب وقياس العكورة بتقنيتي المطيافية الضوئية والتعكرية. تتصف هذه الطريقة بالبساطة والحساسية والسرعة في التقدير. كذلك خلال هذا البحث تمت المقارنة بين التقنيتين. الطريقة تعتمد على تكوين مزدوج ايوني بين المادة الدوائية السايبروفلوكسسين والكاشف تنكستات الصوديوم بالوسط الحامضي اذ يتكون راسب ابيض مصفر. تم دراسة كافة المتغيرات الكيميائية والفيزيائية للتفاعل. كان المدى الخطي لتقدير الدواء السايبروفلوكسسين هيدروكلورايد (0.05−1.25) مللي مول.لتر$^{-1}$ و (0.05−3.0) مللي مول.لتر$^{-1}$ وبمعامل الارتباط (r) 0.9956 و 0.8841 للقياسات الطيفية والتعكرية على التوالي. اما حد الكشف فقد كان 7×10^{-5} مول.لتر$^{-1}$ للتقنية الطيفية و 5×10^{-5} مول.لتر$^{-1}$ للتقنية التعكرية. الطريقة المقترحة تعد طريقة بديلة لتقدير السايبروفلوكسسين في المستحضرات الدوائية.

1. Introduction

Ciprofloxacin belongs to the antibacterial agents group called Fluoroquinolones. These agents have antimicrobial activity against Gram-positive and Gram-negative aerobic bacteria beside to some activity against rickettsias, mycobacteria, mycoplasma and Plasmodium Falciparum. Ciprofloxacin (CIP) [1-cyclopropyl-6-fluoro-1,4dihydro-4-oxo-7-(piperazinyl) quinolone-3-car-boxylicacid]Figure-1 was developed in research laboratories in 1985.[1,2].

*Email: mohki_276@yahoo.com

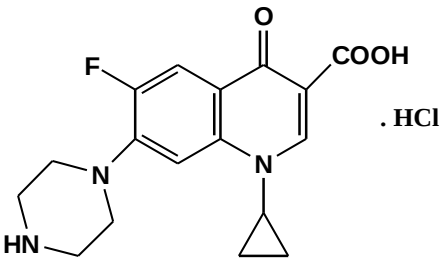

Figure 1- Structure of Ciprofloxacin HCl

This group of antibiotic which considers a synthetic antibiotic has lots of clinical applications in human life such as treatment of digestive, urinary and pulmonary infections. However, the applications of the synthetic antibiotic are not limited only for human but also, it have broadly used in treatment of veterinary diseases of animals [3]. Because of its widely used, many of antibiotic agents were developed for veterinary such as sparfloxacin, danofloxacin and enrofloxacin which are used in treatment of respiratory and enteric bacterial in chicken, cattle and turkey [1]. This compound exhibits antimicrobial activity more than quinoline carboxylic acids compounds, thus using the fluoroquinolones have become widely used to treat many infections [4]. In blood, serum and other biological samples, several techniques have been used to determine the fluoroquinolones compounds, for example: liquid chromatography (LC) [4, 5] and capillary electrophoresis (CE)[6]. The latter technique has very good sensitivity than liquid chromatography but less than high-performance liquid chromatography (HPLC) especially, the CE based on concentrations of compounds [7]. Ciprofloxacin has been determined in drugs using different techniques such as voltammetry [8, 9], biosensor [10] and high-performance liquid chromatography (HPLC) [11, 12]. In addition to another technique called extraction spectrophotometric which used tetrachloro-benzoquinone, p-nitrophenol, bromothymol blue and finally supracenviolet 3B [13, 14]. In this paper a developed method is described to determine trace amounts of Ciprofloxacin in drugs using spectroscopy and turbidity. In addition to comparison between the used techniques which conducted in determination. Also, the proposed method has satisfactorily applied to determine the CIP in tablets.

2. Materials and Methods

2.1. Chemicals, Solutions and Reagents

All chemicals were of analytical grade and distilled water was used in all dilution processes to maintain the purity.

