Levofloxacin Determination in Pharmaceutical Tablets by Sensitive Spectrofluorometric Method with L-Tryptophan as a Fluorescent Probe

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***Abstract*—Proper dosage, therapeutic effectiveness, patient safety, and quality control throughout manufacture and storage can only be achieved by closely monitoring the concentration of pharmaceutical products. A precise and reliable spectrofluorometric approach for quantitative analysis and detection of levofloxacin (LEVO) in various pharmaceutical products was developed in this work using the fluorescent reagent L-tryptophan. When L-tryptophan, which has its inherent fluorescence signal quenched by LEVO, is mixed with Britton-Robinson buffer solution (pH 9.0), a stable ion-associated complex forms. The fluorescence intensity of L-tryptophan decreased at 365 nm after excitation at 281 nm. The method showed linearity for LEVO concentrations from 0.3 to 18.0 μg/mL, with a minimum detectable value of 0.10 μg/mL. An effective linear relationship ($R^2 = 0.9985$) between the concentration and fluorescence intensity (ΔF) was obtained. This technique has been well-proven to be minimally affected by impurities commonly found in pharmaceutical formulations. The results were validated through comparative analyses with high-performance liquid chromatography. The study revealed that both equivalence levels and analytical quality (as measured by precision and accuracy) are very satisfactory. This study addresses the increasing demand for established and reliable methods in the quality control of pharmaceutical products.**

***Index Terms*—L-tryptophan, Levofloxacin, Pharmaceuticals, Quenching, Spectrofluorometric.**

I. Introduction

Levofloxacin (LEVO) is a 3rd-generation fluorine class antibiotic. It works extremely well against a lot of germs (El-Yazbi, et al., 2020). The molecular mass is 361.37°g/mL as represented in Fig. 1 9-fluoro-2,3-dihydro-3-methyl-1,2,3-de-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido. It has strong antibacterial properties, such as inhibiting the growth of pathogens outside and within body cavities *in vitro* (in a test tube). It is effective to treat urinary tract infections (UTIs), lung infections, and gastrointestinal problems (Bano, et al., 2014). LEVO is one of the most commonly administered bacterial infection-specific medications in hospital outpatient settings. LEVO is used to treat acute and moderate illness, chronic bacterial prostatitis, infections of the skin and soft tissue with the UTIs, community-acquired pneumonia, and other mild-to-severe diseases (Elgendy, et al., 2024). A subgroup of quinolones designated fluoroquinolones is characterized by a fluorine substituent present on the sixth position of the naphthyridine ring (Bano, et al., 2014)

In the United States, LEVO is the only Food and Drug Administration -accepted respiratory fluoroquinolone for the treatment of patients with nocomial pneumonia. LEVO, the optical S-(-) isomer of ofloxacin, was created by Daiichi Seiyaku Pharmaceutical Co., Ltd. of Japan. Despite the fact that the two form a racemic mixture, the S-isomer of ofloxacin is 32–128 times more efficient against bacteria than the R-isomer; hence, the S-isomer is primarily responsible for the drug's antibacterial activities. Combining the strong antibacterial properties of ofloxacin with the relatively moderate toxicity profile of LEVO suggests that the former may be equally effective at half the usual dose (Une, et al., 1988; Tanaka, et al., 1992; Inage, et al., 1992; Nakamori, et al., 1995). The antibacterial spectrum of the novel broad-spectrum antibiotic LEVO surpasses that of the more recognized quinolones, including ciprofloxacin and norfloxacin. Anaerobes, atypical pathogens, and specific species among microorganisms that are Gram positive and Gram negative (Bano, et al., 2014).

Accurate quantification of LEVO is critically important to ensure the safety, efficacy, and regulatory compliance of pharmaceutical formulations. Over the years, numerous analytical techniques have been developed and refined for this purpose. Among them, ultraviolet (UV) spectrophotometry, fluorescent methods, and high-performance liquid chromatography (HPLC) have been widely employed (Ashour and Bayram, 2020; Da Silva, et al., 2012; Radi and El-Sherif, 2002; Devi and Chandrasekhar, 2009; Koeppe, et al., 2011; Ocaña, Callejón and Barragán, 2001; González, Mochón and De La Rosa, 2000).

