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Spectrophotometric Method Enhanced by Cloud Point Extraction for the Determination of Levofloxacin in Pharmaceutical Preparations

Talal Abdullah Attawi*, Shahla Jamal Shakkor

Department of Chemistry, College of Sciences, University of Kirkuk, Kirkuk, Iraq.
*Corresponding author : sccm24004@uokirkuk.edu.iq

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Abstract

This study presents a simple and accurate analytical determination of the quantity of certain anti-inflammatory drugs of the fluoroquinolone class, specifically Levofloxacin, in its pure form and in pharmaceutical preparations. This study uses the cloud point extraction method, which is the reaction of the drug with namely Ferric (III) ions in $FeCl_3$ salt, in the presence of the active surface Triton X-114. The used method showed excellent linearity in the range of 5-45 μg ml^{-1} at a wavelength of 386 nm, with high accuracy and sensitivity. This is evident from the limit of detection (LOD) of 1.346 μg ml^{-1} and the limit of quantification (LOQ) of 4.055 μg ml^{-1}. The application of this method to commercial tablet formulations and intravenous injections resulted in high recovery rates RSD%.

1. Introduction:

Fluoroquinolones were discovered through gradual development that began in the 1960s, with a breakthrough in the 1980s. The first quinolone, nalidixic acid [1], was extracted from the antimalarial drug chloroquine [2]. Fluoroquinolones are broad-spectrum compounds known for their activity against Gram-positive and Gram-negative bacterial pathogens [3]. Levofloxacin is a third-generation antibiotic and one of the fluoroquinolones listed in Group A of the World Health Organization's standard guidelines for the treatment of multidrug-resistant tuberculosis [4, 5].

Levofloxacin can be dissolved in water slowly and other solvents. The molecular weight of Levofloxacin is 361.368 g mol^{-1}, its molecular formula is $C_{18}H_{20}FN_3O_4$, and its scientific name is 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro7H [1,4] oxazino [2,3,4-ij] quinoline-6-carboxylic acid [6]. Levofloxacin is a broad-spectrum antimicrobial agent that works by inhibiting bacterial DNA polymerase, which is essential for DNA replication [7], leading to the degradation of bacteria. The addition of 6-fluoro and 7-piperazinyl groups to the molecule greatly increases its antibacterial activity [8].

It is commonly referred to as a second-generation fluoroquinolone antibacterial agent and is highly effective against

Gram-negative and Gram-positive bacteria that are resistant to other antibacterial drugs [9]. Its chemical structure [10] is as shown in Figure 1.

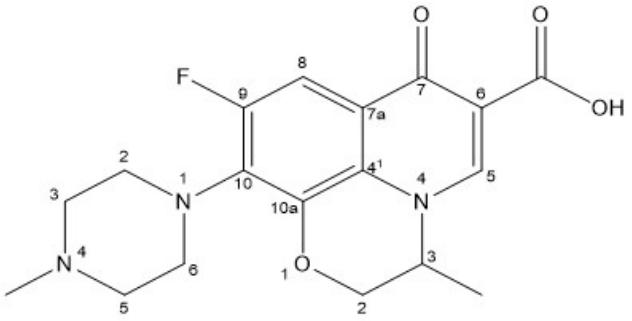


Figure 1. Chemical structure of Levofloxacin.

Levofloxacin is a highly effective [11] antibiotic for treating lower respiratory tract infections, as well as skin and soft tissue infections [12]. It is also used to treat ear infections, sore throats, pneumonia, and various skin infection [13]. It may also be prescribed in special cases, such as to treat anthrax infection after exposure through inhalation [14]. There are several methods for estimating Levofloxacin which have been applied to determine Levofloxacin in different real samples, including spectrophotometry, high-performance liquid chromatography (HPLC) [15], capillary electrophoresis (CE) [16], and high-performance liquid chromatography with ultraviolet detection (HPLC–UV) [17].

The proposed method has clear advantages in the rapid determination of Levofloxacin in its pure form and in pharmaceutical preparations. This study implemented a simple, sensitive and rapid spectroscopic method for the estimation of Levofloxacin, which proved to be low cost, highly selective and accurate compared to some other methods [18, 19, 20].