Sodium tungstate ($Na_2(WO_4).2H_2O$) was obtained from Sigma-Aldrich (0.05 $mol.L^{-1}$) was prepared by dissolving 4.1241 g in 250 mL double distilled water, the solution was kept in dark container to protect from light and kept under room temperature.

Pure grade Ciprofloxacin HCl was supplied by Samarra drug industry. Standard stock solution of ciprofloxacin HCl (0.05 $mol.L^{-1}$) was prepared dissolving 0.9645 g in 50 mL double distilled water and stored in dark container at 4°C to maintain the stability for at least one week.

The tablets were commercially purchased containing 500 mg of Ciprofloxacin HCl (Jordan, China). 2-Propanol (anhydrous, 99.5%) (BDH) was used as (10%) solution by transfer 10 mL and completed to 100 mL with water, glycerin (refractive index=n20/D 1.474(lit.), 1.25 g/mL(lit.)), gelatin (BDH) (10%) was prepared by dissolving 10 g in 100 mL water. Hydrochloric acid (ACS reagent, 37%) (1 $mol.L^{-1}$) was prepared by diluting suitable amount in double distilled water and standardized with Na_2CO_3 solution.

2.2. Apparatus and Software

Turbidity Benchtop Meter (HANNA Instruments LP2000, Italy) with cells with 10 mL size was used to conduct all the turbidity measurements. A digital pH meter (HANNA Bench type) was used to make sure that samples have reached to the required pH. While in the timing experiments, a magnetic stirrer was required to shake the samples for a certain time. For spectroscopic measurements, UV-visible double beam spectrophotometer device (Shimadzu, type 1800, Japan) was used to measure the samples absorbance. All the Figures were plotted using the software Origin Pro 9.1(Microcal) which allows interactive data analysis and scientific graphing.

2.3. General Procedure

The determination of CIP was depended on precipitation reaction with sodium tungstate in an acidic media to form yellowish white precipitate as an ion pair complex.

In a set of experiments, aliquots (0.05-3 mL) of a stock solution of CIP (10 mmol.L^{-1}) were transferred into 25 mL volumetric flask, then sodium tungtstate 4 mL of 10 mmol.L^{-1} was added to each flask followed by adjusting the pH solution to 1.4 using dilute HCl solution. The turbidity was measured after one minute in turbidimeter and in spectrophotometer at 420 nm. Each solution was assayed in triplicate. The proposed suggested mechanism for ion-pair complex formation is shown in Figure-2.

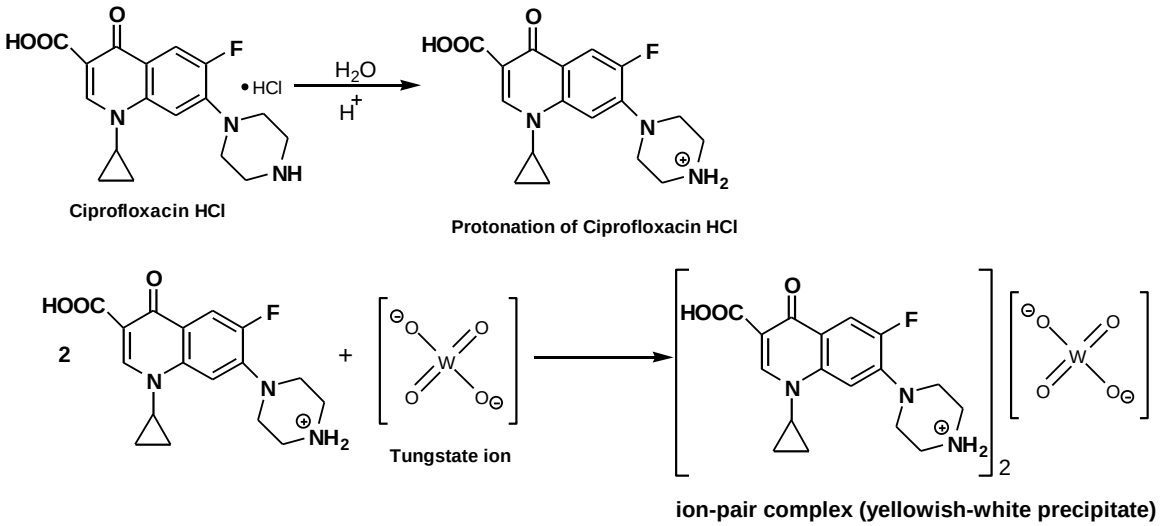

Figure 2- The proposed mechanism of reaction (CIP-Na(WO_4).2H_2O)