ARO-The Scientific Journal of Koya University
Vol. XIII, No. 1 (2025), Article ID: ARO.12197. 11 pages
DOI: 10.14500/aro.12197
Received: 14 April 2025; Accepted: 04 June 2025
Regular research paper; Published: 24 June 2025
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http://dx.doi.org/10.14500/aro.12197

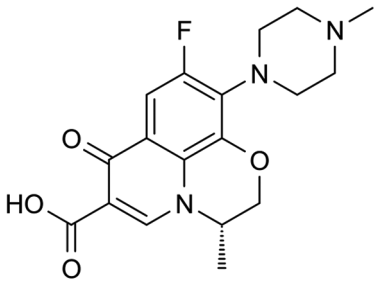

Fig. 1. Structure of levofloxacin in chemical terms.

In addition to pharmaceutical formulations, the analysis of LEVO in biological matrices such as blood, bile, soft tissues, and bones has been performed using microbiological tests and HPLC methods to determine drug concentrations (Aman, et al., 2010; Maurya, et al., 2021; Böttcher, et al., 2001). High-performance thin-layer chromatography (HPTLC) has also been explored for LEVO quantification in pharmaceutical samples (Davit, et al., 2009; Meyyanathan, et al., 2003). More recently, HPLC coupled with tandem mass spectrometry has been applied for precise measurement of LEVO levels in human plasma, providing enhanced sensitivity and specificity (Une, et al., 1988; Lu, et al., 2022; Szerkus et al., 2017; Zheng et al., 2020; Notario, et al., 2017). A considerable body of research has also addressed the detection of LEVO metabolites and its presence in various biological fluids (Elgendy, et al., 2024).

Despite these advances, many existing methods face practical limitations because of the complexity of mobile phases (buffers) and detection techniques, such as fluorescence and HPTLC, which restrict their routine applicability. Furthermore, while numerous analytical procedures have been established for LEVO in biological samples, there is a notable lack of stability-indicating methods designed for bulk LEVO samples contaminated with degradation products and process-related impurities (Nakamori, et al., 1995; Parys, Dołowy and Pyka-Pająk, 2022; Mehta, et al., 2010; Szerkus, et al., 2017; Elgendy, et al., 2024; Maurya, et al., 2021; Notario, et al., 2017). Our work addresses this gap by developing a reliable, stability-indicating analytical method that can accurately quantify LEVO in bulk samples, thereby enhancing quality control and ensuring pharmaceutical safety.

These accomplishments underscore the ongoing need for sensitive and versatile analytical techniques in pharmaceutical quality control. The objective of this study is to develop a simple, sensitive, and cost-effective spectrofluorometric method for quantifying LEVO in pharmaceutical tablets. By utilizing L-tryptophan as a novel fluorescent reagent, this approach aims to offer an efficient and reliable alternative to existing fluorescence probes and standard methods, enhancing the accuracy and practicality of LEVO analysis in quality control applications.

II. Materials and Methods

A. Instruments

Fluorescence spectra were recorded using a Cary Eclipse Fluorescence Spectrophotometer equipped with instrumentation from Agilent Technologies, USA. The system featured a xenon lamp as the light source and a grating monochromator for excitation and emission measurements. Both the excitation and emission monochromators utilized slits with a width of 5.0 nm, and measurements were carried out in a 1.0 cm quartz cell. The pH of the buffer solutions was measured using a digital pH meter (EUTECH, Thermo Fisher Scientific, USA).

B. Chemicals and Reagents

The study exclusively used high-purity chemical compounds provided by the suppliers, without any further purification performed. The tests were carried on with solutions prepared as solvents by using pure water, methanol, and sodium hydroxide.

To prepare a 0.1 moL/L NaOH solution, 0.2 g of NaOH (Merck, Germany) was dissolved in 50 mL of distilled water. The aqueous solution of L-Tryptophan 4.0×10^{-4} moL/L (Sigma-Aldrich) was prepared by dissolving 8.2 mg in 50 mL of 0.1 moL/L NaOH.

To make the Carbonate-Bicarbonate buffer (pH 9.2–10.6), 0.1 moL/L sodium bicarbonate ($NaHCO_3$) (Schallau, Spain) was mixed with sodium carbonate (Na_2CO_3) (Schallau, Spain) in the proper proportions. More carbonate increases pH, whereas more bicarbonate reduces it.

To prepare an ammonia-ammonium chloride buffer (pH 9–12) in water. The components were 0.1 moL/L ammonium chloride (NH_4Cl) and 0.1 moL/L ammonia solution (NH□OH) from Scharlau, Spain. They use an ammonia solution to elevate the pH, and ammonium chloride to lower it.