2. Experimental Part:

2.1 Apparatus:

All spectrophotometric measurements were made using a T92+Spectrophotometer double beam, China with a 1.0 cm quartz cell and all weight measurements were made using a Sartorius Balance BL210 SAG, Germany, centrifuge from the German company Zentrifugen Hetticha set of glassware, water bath from the South Korean company Daihan Labtech and an ice bath.

2.2 Reagents and Chemical Materials:

The Levofloxacin (LEVO) pure standard powder was supplied by the State Drug Industries and Medical Appliances Company (SDI), Samarra, Iraq. All other analytical-grade reagents (including ferric chloride $FeCl_3$ and the surfactant Triton X -114) were obtained from Fluka and BDH, as shown in Table 1, and distilled water was used throughout the experiment. The pharmaceutical formulation Levoshr 500 mg, manufactured by the Indian company SAGA, was used as the dosage form. All chemicals and reagents were analytical-grade, and all solutions were freshly prepared.

Table 1. Chemicals and reagents used in this study.

Chemical Compounds	Chemical Formula	Molar mass g mol^{-1}	Purity	Provenance
Levofloxacin	$C_18H_{20}FN_3O_4$	361.373	99.9%	SDI-Iraq
Ferrous chloride	$FeCl_3$	162.19	98%	Fluka
Triton X-114	$C_{14}H_{22}O(C_2H_4O)_n$	563	99%	BDH
Ethanol	C_2H_5OH	64.07	98%	BDH

2.3 Preparation of solutions:

2.3.1 LEVO solution (250 μg ml^{-1}):

It was prepared by dissolving 0.0250 g of LEVO in amount of distilled water, and then completing the volume to 100 mL with distilled water using a volumetric flask to obtain a solution with a concentration of 250 μg ml^{-1}

2.3.2 Preparation of LEVO tablet solution:

In the pharmaceutical formulation (Levoshr 500 mg), each tablet contains 500 mg of Levofloxacin and weighs 0.879 g. Ten tablets were weighed accurately, grounded, and mixed well to obtain a homogeneous powder. A portion equivalent to 0.01758 g of the powdered tablets, corresponding to 20 mg of Levofloxacin, was transferred into a 100 mL volumetric flask. The powder was dissolved in about 5.0 mL of distilled water under gentle stirring to achieve complete dissolution. The solution was filtered to remove any insoluble materials and topped up with distilled water to the mark to obtain a Levofloxacin concentration of 250 μg ml^{-1}.

2.3.3 Ferric chloride $FeCl_3$ 250μg ml^{-1} :

The solution was prepared by weighing 0.0250 g of $FeCl_3$ and dissolving it in 100 mL of distilled water, in a 100 ml volumetric flask.

2.3.4 Effective surface Triton X-114 (10%v/v):

Take 10 ml of the active substance from the surface (Triton X-114) in a 100 ml volumetric flask and distilled water was added gradually with continuous stirring up to the mark, until the active ingredient was completely dissolved. The concentration of the prepared Tritonx-114 solution was 10% (v/v).

3. Results and Discussion:

3.1 General idea of the method:

In a 25 ml volumetric flask, 3 ml of ($FeCl_3$) is mixed with 3 ml of Levofloxacin and 1.8 ml of (Triton X-114 10% V/V) with continuous stirring, and the volume is made up to the mark with distilled water. During this period, the compound solution turns yellow. The solution is then heated in a water bath, transferred to test tubes, placed in a centrifuge, cooled in an ice bath, and separated into two phases (an aqueous phase that is discarded and a phase rich in surfactants and complexes). The ethanol-rich material is dissolved, and the resulting solution is measured by UV-Vis, which shows a maximum absorbance at 386 nm.

3.2 Study of optimal reaction conditions:

The effect of various variables on the absorption intensity of Levofloxacin and the optimal conditions were selected as follows:

3.3 Selection of the best metal ion:

Different salts were used to form a colored complex with Levofloxacin. In the presence of the surfactant Triton X-114, a stable yellow complex was formed with the highest absorbance at 386 nm, indicating it most effectively mediates the LEVO-$FeCl_3$ interaction. Therefore,$FeCl_3$was selected as the optimal salt for all subsequent experiments. The results are shown in Table 2.

Table 2. Selection of the best metal ion.