2.4. Absorption Study

A dilute aqueous solutions of CIP starting from (0.5 to 2 mmol.L^{-1}) were mixed with sodium tungstate (4 mmol.L^{-1}) as precipitating agent, an intense white precipitate product was formed immediately, the absorbance spectrum showed maximum peak at 420 nm against reagent blank.

2.5. Optimization Conditions

1- Effect of the Reagent (Sodium Tungstate)

For the purpose of finding the optimum concentration of reagent, a series of 0.1, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 mmol.L^{-1} were prepared from 10 mmol.L^{-1} of reagent and added to the 2 mmol.L^{-1} of the Ciprofloxacin HCl solution. The results showed that the optimum concentration of reagent is 4.0 mmol.L^{-1} as shown in Figure-3.

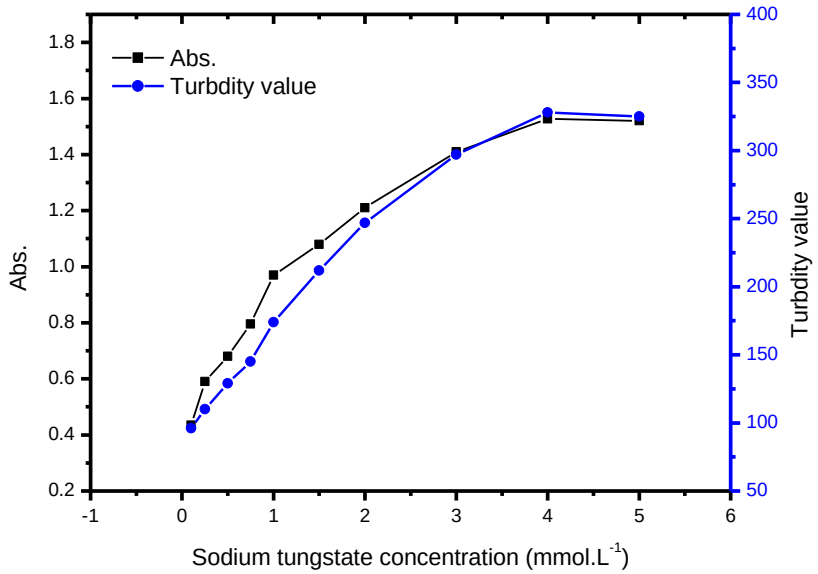

Figure 3- The optimum concentration of sodium tungstate effect for CIP absorbance

2- pH Value Effect

Studying the acidity effect on the precipitation reaction was investigated in this paper. A range of hydrochloric acid concentrations were prepared (0-40 mmol.L^{-1}) and added to the 2 mmol.L^{-1} of CIP with optimum concentration of sodium tungstate at 4 mmol.L^{-1}. Then the pH values were recorded. The results showed that the values of turbidity and absorbance have increased with decreasing of pH values till the range (1.25-1.4) due to acidity which play an important role in protonation of CIP in starting step, but any increase in medium acidic (pH < 1.25) might lead to dissolution of precipitate. Summarizing from above results that the optimum pH range is 1.25-1.4 Figure-4.

