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Spectrophotometric Estimation of Levofloxacin Via Ion-Pair Complex Formation with Erythrosine B

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ABSTRACT

In this study, a simple, rapid, and cost-effective analytical method was described for the quantitative determination of levofloxacin (LEV) in its pure state and in therapeutic doses. The method relies on forming an ion-pair complex between LEV and erythrosine dye (ERY) at pH=4.0. The spectrum of the coloured product exhibits maximum absorption at 558 nm against the blank. A linear relationship, was obtained in the concentration range of 1.0-15.0 µg/ml with a quantification coefficient of 0.9991 under the optimum conditions. The molar absorption coefficient and Sandell's sensitivity index values were 1.91×104 L/mol.cm and 0.0189 µg/cm^2 respectively, whereas the values of the detection limit and the quantification limit were 0.200 and 0.666 µg/ml respectively. The developed method was effectively applied to estimate LEV present in pharmaceutical dosage. The accuracy and reliability of the suggested method were ascertained by recovery study via the standard addition method.

Keywords: Levofloxacin, ion-pair complexation, determination, spectrophotometric, erythrosine.

INTRODUCTION

Levofloxacin (LEV) is white to off white, powder, it is partially soluble in water but good soluble in methanol, its chemical name is (−) -(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-OXO-7h-pyrido[1,2,3-Di]-1,4-benzoxazine-6-carboxylic acid), (WHO, 2019). The chemical structure of LEV is given in Fig. (1) and its molecular formula is $C_{18}H_{20}FN_3O_4$ with molecular weight of 361.373 g/mol (Drugbank, 2021).

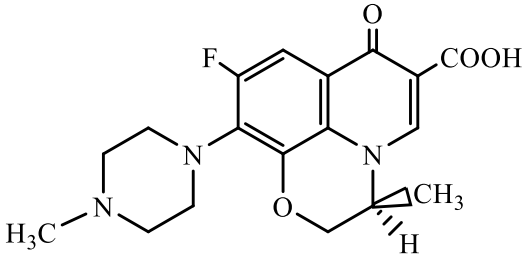


Fig. 1: Chemical structure of LEV.

LEV is a widely used and effective antibiotic that works by preventing bacterial DNA from twisting, causing the bacteria to die, LEV is mainly used to treat urinary tract and respiratory tract infections (Bhatt and Chatterjee, 2022). Its uses also include treating chronic bronchitis, conjunctivitis, chronic prostatitis, mastitis, abdominal infections, gastroenteritis, local infections, acute bacterial sinusitis, and acute pyelonephritis (Jia and Zhao, 2021).

LEV is one of the main drugs used to treat multidrug-resistant tuberculosis. (MDR-TB) (Ghimire *et al*., 2019). LEV belongs to the third generation of synthetic fluoroquinolones, commonly referred to as respiratory quinolones. (Sweetman, 2012). LEV shows a high tendency to migrate towards Gram-positive bacteria and a lower tendency towards Gram-negative bacteria, but it has bactericidal activity towards both Gram-positive and Gram-negative aerobic bacteria, (Asseray *et al*., 2016; Hayakawa *et al.,* 1986).

Given the widespread use of LEV and its therapeutic importance, there was an urgent need to accurately estimate it in the various models containing it. Therefore, a number of different methods published in the previous scientific literature have been monitored to estimate LEV in drug doses and biological fluids, using different techniques, including spectrophotometry (Elgendy *et al*., 2024; Ravi *et al*., 2022; Czyrski, 2022; Talpur *et al.*, 2020; Qassim, 2015; Singh *et al.*, 2015); electrochemical methods (Bhimaraya *et al.*, 2023; Koçak *et al.*, 2022; Tigari *et al.*, 2022; de Farias *et al.,* 2020; Mittal *et al.*, 2017); HPLC (Sen *et al.*, 2023; Peikova *et al.,* 2022; Toker *et al.,* 2021; Yıldırım *et al.,* 2020).

Some of the above procedures require expensive equipment and others require skilled operators, but in this research, we have developed an easy, sensitive and low-skilled spectroscopic method for the determination of LEV in its pure state as well as in its pharmaceutical preparations that involves the formation of an ion complex between erythrosine dye and LEV in an acidic medium.