Metalμg ml^{-1}	Chemical formula	Absorbance nm
Ferric chloride	$FeCl_3$	0.559
Cobalt chloride	$CoCl_2$	No conjugation
Copper chloride	$CuCl_2$	No conjugation
Ferrous chloride	$FeCl_2$	0.345
Calcium chloride	$CaCl_2$	No conjugation

3.4 The effect of the volume of $FeCl_3$:

The effect of ferrous ion on complex formation was studied by measuring the absorption at 386 nm for Levofloxacin. Different volumes of ferric ions (0.5-4 ml, 250 μg ml^{-1}) were tested to determine how the amount used affected absorption. Absorption was highest at 3 ml, while higher volumes (¿3 ml) markedly reduced absorption, as shown in Table 3.

Table 3. The effect of the volume of $FeCl_3$ on Absorption Intensity.

Vol. (ml) of $FeCl_3$ 250 μg ml^{-1}	Absorbance
0.5	0.170
1	0.280
1.5	0.302
2	0.325
2.5	0.526
3	0.559
3.5	0.512
4	0.490

3.5 The influence of the effective surface volume of Triton X-114:

The influence of the effective surface area is an important factor in maximizing extraction efficiency [21]. To evaluate the impact of Triton X-114 volume on the absorption of the resulting complex [22], separate extractions were performed with varying volumes of Triton X-114 (0.4–2.4 ml) at a fixed concentration (10% V/V). After extraction, the absorbance of the resulting Levofloxacin complex was measured at 386 nm. The results shown in Table 4 indicate that the use of (1.8) ml gives the highest absorbance for the complex formed.

Table 4. Effect of Triton X-114 surface volume on Absorption Intensity.

Vol.(ml) of Triton X -114 10% V/V	Absorbance
0.4	0.060
0.6	0.075
0.8	0.140
1	0.155
1.2	0.473
1.4	0.478
1.6	0.538
1.8	0.567
2	0.532
2.2	0.513
2.4	0.408

3.6 The effect of temperature:

The effect of temperature (25-90 °C) on the cloud point extraction reaction (CPE) was studied using the optimal conditions obtained from previous experiments. In this study, we

observed that absorbency reached its maximum at a temperature of 60 °C, for 20 minutes so this temperature was used in subsequent experiments. The results are shown in Table 5 and Table 6.

Table 5. Efect of Temperature and Heating Time on Absorption Intensity.

Parameter	Value	Absorbance
Temperature C	25	0.445
	40	0.504
	50	0.511
	60	0.566
	70	0.520
	80	0.497
	90	0.301
Heating Time (min)	10	0.409
	20	0.566
	30	0.528
	40	0.501
	50	0.426

3.7 Effect of time and centrifugal speed:

A study of the effect of centrifugal speed and time on the efficiency of complex formation (LEVO-Fe+3) showed that 4000 rpm for 15 minutes is sufficient to complete the complex. The results are shown in Table 6.

Table 6. Effect of centrifugal speed and centrifugation time on Absorption Intensity.

Centrifugal Speed (rpm)	Absorbance	Time (min)	Absorbance
1000	0.352	5	0.373
2000	0.498	10	0.525
3000	0.508	15	0.567
4000	0.558	20	0.549
5000	0.537	30	0.538
6000	0.499	40	0.500
		50	0.483
		60	0.370

3.8 Influence of addition order:

The sequence in which reagents are mixed can affect the kinetics and pathway of the complex formation. The results show that adding $FeCl_3$ to LEVO solution then Triton X -114 (denoted as D+M+T) yielded a significantly higher absorbance compared to the reverse order, establishing it as the optimal sequence, as shown in Table 7.

Table 7. Effect of reagent addition sequence on absorption intensity.

No.	Addition Order	Absorbance
1	D+M+Triton X-114	0.566
2	Triton X-114+D+M	0.519
3	M+Triton X-114+D	0.469

3.9 Influence of stability time on the product characteristics:

The stability study demonstrated that the compound formed by cloud point extraction exhibits excellent stability over time. It maintained this stability with only slight changes for more than 60 minutes. This long stability period indicates a strong interaction between the drug and $FeCl_3$ in the complex formation. The results are shown in Table 8.