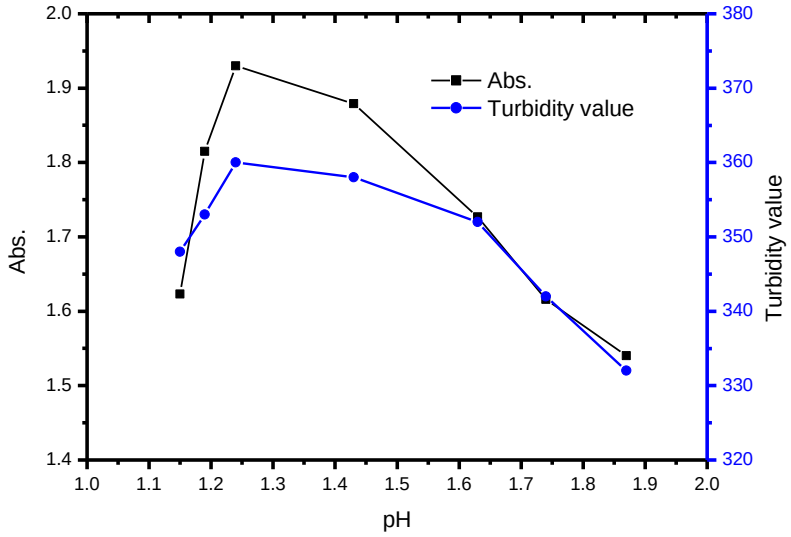

Figure 4- The optimum range of pH.

3- Time Effect

All the previous parameters (2 mmol.L^{-1} CIP, 4 mmol.L^{-1} reagent and pH range (1.25-1.4)) were applied in the time experiment. After addition of precipitating reagent to the standard solution of CIP, turbidity values were measured at interval time (1-60 minutes). The results showed that at time 1 minute, the values of turbidity and absorbance have recorded highest values while after 1 minute time, all the values were decreased with time goes as shown in Figure-5.

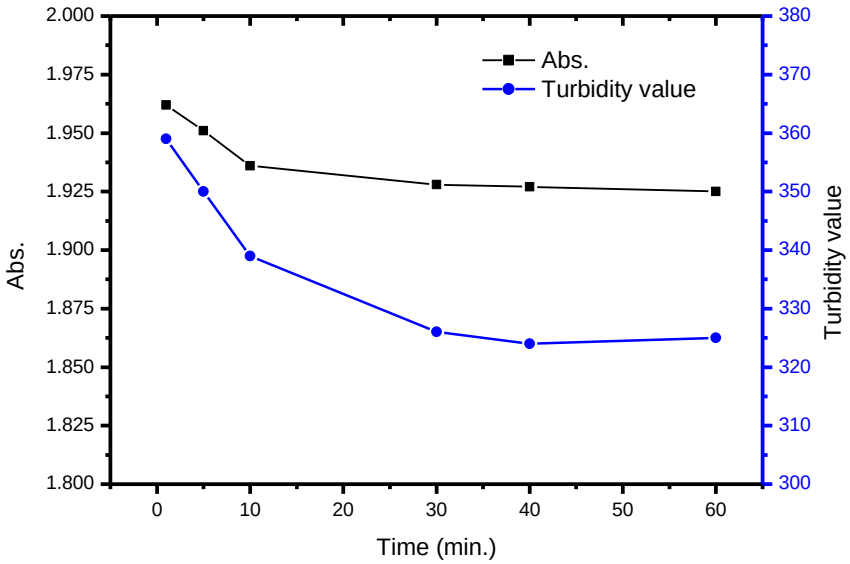

Figure 5- Effect of time on turbidity measurements

4- Effect of Colloidal Protectors

The literature has mentioned using of many chemical materials as protectors for the suspension from sticking at the inner walls of cells which probably lead to error during measuring turbidity values

and help to stabilize the precipitate. Therefore, three types of colloidal protectors 2-propanol, Glycerin and Gelatin were used. The results showed a decrease in turbidity values perhaps due to aggregation of small particles on the colloidal protectors leading to increase light passing to detector Table-1. Thus, deionized water was chosen as optimum solvent for dilution.

Table 1- Values of turbidity and absorbance in presence of colloidal materials

Colloidal material	Absorbance Values			Turbidity Values		
	0.1% v/v	0.5% v/v	1.0% v/v	0.1% v/v	0.5% v/v	1.0% v/v
2-propanol	1.960	1.960	1.950	340	340	333
Glycerin	1.818	1.818	1.822	298	298	280
Gelatin	1.929	1.929	1.906	354	354	347

5- Reaction Stoichiometric

The stoichiometry of the reaction of CIP with $Na_2(WO_4).2H_2O$ was investigated by varying the concentration of drug and reagent using the Job's method [15] under the optimum reaction conditions. The obtained results of these studies are reported in Figure-6, that show the ratio of the reaction between CIP and reagent is (2:1).