EXPERIMENTAL

Apparatus

Absorbance measurements were taken using a Shimadzu1900i UV-Vis dual-beam spectrometer with 1.0 cm glass cells, and a professional pH meter BP3001 was used to measure pH.

Chemical reagents

High purity chemicals were used in this procedure, and pure levofloxacin was prepared by the Samarra state pharmaceutical industry company (SDI).

Levofloxacin working solution 100 µg/ml 2.76 ×10^{-4} mol/L: This solution prepared by dissolved 0.010 g of pure LEV in 10 ml of distilled water with shaking and slight heating, then diluted by distilled water to 100 ml using a volumetric flask.

Erythrosine B (ERY) solution 8×10^{-4} mol/L: It was prepared by dissolving 0.050 g of dye powder in distilled water and diluting it to 100 ml with the distilled water in a volumetric flask.
Hydrochloric acid solution (1M): It prepared by adding 8.4 ml of concentrated hydrochloric acid (11.8 M) into 100 ml volumetric flask and fill it with distilled water to mark.
Recommended procedure and calibration curve

The standard curve was drawn according to the previously established optimum conditions, by adding increasing volumes of the working solution of levofloxacin (100 ppm) covering a range of concentrations 1-20 µg/ml to a series of 10 ml volumetric flasks containing 1.5 ml of acetate buffer-solution at pH=4, Then followed by adding 1.5 ml of ERY dye at 8×10^{-4} M with shaking and waiting for 3 min at room temperature to complete the reaction, after which completed the volume by distilled water to the mark. The absorbance of the resulting-colored solutions was measured at 558 nm versus the blank solution. (Quantitation) Fig. (2) shows the standard curve of the method which indicates that the linear relationship of the developed method followed the Beer-Lambert law in the concentration range 1-15 µg/ml of LEV with an estimation coefficient R^2=0.9991. The molar absorption coefficient was calculated from the straight-line equation and was 1.91×10^4 L/mol.cm while the Sandell's sensitivity index equal to 0.0189 µg/cm^2, which indicates the excellent sensitivity of the proposed method. The detection limit (LOD) and quantification limit (LOQ) were 0.200 and 0.666 µg/ml respectively.

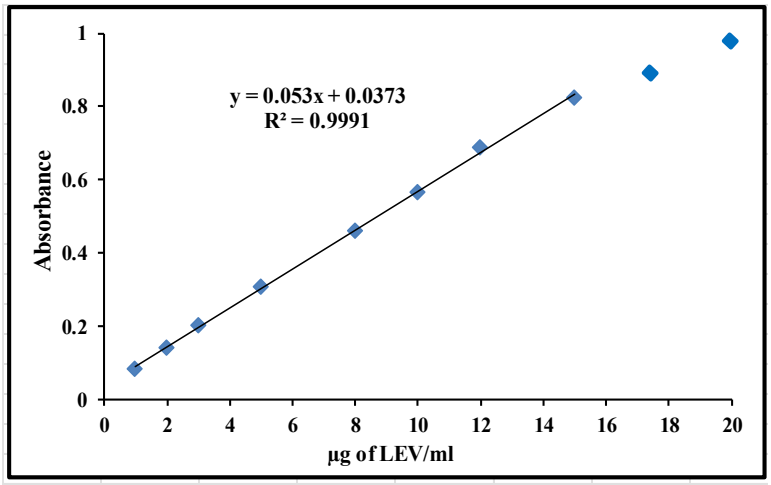


Fig. 2: Calibration curve for determination of LEV.

pharmaceutical preparations of LEV
Tablet solution (100 µg/ml): Ten tablets of Levosol tablets (supplied by Pharma Solutions LLC-Jordan) (each tablet containing 500 mg of levofloxacin) were weighed, the total weight of the tablets was 7.0988 g, crushed and mixed well, 0.0142 g, equivalent to 0.01 g of the pure compound, were weighed, completely dissolved in 30 ml of distilled water, filtered and transferred to a 100 ml volumetric flask and filled with distilled water to the mark.
Intravenous solution (100 µg/ml): 2 ml of Levonir Intravenous Solution (each 100 ml vial contains 500 mg of levofloxacin) (supplied by Pioneer Pharmaceuticals - Iraq) was diluted with distilled water to a 100 ml volumetric flask.