Table 8. Effect of time on compound stability.

Time (min)	Absorbance
5	0.566
10	0.564
20	0.561
30	0.560
40	0.554
50	0.550

3.10 Final absorption spectrum:

After reaching optimal conditions, the final absorption spectrum was measured using 3.0 ml of LEVO (250 μg ml^{-1}), 3 ml of $FeCl_3$ (250 μg ml^{-1}) and 1.8 ml of 10% Triton X-114 surfactant were added. The volume was made up to the mark using a 25 ml volumetric flask, a yellow compound (LEVO-$FeCl_3$ complex) was formed. The mixture was heated in a water bath at 60 °C for 20 minutes, then transferred to test tubes and placed in a centrifuge at 4000 rpm for 15 minutes. The solutions were cooled in an ice bath. The product rich in the active surface was then dissolved in 1 ml of ethanol and the absorbance of the resulting compound (LEVO + $FeCl_3$)

was measured at 386 nm, while the blank solution gave negligible absorbance at the same wavelength. The results The results are shown in Table 9, Figure 2.

Table 9. The optimum conditions for the determination of LEVO.

Parameters	Value
Conc. of LEVO	250μg ml^{-1}
Amount of LEVO	3 ml
Type of metal	$FeCl_3$
Conc. of $FeCl_3$	250 μg ml^{-1}
Amount the metal	3 ml
Amount Triton X-114	1.8 ml
Temperature	60 C
Centrifugal force	4000 rpm
Time of centrifuges	15 min
λ max	386 nm

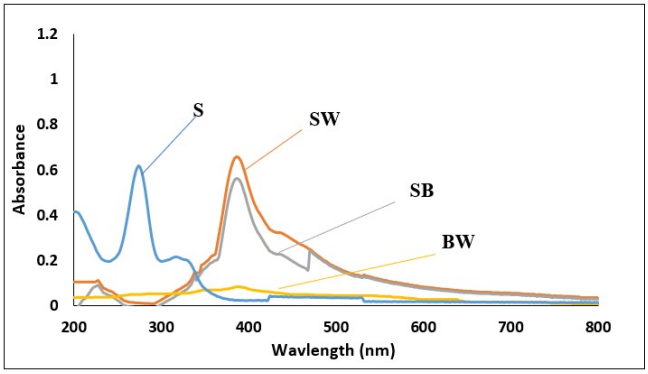


Figure 2. Final absorption spectra of LEVO determination versus blank solution (SB), sample versus distilled water (SW), blank solution versus distilled water (BW), and (S) Pure Levofloxacin.

3.11 The calibration graph:

After determining the optimal experimental conditions, a calibration curve was created between the concentration of (LEVO) and the change in absorbance at 386 nm. The calibration curve (Figure 3) showed a linearity of the method in the range of 5–45 μg ml. Higher concentrations showed a negative deviation from Beer's law, most likely due to saturation effects or aggregation of $FeCl_3$ at high drug concentrations. The regression equation for the linear range was determined to be $Y = 0.0183X + 0.1911$, with determination coefficient (R^2) of 0.9988. The molar absorptivity (ε) was calculated as 6.6×103 L.mol^{-1} cm^{-1}.

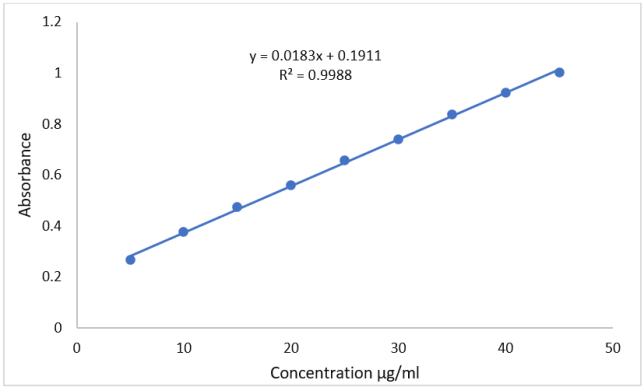


Figure 3. TThe calibration graph of the LEVOs. estimation using the suggested method.