The Britton-Robinson buffer solution consists of a combination of 0.04 moL/L acetic acid (Scharlau, Spain), 0.04 moL/L boric acid (Sigma-Aldrich, USA), (Qader and Fakhre, 2017), 0.04 moL/L phosphoric acid (Merck, Germany). The addition of sodium hydroxide at a concentration of 0.2 moL/L is used to adjust the pH levels by using the pH meter (Abd Ali, et al., 2019).

C. Standard Stock Solution

The standard for LEVO was made in the Erbil-Kurdistan Region of Iraq, by Kurdistan Medical Control Agency (KMCA). A solution with a concentration of 100 μg/mL of LEVO is prepared by dissolving 5 mg of the drug in 50 mL of methanol, and the container should be stored in the refrigerator (Kaczmarek, Staninski and Stodolny, 2021).

D. Sample Preparation

Six different brands of medicinal pills were investigated. The weight of 10 individual pills from each brand was measured. The pills were then crushed into a fine powder using a mortar. The average weight of a single pill was calculated. Exactly 500 mg of LEVO was dissolved in 40 mL of methanol in a beaker. To enhance solubility, the mixture was stirred for 15 min. After filtration, the solution was transferred to a 50 mL volumetric flask, and the volume was adjusted to 50 mL precisely. This resulted in a solution with

a concentration of 10,000 µg/mL. The initial solution was then diluted stepwise to obtain a final concentration of 100 µg/mL. The same procedure was applied to all six brands of pills. Finally, the proposed method was used to analyze the diluted solutions.

Each LEVO tablet contains 500 mg, were received from a local pharmacy, and included the following brands: Brand 1: Levobest (Syria); Brand 2: Levoneer (Iraq); Brand 3: Levoking (Ukraine); Brand 4: Nevotek (Turkey); Brand 5: Levopyx (India); Brand 6: Advaquin (Oman).

E. Spectrofluorometric Assay for the Quantification of LEVO

Preparing all the standard and sample solutions separately, 0.235 mL of L-tryptophan solution was placed into a 10-mL volumetric flask. Next, 1.0 mL of Britton-Robinson buffer solution (pH 9.0) was added. Then, distilled water was added to complete the volume to 10 mL. The mixture was stirred for 15 min at 25 ± 5°C. A 1.0 cm quartz cell was used to measure the intensity difference of the fluorescence signal at λex = 281 nm and λem = 365 nm for each sample, standard solution, and blank solution (Tahir, et al., 2019).

III. Results and Discussion

A. Optical Response Spectroscopy

A 10 mL volumetric flask was employed to dilute 0.15 mL of the fluorescent reagent L-tryptophan (4.0×10^{-4} mol/L), and the emission spectra showed significant fluorescence at excitation 281 nm. Figure 2 shows A new ion-associated complex is formed along with its interaction with the target analyte, LEVO (5.0 µg/mL). The formation of this complex resulted in the complete loss of fluorescence intensity of L-Tryptophan. Consequently, to ascertain the quantity of LEVO, fluorometric studies were conducted at 365 nm after excitation at 281 nm, which involved quenching the fluorescence spectra of L-Tryptophan. The data from the spectrofluorometric analysis demonstrate a progressive reduction in L-Tryptophan fluorescence with increasing concentrations of LEVO, thereby validating the establishment of a stable complex.

The unique absorption outcome of the UV-visible spectrophotometry experiment is indicative of Bell-shaped dose–response curve of L-Tryptophan, LEVO, and their combination (Fig. 3). The L-Tryptophan molecule shows a significant peak in the UV-V range, especially between 250 and 300 nm, with a maximum absorbance of approximately 0.40.

In addition, there is another peak at approximately 230 nm, though this is less pronounced. In contrast, LEVO presented a small peak at 250 nm and a high absorption in between 270 and 300 nm. Absorbance increases dramatically from 270 to 300 nanometers due to interactions between the two chemicals. Based on spectrophotometric studies, the interaction of L-Tryptophan and LEVO is significant with time, which has been fluorescently evidence from a stable complex formation. It strongly suggests that L-Tryptophan may be used as a LEVO test reagent.