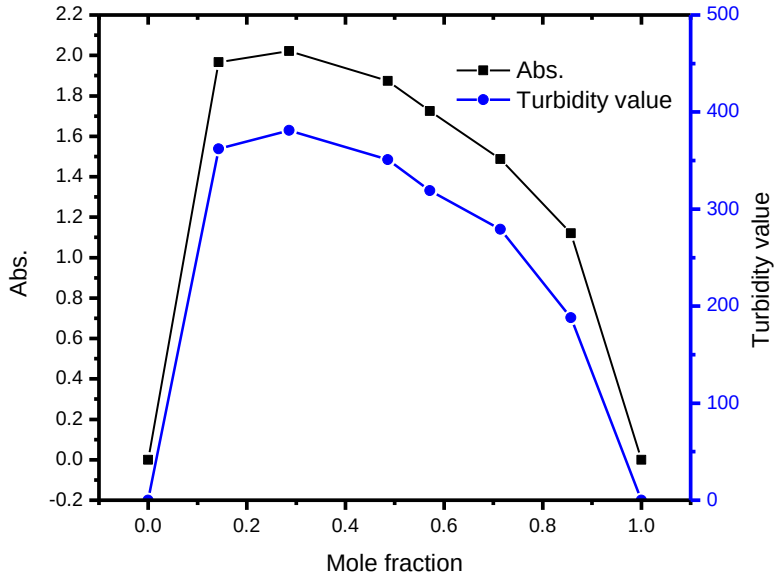

Figure 6- Stoichiometric ratio for CIP with sodium tungstate by using Job's method [12]

3. Results and Discussion

3.1. Determination of Ciprofloxacin HCl

The effect of CIP concentration on the turbidity and absorbance values under the optimum conditions was evaluated in the range 0.05–3.5 mmol.L^{-1} for the preparation of scatter plot diagram followed by the choice of calibration graph. Turbidity increased linearly with the increase in CIP concentration from 0.05 to 2 mmol.L^{-1} and 0.05 to 3.0 mmol.L^{-1} for spectrophotometry and turbidity method as shown in Figure-7. The difference in calibration graph range for both techniques may be attributed to the basic principles of techniques. The absorbance depends on the amount of light that reaches to the detector. While the turbidity depends on measuring the scattering or attenuation of light. So, the absorbance consists of light interference and scattering where as the turbidity depends only on the scattering. Linear dynamic range, correlation coefficient, the calculated t-value at 95% confidence interval and linear percentage are shown in Table-2.

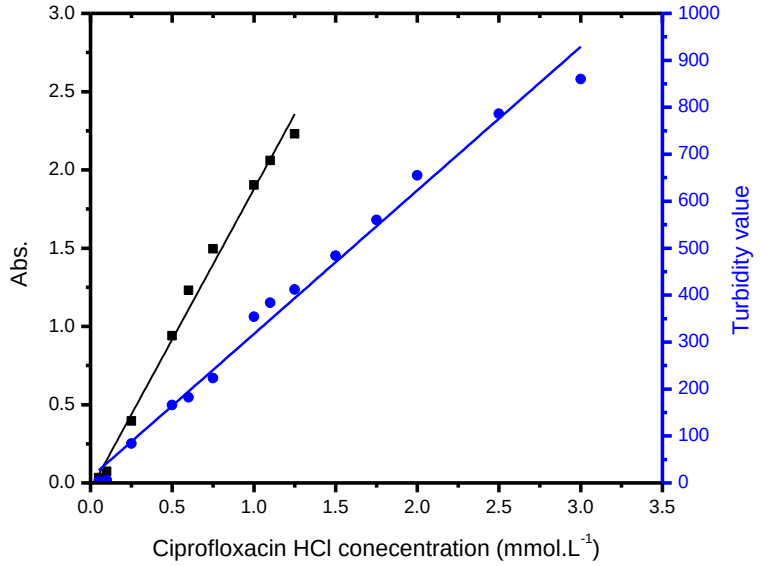

Figure 7- Calibration graph for the determination Ciprofloxacin HCl in mmol.L^{-1}