3.12 Techniques used in diagnosing resulting complex (FE-SEM Analysis):

The (SEM) image of pure LEVO (Figure 4 A) shows that it is characterized by a regular semi-crystalline surface and fine grains of similar size. The particles are not completely spherical and tend to clump together as a result of interactions between the crystals. With an average compound diameter of 20 nm, this can be attributed to the different crystalline nature of the compound.

The SEM image of the complex (LEVO+$FeCl_3$) (Figure 4 B) indicates a clear change in the surface appearance of the drug after the cloud point extraction process, where small, relatively spherical particles with a relatively uniform distribution appeared within the phase rich in the active surface. This change indicates the formation of complexes between iron ions and drug molecules within the tail. Their average diameter is 31 nm. The increase in particle size upon complexation of Levofloxacin with Fe^{3+} ions is attributed to increased molecular weight because of coordination bond formation, possible formation of polymeric or network structures and reduced surface charge leading to aggregation, resulting in larger and less uniform particles compared to the free drug.

The findings confirmed the binding of the drug to iron ions and the subsequent formation of a new surface layer that differed morphologically from the pure drug. These changes provide evidence for the formation of a drug–iron complex with distinct surface characteristics and physicochemical properties.

3.13 The Nature of the formed product:

To determine the nature of the light-yellow product (chemical equivalence between the drug and salt), the Job's and the molar ratio methods were applied. In both methods, the concentration of the LEVO solution and the $FeCl_3$ salt solution was equal to 6.91×10^{-4} M.

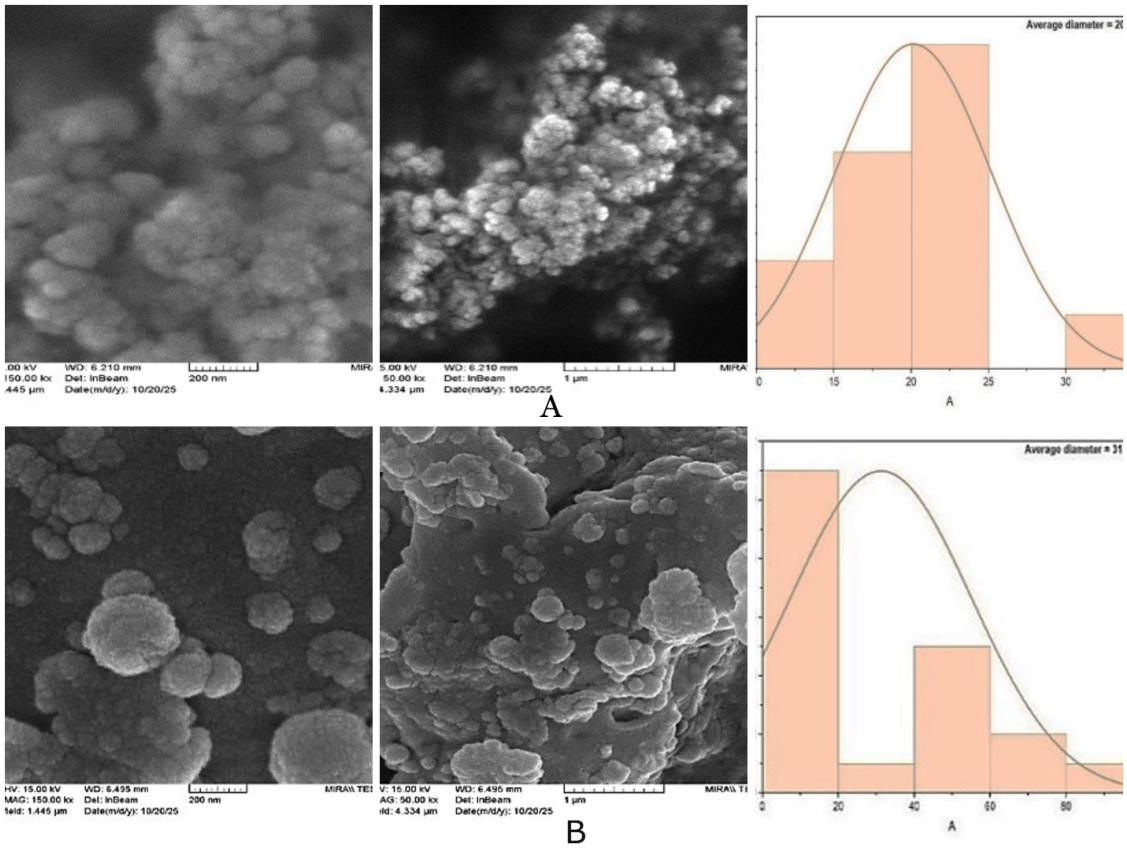


Figure 4. FE-SEM images and particle size distribution of **(A)** pure LEVO and **(B)** the LEVO -FeCl3 complex.