RESULTS AND DISCUSSION

principle of the proposed method

The chemical reaction of the method involves a single-step, the reaction of LEV with a known excess amount of erythrosine dye in acidic medium, resulting in the formation of a colored ion pair complex, which exhibits maximum absorbance at 558 nm and it is directly proportional to the amount of LEV present in the sample as shown in Scheme (1) and Fig. (3).

$$\underset{\text{(excess)}}{\text{LEV} + \text{ERY}} \xrightarrow{H^+} \underset{\text{(colored product)}}{\text{LEV-ERY}}$$

Scheme 1: Chemical reaction.

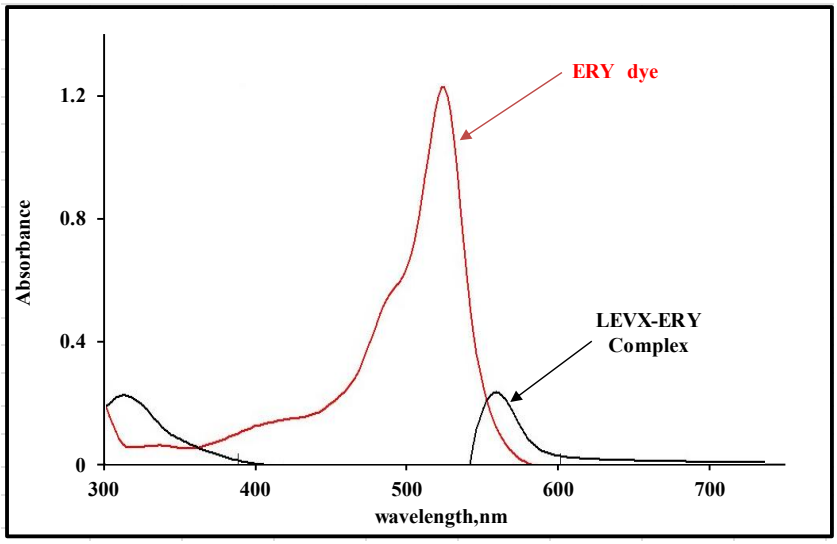


Fig. 3: Initial absorption spectrum of the method.

Optimization of reaction conditions

All experiments were performed using 100 µg of LEV in a final volume of 10 ml and absorbance values were measured at 558 nm, and the reaction conditions were optimized.

Effect of pH value

In order to obtain the best absorption value for the colored product, the use of a number of available acid solutions with a concentration of 0.01 M and in a different volume was studied and the results are shown in (Table 1).

Table 1: The acidity function effect.

Type of acid (0.01 M)	Absorbance/ml of acid					
	0.5	**pH**	**1.0**	**pH**	**1.5**	**pH**
HCl	0.277	4.73	**0.318**	**4.04**	0.251	3.37
H_2SO_4	0.284	4.21	Turbid	----	Turbid	----
HNO_3	0.269	4.46	0.248	4.12	0.233	3.84
CH_3COOH	0.226	5.12	0.237	4.68	0.245	4.17

From the results in (Table 1), 1 ml of 0.01 M HCl was selected, and due to the instability of the absorption values, the effect of different buffer solutions on the absorption was studied.

Effect of buffer solutions

A number of buffer solutions with a pH ≅ 4 were prepared, and their effect of adding them in different volumes on the absorption value and stability was studied. The results are listed in (Table 2).

Table 2: Choose of type and volume of buffer solution.

Type of buffer solution (pH=4)	ml of buffer	Absorbance	Final pH
With out (Water)		0.215	5.76
Acetate buffer	**0.5**	0.301	4.08
	1	0.337	4.00
	1.5	0.344	4.01
	2	0.335	4.00
Citrate buffer	**0.5**	0.290	3.94
	1	0.323	3.97
	1.5	0.328	4.02
	2	0.330	4.00
KH-phthalate buffer	**0.5**	0.315	3.91
	1	0.334	3.98
	1.5	0.337	4.07
	2	0.331	4.03

The results in Table 2, show that using 1.5 ml of acetate buffer solution gave the best absorption, so it was chosen and fixed in the proposed method.