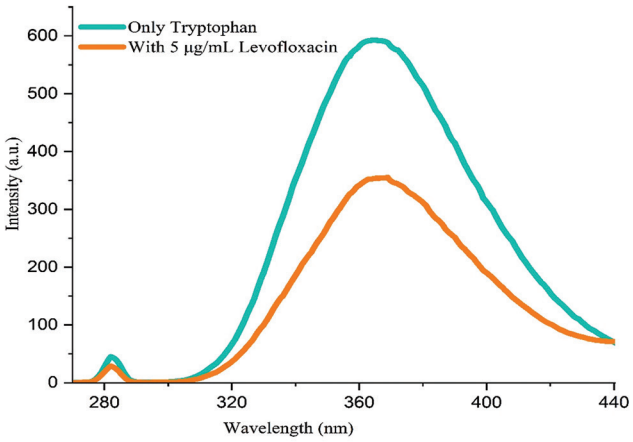

Fig. 2. Demonstrates the emission spectra of L-Tryptophan (0.15 mL) with a concentration of 4.0×10^{-4} mol/L and the reaction product of L-Tryptophan with levofloxacin (5.0 µg/mL) after being excited at 281 nm.

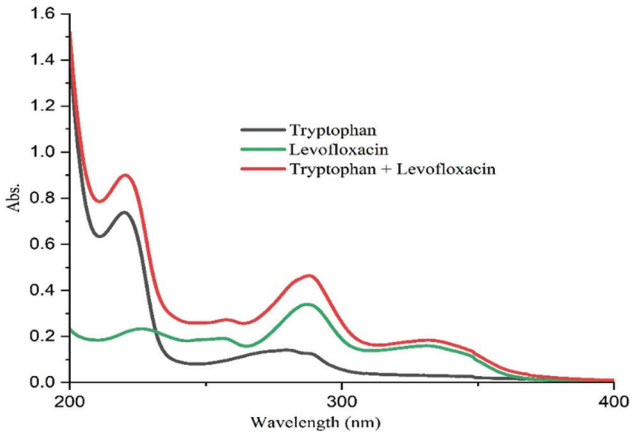

Fig. 3. The absorption spectra of L-Tryptophan, levofloxacin, as well as the combination of the two chemicals, emphasizing the different interactions and ultraviolet-visible features of both chemicals.

B. Optimization of Experimental Conditions

Chemical parameters associated with the reaction were systematically assessed for standardizing optimum sensitivity. Several different types of scenarios were considered to determine the most useful criteria for the proposed strategy. To obtain the maximal quenching fluorescence intensity (ΔF), the experimental conditions for fluorescence reactions were optimized. We meticulously varied the pH of the medium, concentration of L-Tryptophan, reaction duration, and the presence or absence of coexisting materials to acquire the optimal conditions for the proposed strategy.

C. Effects of Varied Buffers and Buffer Volume

LEVO (5.0 µg/mL) and fluorescent L-Tryptophan reagent (0.15 mL from 4.0×10^{-4} moL/L) were tested in several buffer solutions, including ammonia-ammonium, carbonate-bicarbonate, and Britton-Robinson buffers, to determine the best pH buffer for the fluorescence quenching. Fig. 4a shows that the fluorescence intensity was optimally quenched using

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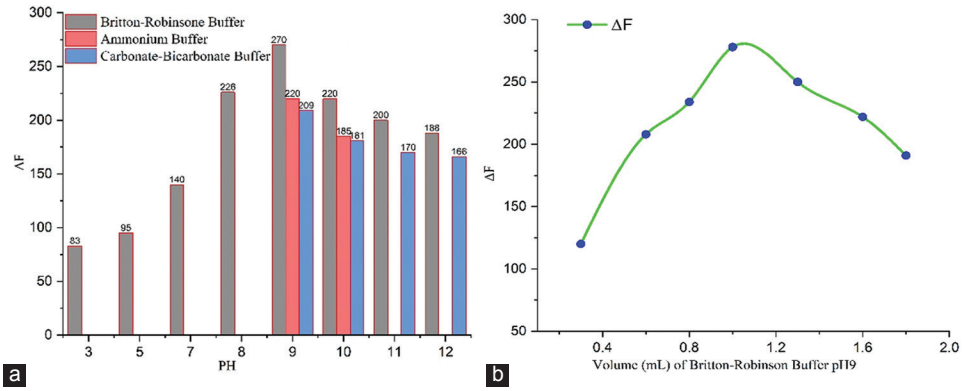

Fig. 4. (a) Different buffer solutions affect the ΔF intensity of L-Tryptophan and levofloxacin (5.0 µg/mL), (b) Changes in the volumes of Britton-Robinson buffer solutions influence the ΔF intensity of L-Tryptophan and levofloxacin.

the Britton-Robinson buffer solution at pH 9.0. Consequently, the following investigations will employ the Britton-Robinson solution, which has a pH of 9.0. This pH promotes a favorable ion-association complex between L-tryptophan and LEVO, diminishing the fluorescence response (Üçer, Ertekin, and Dinç 2024). The best buffer quantities were carefully examined; several amounts of Britton-Robinson buffer (0.3, 0.6, 0.8, 1.0, 1.3, 1.6, and 1.8 mL) were added to 5.0 µg/mL LEVO (Fig. 4b). The fluorescence signal produced for 1.0 mL of Britton-Robinson medium solution shows the most notable difference. This volume was chosen for additional experimental examination as a consequence.