3.13.1 Continuous Variation Method (Job's Method):

In a series of 25 ml volumetric flasks, different volumes of Levo solution ranging from 0.5 to 4.5 ml and different volumes of $FeCl_3$ (4.5-0.5) ml was taken in the present of 10% Triton X-114 surfactant then the volumes were completed to 25 ml. The same ideal conditions were applied, and then the absorbance was measured at 386 nm against the blank solutions. The graph in Figure 5 shows that the binding ratio between the drug and $FeCl_3$ is 1:1.

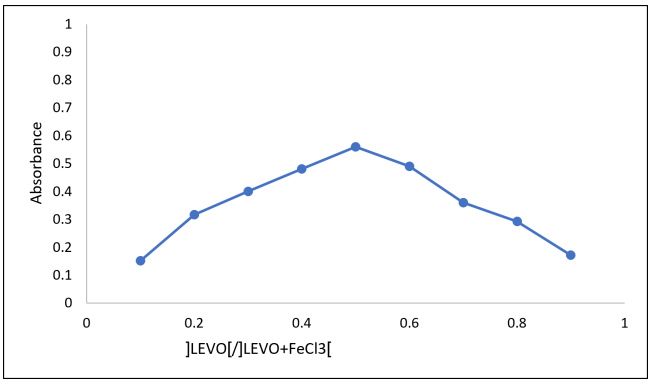


Figure 5. Job's method for the LEVO-$FeCl_3$ complex. .

3.13.2 Molar ratio method:

3.0 mL of the standard drug solution was transferred into a series of 25 mL volumetric flasks, and different volumes (0.5-5.0) mL of $FeCl_3$ salt solution were added. The volumes were made up to the mark with distilled water, and the absorbance was measured at 386 nm against the blank solution. The results are shown in Figure 6, which are consistent with the results of the Job's method.

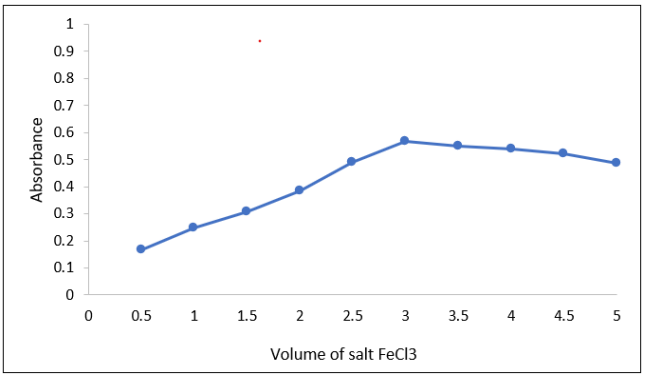
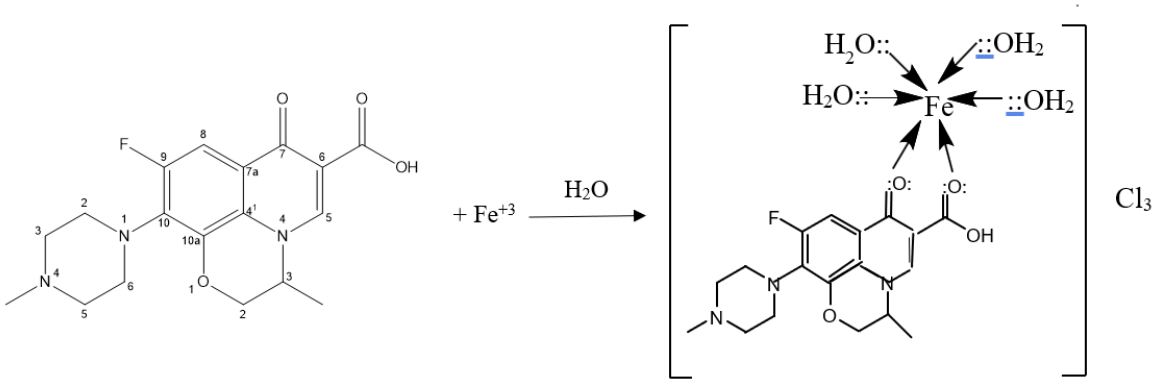


Figure 6. Molar ratio method for the Levo $FeCl_3$ complex.