Effect of reaction time

In order to investigate the influence of time on the completing the reaction, varying time were allowed before diluting with distilled water. The results in Fig. (4) show that the reaction reaches completion and the stabilize absorption value is within 3 minutes, so it was fixed in the suggested method.

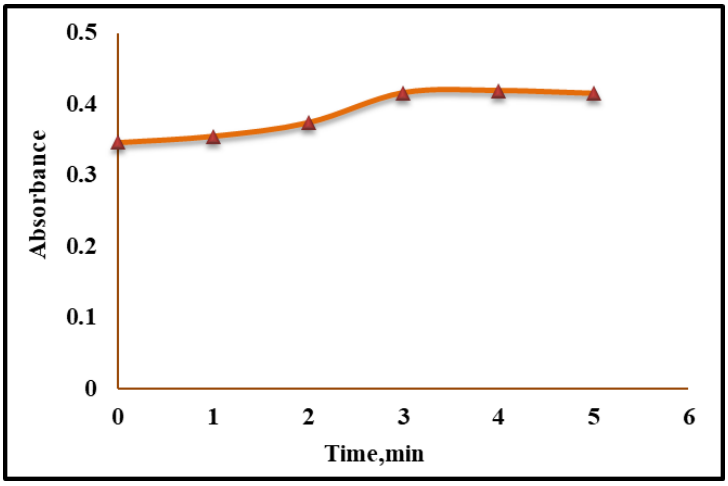


Fig. 4: Time required for reaction completion.

Effect of ERY dye concentration

Different concentrations (2×10^{-4}-1×10^{-3} M) of ERY dye were prepared and 1 ml of each concentration was added to a number of volumetric flasks containing the remaining components of the method. The 8×10^{-4} M gave the best result, so it was adopted in subsequent experiments.

Effect of ERY dye volume

Different volumes of ERY dye at a concentration of 8×10^{-4} M were used against increasing volumes of LEV followed by the estimation of the coefficient's values. The results listed in (Table 3), indicate that 1.5 ml of erythrosine dye was the best, therefore was selected and fixed in subsequent experiments.

Table 3: Effect of ERY dye amount.

ml of 8×10^{-4} M ERY dye	Absorbance, µg/ml of LEV					
	2	5	8	10	12	R^2
0.5	0.112	0.174	0.202	0.226	0.255	0.9793
1.0	0.121	0.272	0.363	0.418	0.529	0.9858
1.5	0.127	0.291	0.435	0.563	0.656	0.9985
2	0.129	0.286	0.428	0.541	0.669	0.9972

Effect of using surfactants

To evaluate the effect of adding surfactants (neutral, negative and positive) at a concentration of 1% for each one, on absorbance different volumes of them were added to the reaction components. The results in (Table 4) indicated that adding them led to a decrease in the absorption value, so they were excluded.

Table 4: Effect of adding surfactants.

Surfactant (1%)	Abs./ml of surfactant			
	Without	1	2	3
SDS	0.567	0.089	0.157	0.132
CPC		0.116	0.179	0.165
CTAB		0.098	0.076	0.031
Triton-x 100		0.263	0.236	0.223

SDS: Sodium dodecyl sulphate.
CTAB: Cetyl trimethyl ammonium bromide.
CPC: Cetyl pyridinium chloride.
Triton X-100: Iso ocytylphenoxy polyethoxyethanol.

Effect of temperature and time

The proposed method was applied at different temperatures (10, RT and 40 °C) with monitoring the absorption values of the colored product during different time periods at each temperature. Room temperature (25±2 °C) was determined as the best temperature at which the proposed method can be applied.

Effect of the order of addition

A number of different addition sequences were applied. The results in (Table 5) reveals that the sequence I is the best sequence that gives the highest absorbance, so it was kept for subsequent experiments.

Table 5: The effect of changing the order of the additions.

Order of addition	NO.	Abs.
B+ LEV + ERY	I	0.568
LEV+ B + ERY	II	0.503
ERY +B + LEV	III	0.492

*LEV= Levofloxacin, B= buffer solution, ERY = Erythrosine dye.