D. Effect of L-Tryptophan Concentrations

To evaluate the effect of varying L-tryptophan reagent concentrations, the quenching signal was examined for volumes of 0.050, 0.100, 0.150, 0.200, and 0.235 mL from (4.0×10^{-4} moL/L). Fig. 5 shows the maximum fluorescence response, indicating that 0.235 mL of L-tryptophan was determined to be the most effective amount for further tests. This resulted in quenching because, following the addition of L-tryptophan, the two substances combine to create an ion-association complex that does not fluoresce and lowers the L-tryptophan fluorescence.

E. Impact of Response Duration

The effect of reaction time on fluorescence response was studied at room temperature (25.0 ± 5.0°C). After completely combining all of the reactants, the quenching signal gradually decreased (Fig. 6). The findings show that the quenching of the fluorescence signal decreased gradually over time, peaking at 15 min after mixing and remaining stable for at least 70 min. As a result, the quenching fluorescence signal for this process was examined 15 min later.

F. Analysis of Materials that Coexist

To determine how effectively the proposed fluorescence approach functioned analytically with real samples examined the impacts of common coexisting components, such as those found in medication tablets. LEVO and 0.235 mL of (4.0×10^{-4} moL/L) L-Tryptophan were introduced to 10.0 mL

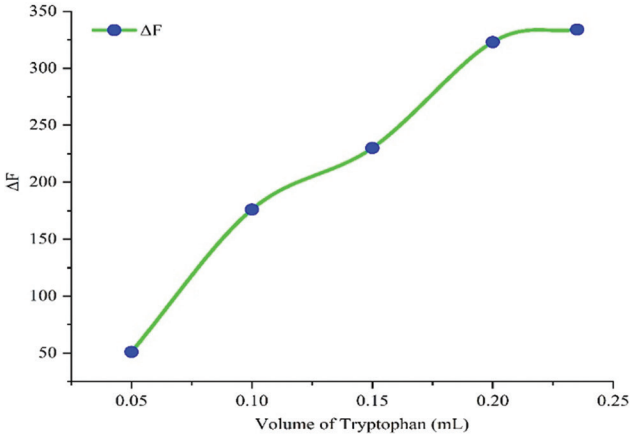

Fig. 5. The effect of different doses of L-Tryptophan on the ΔF fluorescence intensity reaction between L-Tryptophan and levofloxacin (5.0 µg/mL) at pH 9.0.

of a volumetric flask holding a number of coexisting compounds at concentrations of 500 µg/mL under optimum experimental conditions. After 15 min of stimulation at 281 nm, the decrease in L-Tryptophan fluorescence intensity was measured at 365 nm. Fig. 7 illustrates the results. With the permitted limit established at the concentration of the coexisting species, the quantitative examination of the quantity of LEVO revealed an error of no more than 3.0%. (Average of five determinations). Based on the data gathered, it was determined that there was no interference or influence since the ingredients – starch, lactose, stearic acid, magnesium stearate, cellulose, and silicon dioxide – were present in the standard ratios used to manufacture pharmaceutical tablets.

G. LEVO Detection and the Calibration Graph

Fig. 8a depicts the fluorescence intensity interaction of L-tryptophan with varying amounts of LEVO after optimal circumstances have been established. The results showed that when the quantity of LEVO in the solution increased, the fluorescence signal gradually decreased, with the intensity of the quenched signal exactly proportional to the concentration of LEVO. As a result, the L-Tryptophan reagent serves as a

fluorescent agent for the quantitative assessment of LEVO. Fig. 8b shows a calibration curve based on the fluctuation in different fluorescence intensity (ΔF) versus LEVO concentration under optimum circumstances. The calibration curve showed a linear correlation ($\Delta F = 40.888\ C_{Levo}$ (μg/mL) + 47.139) at concentrations ranging from 0.3 to 18.0 μg/mL, with a correlation value of 0.9985. The International Union of Pure and Applied Chemistry standards specify that the limit of detection (LOD) is computed as LOD = 3.3 standard deviation (SD)/P, while the limit of quantification (LOQ) is established as LOQ = 10 SD/P (Qader, 2024). In this case, SD is the SD obtained from five reagent blank measurements, whereas P represents the slope of the calibration curve. The limits of detection (LOD) and LOQ for LEVO were established at 0.100 μg/mL and 0.294 μg/mL, respectively.