Scheme 1. Proposed equation for the formed complex.

In Scheme 1 it is clear that H_2O completes the coordination number because it is present in the reaction medium as a solvent, and it participates as a co-ligand in completing the coordination number of the ferric ion, without being a principal reactant in the formation of the complex and it works to increase the stability of the complex in addition to it can be easily replaced, without it, the complex is weak or incomplete.

3.14 Direct method:

Three different concentrations (10, 25 and 40μg ml^{-1}) of the active compound (LEVO) were prepared from a 250μg ml^{-1} syrup stock solution and analyzed according to the calibration procedure. The absorbance was measured at the selected wavelength, the recovery percentage, and the relative error (RE%), moreover, the relative standard deviation (RSD%) was calculated. The results are presented in Table 10.

Table 10. Accuracy and precision of the proposed method.

Drug	Amount μg ml^{-1}	Found μg ml^{-1}	RE%	Recovery %	Average	RSD%
LEVO	10	10.158	1.58	101.58	100.10	0.238
	40	40.049	0.122	100.122		0.178
	45	44.366	-1.40	98.6		0.268

*Average of six times

3.15 Limits of detection and quantitation:

The limits of detection and quantitation were determined by measuring the absorption of the blank solution [23] six times at a wavelength of 386 nm under optimal conditions. The results are shown in Table 11.

Table 11. LOD and LOQ values of the blank solution

LEVO	
*Absorbance of Blank	0.083
Slope	0.0183
SD	0.0022
LOD μg ml^{-1}	0.402
LOQ μg ml^{-1}	1.092

*Average of six times

3.16 The applications part:

The proposed method was successfully applied for the determination of (LEVO) in its pharmaceutical formulation. The pharmaceutical formulation used was LEVOshr 500 mg, manufactured by the Indian company SAGA each tablet containing a labeled amount of 500 mg of Levofloxacin.

3.16.1 Direct method:

Three different concentrations (10.25 and 40 μg ml^{-1}) of the active compound (LEVO) were prepared from a 250μg ml^{-1} syrup stock solution and analyzed according to the calibration procedure. The absorbance was measured at the selected wavelength, and the recovery percentage, relative error (RE%), and relative standard deviation (RSD%) were calculated. The results are presented in Table 12.

3.16.2 The standard addition method:

To validate the accuracy of the developed method and confirm the absence of matrix interferences from the pharmaceutical formulation, the standard addition method was applied for the determination of LEVO in the tablets. The absorbance was measured against a reagent blank at the analytical wavelength of 386 nm. The results are presented in Table 13 and Figure 7.

Table 12. The results of the direct method for the determination of LEVO in pharmaceutical preparations.

Pharmaceutical Formulation	taken (μg ml^{-1})	Found μg ml^{-1}	*RE%	*Recovery %	RSD%
LEVOshr 500 mg	5	4.967	-0.655	99.344	0.317
	10	9.830	-1.693	98.306	0.364
	15	15.2	1.602	101.602	0.578