Final absorption spectrum

The proposed method was applied according to the optimized conditions and addition order mentioned above, and the absorption spectra were plotted. Fig. (5) shows the final absorption spectrum, where it was found that the ion-pair complex achieved maximum absorption at a wavelength of 558 nm, while the blank solution gave maximum absorption at 527 nm, and no absorption was recorded at λ_{max} for the complex.

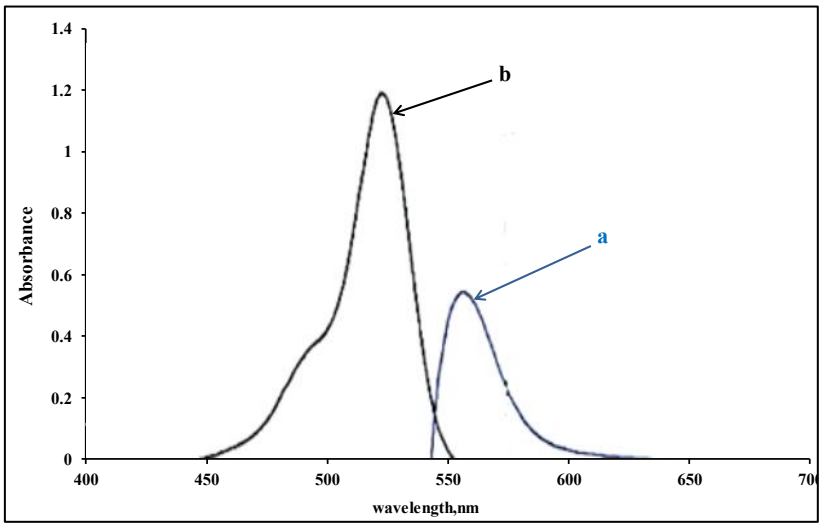


Fig. 5: Absorption spectra for: (a) complex containing 10 µg/ml LEV Vs reagent blank, and (b) reagent blank Vs water.

Nature of the reaction between LEV and ERY dye

The molar ratio method (Delevie, 1997) was applied to determine the reaction ratio between LEV and ERY dye, Fig. (6) indicate that the reaction ratio was 1:1.

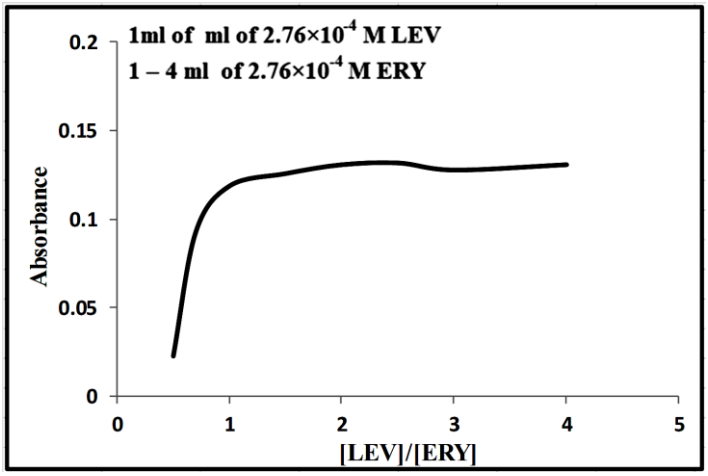
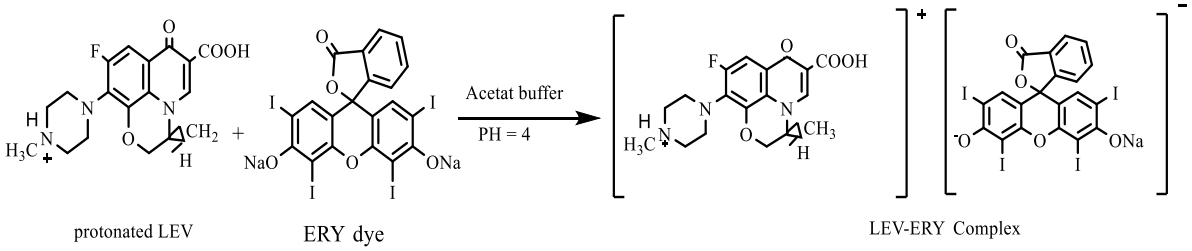


Fig. 6: Plot of applying the molar ratio method.