Table 2- Summary of linear regression equation [16, 17] for analysis of Ciprofloxacin HCl

Measurement method	**Measured range mmol.L^{-1}**	**Linear range mmol.L^{-1}**	**y^(mV)=a±S_at+b±S_bt [X] mmol.L^{-1} at confidence interval 95%, n-2**	**r, r^2, r^2%**	**$t_{tab.}$**	**$t_{cal} = \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$**
					at 95%, n-2	
Abs.	0.05-3.5	0.05-1.25 (n=10)	-0.042 ± 0.109 + 1.919 ± 0.146 [CIP] mmol.L^{-1}	0.9956 0.9913 99.13	2.160 << 28.39	
Turbidity	0.05-3.5	0.05-3.0 (n=15)	11.39 ± 32.10 + 305.83 ± 22.08 [CIP] mmol.L^{-1}	0.9941 0.9883 98.33	2.306 << 31.93	

Analysis of variance for spectrophotometry and turbidity method was carried out and recorded in Table-3. The value of calculated F is greater than tabulated value, therefore, it can be concluded that there is a significant relation between the concentrations of CIP and the response obtained.

Table 3- Analysis of variance (ANOVA) for linear equation results

Source of Variation (Abs.)	DF	Sum of Squares (SS)	Mean Square (MS)	F Value S_1/S_2
Regression $(\hat{y}_i - \bar{y})^2$	1	5.6573	5.6573	806.106
Residual error $(y_i - \hat{y}_i)^2$	7	0.0491	0.0070	
Total $(y_i - \bar{y})^2$	8	5.7064		

DF: Degree of freedom. S_1: Regression mean square. S_2: Mean square error

Source of Variation (Turbidity)	DF	Sum of Squares (SS)	Mean Square (MS)	F Value S_1/S_2
Regression $(\hat{y}_i - \bar{y})^2$	1	980998.4073	980998.4073	1019.892
Residual error $(y_i - \hat{y}_i)^2$	12	11542.3705	961.8642	
Total $(y_i - \bar{y})^2$	13	992540.7779		

DF: Degree of freedom. S_1: Regression mean square. S_2: Mean square error

3.2. Limit of Detection and Quantification

Limit of detection (LOD) and Quantification (LOQ) for CIP were presented in Table-4. LOD was calculated using three different approaches (gradual dilution of lowest concentration, numerical value of slope and linear regression).

Table 4- LOD and LOQ for Ciprofloxacin HCl at conditional parameters

Measurement method	Gradual dilution for the minimum concentration in calibration graph	Based on the value of slope $x = \frac{3S_B}{slope}$	Linear equation $y^{(mV)} = y_B+3S_B$	Limit of Quantification $y^{(mV)} = y_B+10S_B$
Abs.	7×10^{-5} M/sample 270.06 μg/sample	4.68×10^{-2} M/sample 180.89 mg/sample	1.31×10^{-4} M/sample 0.50 mg/sample	3.04×10^{-4} M/sample 1.17 mg/sample
Turbidity	5×10^{-5} M/sample 192.9 μg/sample	2.94×10^{-4} M/sample 1.13 mg/sample	3.04×10^{-4} M/sample 1.17 mg/sample	1.31×10^{-4} M/sample 0.50 mg/sample

3.3. Precision

Precision of the developed method was achieved for six replicates of a fixed amount (0.5 and 1.25 $mmol.L^{-1}$) of CIP. The percent of relative standard deviation (%RSD) was calculated and presented in Table-5. The repeatability of new method was found to be less than 2%.