*Average of six times

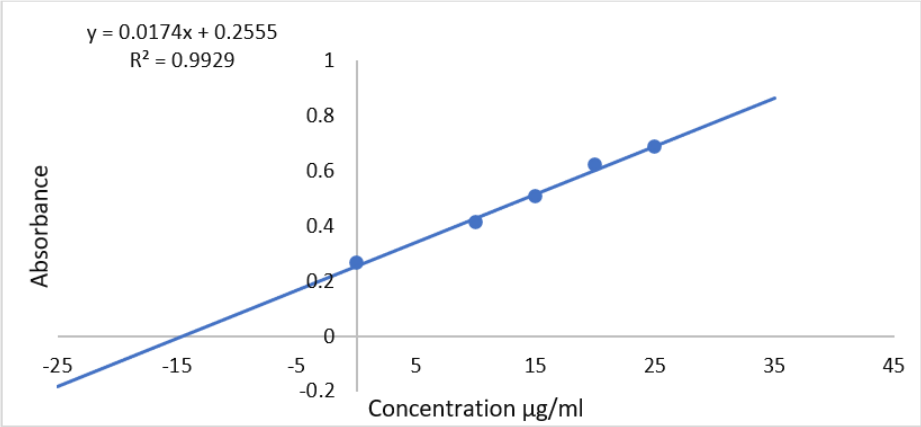


Figure 7. Standard addition curve for the determination of LEVO in tablets.

Table 13. Determination of LEVO in tablets using standard-addition method.

Drug	taken Amount of drug (μg ml^{-1})	Found Amount of drug (μg ml^{-1})	*RE%	Recovery %*
Tablet	15	14.68	-2.107	97.866

*Average of six determinations

4. Conclusion:

The proposed spectrophotometric method, combined with the cloud point extraction method, provides a simple, accurate, and reliable procedure for the determination of Levofloxacin in pure forms and pharmaceutical dosages. Optimal conditions ensured high sensitivity, good stability, and good reproducibility. Its application to commercial dosage formulations confirmed its accuracy and freedom from excipient interference. Due to its cost-effectiveness and simple procedures, this method is recommended for use in quality control of medicines in laboratories and factories and can be adapted for the analysis of similar active pharmaceutical ingredients in complex formulations.

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Data Availability Statement: All data supporting the findings of this study are available from the corresponding author upon request.

Declarations:

Conflict of interest: The authors declare no conflicts of interest.

Ethical approval: This study did not involve human or animal subjects; therefore, ethical approval was not required.

Author contributions: The first author conducted the experiments, collected the data, and wrote the draft. The second author designed the study, provided scientific supervision, and reviewed the research.

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طريقة طيفية محسنة باستخدام الاستخلاص بنقطة الغيمة لتقدير الليفوفلوكساسين في المستحضرات الصيدلانية

طلال عبد الله عطاوي * ، شهلة جمال شكور

قسم الكيمياء، كلية العلوم، جامعة كركوك، كركوك، العراق

* الباحث المسؤول: *sccm*24004@*uokirkuk.edu.iq*

الخلاصة

تقدم هذه الدراسة طريقة تحليلية بسيطة ودقيقة للتقدير الكمي لبعض الأدوية المضادة للالتهابات من فئة الفلوروكينولونات، وتحديدًا الليفوفلوكساسين، في صورته النقية وفي المستحضرات الصيدلانية، باستخدام طريقة الاستخلاص بنقطة الغيمة، والتي تتضمن تفاعل الدواء مع أيونات الحديديك (*III*) في ملح كلوريد الحديد الثلاثي ($FeCl_3$) ، وبوجود السطح النشط *Triton* $X-114$. أظهرت الطريقة المستخدمة خطية ممتازة ضمن النطاق 45 – 5 ميكروغرام عند الطول الموجي 386 نانومتر، مع دقة وحساسية عاليتين. ويتضح ذلك من حد الكشف (*LOD*) البالغ 1.346 ميكروغرام والحد الكمي (*LOQ*) البالغ 4.055 ميكروغرام وطبقت الطريقة على المستحضرات الصيدلانية مثل الأقراص التجارية والحقن الوريدية وبمعدلات استرداد عالية (*RSD%*).

الكلمات الدالة: ليفوفلوكساسين، الاستخلاص بنقطة الغيمة، مطياف ضوئي، قياس الطيف الضوئي للأشعة فوق البنفسجية والمرئية.

التمويل: لا يوجد

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: تضارب المصالح: يقر المؤلفان بعدم وجود تضارب في المصالح

الموافقة الأخلاقية: لم تتضمن هذه الدراسة أي تجارب على البشر او الحيوانات؛ لذلك، لم تكن الموافقة الأخلاقية مطلوبة. الحصول على موافقة أخلاقية

مساهمات المؤلفين: قام المؤلف الأول باجراء التجارب، جمع البيانات و كتابة المسودة كما قام المؤلف الثاني بتصميم الدراسة، الاشراف العلمي ومراجعة البحث وتعديله