Therefore, according to the obtained result in, Fig. (6) the following reaction was proposed for the resulting complex:



Accuracy and precision

Using three different concentrations (4, 8, and 12 µg) of pure LEV solution, the accuracy and precision of the proposed method were studied. The relative error, recovery percentage, and relative

standard deviation were calculated. The results in (Table 6) show that the proposed method is precise and accurate.

Table 6: Accuracy and precision.

LEV (µg/ml)		Recovery %*	RE, %*	RSD, %*
Present	Measured			
4	3.94	98.5	- 1.5	1.13
8	7.62	95.25	- 4.75	2.07
12	11.81	98.41	-1.59	1.56

*Average of five determinations.

Application of the method

The proposed method was applied under optimal conditions for the determination of levofloxacin in available therapeutic drugs (tablets and intravenous solution), using two different concentrations for each preparation (4 and 8 µg/ml). The results listed in (Table 7) demonstrate the accuracy and precision of the method, as the t-exp. values were lower than the table value for four degrees of freedom (N = 4), confirming the successful application of the method for the analysis of LEV present in therapeutic drugs with good accuracy and acceptable agreement with the declared content.

Table 7: Applying the method to available drugs.

LEV formulations	Taken amount of LEV µg/ml	Found amount of LEV µg/ml	Recovery %*	RE%*	RSD%*
Levosol (Tablets) 500mg/ Tab. Pharma solution -Jordan	4	3.92	98.0	-2.0	1.26
	8	7.94	99.25	-0.75	1.09
Levoneer, infusion solution 500mg /100 ml Pioneer -Iraq	4	4.11	102.75	2.75	2.42
	8	8.26	103.25	3.25	1.96

*Average of five determinations.

Evaluation of the proposed method

To demonstrate the selectivity of the proposed method and the absence of interference from additives in the therapeutic drugs, the standard addition method (Harris, 2016) was applied using two concentrations of LEV (4 and 8 µg/ml) for each pharmaceutical preparation. The results in Fig. (7) and (Table 8) show that the standard addition method was in good agreement with the developed method.

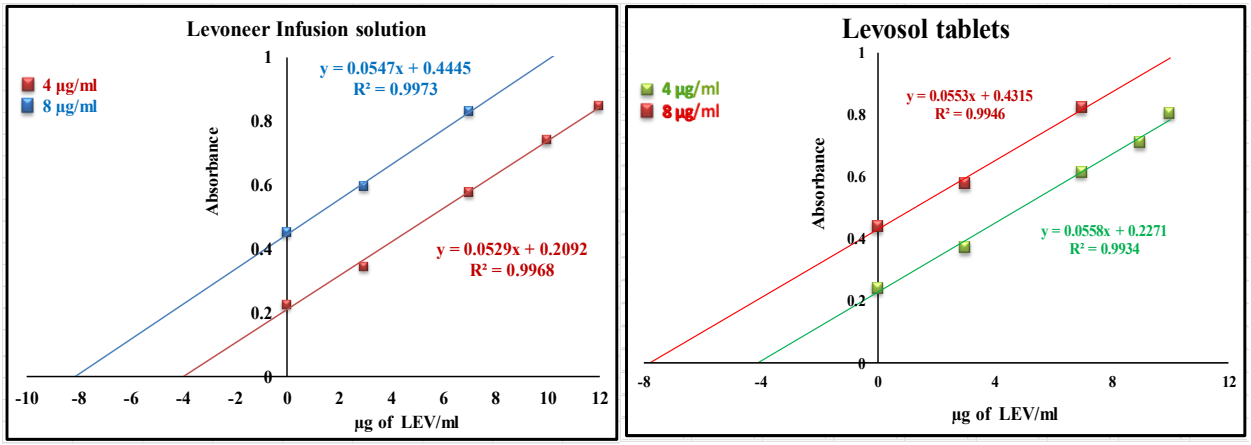


Fig. 7: Standard addition curves for estimation of LEV in pharmaceutical drug.