Table 5- Evaluation of repeatability in determination of CIP by the turbidity method

Measurement method	[CIP] $mmol.L^{-1}$	Number of measuring (n)	Response average $\bar{y}i$ (n=6)	Standard Deviation σ_{n-1}	Repeatability R.S.D.%	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
Abs.	0.5	6	1.074	0.024	2.26	1.074 ± 0.025
	1.25	6	2.310	0.063	2.76	223.33 ± 0.067
Turbidity	0.5	6	158.833	2.483	1.56	158.83 ± 2.606
	1.25	6	482.833	8.773	1.81	482.83 ± 9.208

3.4. Analysis of Tablets

Forty commercial tablets (500 mg) of CIP from two different drug companies (Ciprofloxacin, 500mg, China and Cipropharm, 500mg, Jordan) (twenty tablets from each company) were accurately weighed and the average weight of one tablet of Ciprofloxacin HCl was determined. All tablets were triturated and the weighting (0.1286g and 0.1538g) of CIP and dissolved in 25 mL of deionized water to prepare 10 $mmol.L^{-1}$. The mixture was shaken on magnetic stirrer at room temperature for 60 minutes. After stirring the prepared solution was filtered.

A series of standard CIP solutions (0 to 2 mL) were placed in 10 mL volumetric flasks and 0.5 mL of pharmaceutical preparation solution was added to each flask to obtain concentration 0.5, 1, 1.5, 2, and 2.5 $mmol.L^{-1}$. Flask no.1 is the sample flask volume. Then 4 mL of reagent and 0.6 mL of 0.1N Hydrochloric acid was added to the mixture and then the flask was filled to the sign by deionized water. After 1 minute, the absorbance and turbidity of solution were recorded.

The determinations were carried out in triplicate for each of pharmaceutical by standard addition method and the results were mathematically treated. The obtained results of analysis are presented in Table-6. In addition, to check the validity of proposed method, the t-test at 95% confidence intervals was used in comparison with quoted value. The obtained values indicate that the calculated t-test at 95% confidence interval is less than tabulated t-test, therefore, no significant difference between new turbidity methods with quote value Table-7.

Table 6- Summary of determination of CIP in pharmaceutical preparation

Commercial name Content Country	**Confidence interval for average weight at 95%** $\bar{w} \pm 1.96\frac{\sigma_{n-1}}{\sqrt{n}}$ **(g)**	**Weight of sample (g) to obtain 10 mmol.L^{-1} (g)**	**Theoretical content of active ingredient at 95% n=∞ (mg)**	**Found content of active ingredient at 95% n=∞ (mg)**		**% Recovery**
Ciropharm 500 mg Jordan	0.7758±0.00589	0.1286 g equivalence to 500 mg CIP active ingredient	500 ± 0.052	Abs.	554.66±61.32	110.93
				Turb.	483.00±26.17	96.60
Ciprofloxacin 500 mg China	0.9289±0.00424	0.1538 g equivalence to 500 mg CIP active ingredient	500 ± 0.076	Abs.	542.66±46.23	108.53
				Turb.	473.33±38.59	94.66

Table 7- Calculations of paired t-test for developed methods with quoted value using standard additions method

Drug	**Measurement method**	**Practical content (mg)**		**D**	$\bar{x}_d$	σ_{n-1}	**Paired t-test** $t = \frac{\bar{x}_d\sqrt{n}}{\sigma_{n-1}}$	$t_{tab.}$ **at 95% confidence interval n-1**
		Quoted value	**New method**					
CIP – Jordan	Abs.	500	534	-34	-54.66	24.684	|-3.835| < 4.303	
		500	548	-48				
		500	582	-82				
	Turbidity	500	484	16	17	10.535	2.794 < 4.303	
		500	472	28				
		500	493	7				
CIP – China	Abs.	500	560	-60	-42.66	18.610	|-3.971| < 4.303	
		500	545	-45				
		500	523	-23				
	Turbidity	500	456	44	26.66	15.534	2.973 < 4.303	
		500	478	22				
		500	486	14				

4. Conclusion

The results of proposed method study indicate that there is no significant difference between the proposed method with official method or reported methods with regard to precision and accuracy. The main advantage of proposed method is their suitability for routine quality control in tablet and in drug alone without any fear of interference which caused by the excipient of tablets which expected strongly to be present in tablet. Comparison with other reported methods, the proposed method more accurate, rapid and much cheaper.

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