The recovery percentage for each preparation was calculated from the linear equations in Fig. (7), and the results are shown in (Table 8).

Table 8: Results of standard addition method

LEV formulations	Taken amount of LEV µg/ml	Found amount of LEV µg/ml	Recovery %
Levosol (tablets) 500mg/Tab. Pharma solution (Jordan)	4	4.07	101.75
	8	7.80	97.53
Levoneer, infusion solution 500mg/100ml. Pioneer (Iraq)	4	3.95	98.75
	8	8.12	101.5

Comparison of the developed method

Some spectral values and analytical properties of the proposed method were compared with two spectral methods from the published literature. By reviewing the comparison results listed in (Table 9), we find that the proposed method is more sensitive, less risky, and less expensive than other methods.

Table 9: Comparison of some variable the method with literature method.

Variable	Present method	Literature method*	Literature method**
Type of reaction	Ion-pair formation	Chelating complex	UV
Reagent used	Erythrosine dye	Al (III)	------
Maximum wavelength, nm	558	420	287
Linearity range, µg/ml	1.0-15.0	5-45	2.0-20.0
R^2	0.9991	0.9993	0.9998
Σ, l/mol.cm	1.91×10^4	111.1×10^3	2.09×10^4
Sandell's sensitivity index	0.0189	0.0033	0.237
LOD, µg/ml	0.200	0.009	0.063
LOQ, µg/ml	0.666	0.25	1.92
Applications	pharmaceuticals	Pharmaceuticals	pharmaceuticals

*(Qassim, 2015), ** (Gholse, *et, al.,* 2022)

CONCLUSIONS

In this research, a simple, high-accuracy and inexpensive spectrophotometric method was developed for the determination of levofloxacin in its pure state and in its pharmaceutical forms. The method is based on the reaction of ERY dye with LEV in a buffer medium at pH 4 to form a colored ion pair complex that is directly proportional to the amount of LEV present in the sample. The method was successfully applied to the determination of LEV in pharmaceutical doses and gave acceptable results in terms of recovery and accuracy.

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التقدير الطيفي لليفوفلوكساسين عن طريق تكوين المزدوج الأيوني مع الاريثروسين ب

ضياء ثامر عزيز **خالدة محمد عمر**

قسم الكيمياء/ كلية العلوم/ جامعة الموصل/ العراق

الملخص

في هذه الدراسة، تم وصف طريقة تحليلية بسيطة وسريعة وفعّالة وقليلة التكلفة للتقدير الطيفي الكمي لليفوفلوكساسين (LEV) في حالته النقية وفي الجرعات العلاجية. استندت الطريقة إلى تكوين معقد زوج أيوني بين LEV وصبغة الإريثروزين (ERY) عند درجة منظمة من الحموضة =4.0، حيث يُظهر طيف المحلول الناتج الملون أقصى قيمة للامتصاص عند الطول الموجي 558 نانومتر مقابل المحلول الصوري، وقد تم الحصول على علاقة خطية في مدى من التراكيز يتراوح بين 1.0−15.0 مايكروغرام.مللتر$^{-1}$ في ظل الظروف المثلى وبمعامل تقدير كمي يساوي 0.9991، وتم أيضاً حساب قيم معامل الامتصاص المولاري ومؤشر حساسية ساندييل لتكون 1.91×10^{4} لتر.مول$^{-1}$.سم$^{-1}$ و0.0189 مايكروغرام.سم$^{-2}$ على التوالي، في حين كانت قيم حد الكشف وحد القياس الكمي للطريقة المقترحة وقد بلغت 0.200 و0.666 مايكروغرام.مللتر$^{-1}$ على التوالي، وقد تم تطبيق الطريقة المطورة بشكل ناجح وفعال لتقدير LEV الموجود في الجرعات الصيدلانية المختلفة، وتم التأكد من دقة وموثوقية الطريقة المقترحة من خلال دراسة قيم الاسترجاع بتطبيق طريقة الإضافة القياسية.

الكلمات الدالة: الليفوفلوكساسين، المزدوج الأيوني، التقدير الطيفي، الاريثروسين.