H. Exactness and Correctness of the Fluorometric Technique

The calculation of the SD, which measures the degree of variance or dispersion within a collection of values, was part of the assessment of the precision and accuracy of the fluorometric approach. Five repeated aliquots of three standard solutions of LEVO at concentrations of 0.3, 8.0, and 18.0 μg/mL were used to compute the relative SD (RSD %) and percentage error. Table I presents the results. The method exhibits a great degree of accuracy, with the defined ranges of RSD % being 0.48%, 1.01%, and 1.28%. Furthermore, it was found that the percentage error ranged from 3.33 to −1.7, suggesting that the established approach exhibits a high degree of accuracy.

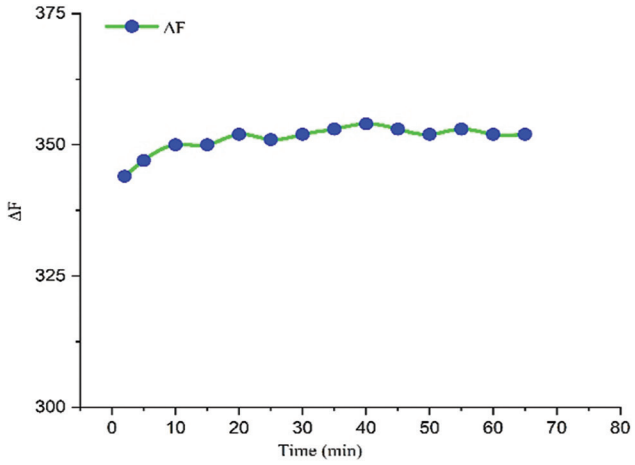

Fig. 6. The duration of the reaction influences the ΔF intensity in the fluorescence reaction involving 0.235 mL of (4.0×10^{-4} moL/L) L-Tryptophan and 5.0 μg/mL of levofloxacin at pH 9.0.

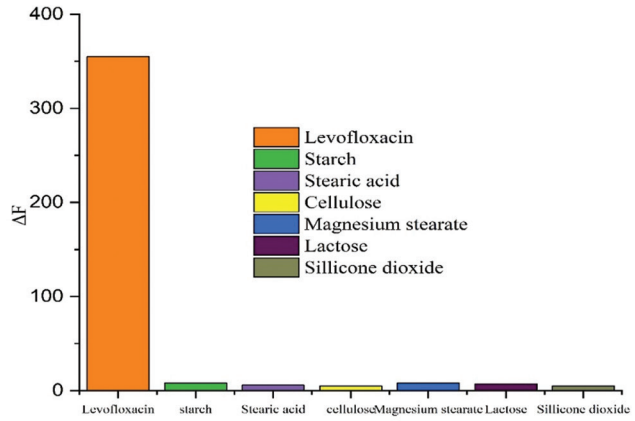

Fig. 7. The impact of standard excipients in pharmaceutical tablets on the fluorescence detection of levofloxacin reveals interference from coexisting substances, including lactose, starch, stearic acid, magnesium stearate, cellulose, and silicon dioxide.

I. Fluorometric Reaction Stoichiometry

To determine the stoichiometry reaction between the fluorescent reagents L-tryptophan and LEVO, the limiting logarithmic approach was applied (Qader and Fakhre, 2017; Tahir, et al., 2019). Fig. 9a illustrates a graph depicting the relationship between log (LEVO) and log ΔF, with L-Tryptophan concentration held constant. Conversely, (Fig. 9b) depicts log (L-Tryptophan) in relation to log ΔF while maintaining a constant concentration of LEVO. The analysis of both plots revealed linear segments, and by evaluating the slopes of these lines, the results indicated that the LEVO -L-tryptophan complex had a 1:1 reaction ratio (Fig. 10).

J. Fluorescence Quenching Mechanism

Fluorescence quenching refers to the interaction of fluorescent compounds with quenching agents. Two basic mechanisms impact the decline in fluorescence intensity: static quenching and dynamic quenching. When the fluorescent species in its ground state interacts with the quencher species, a complex structure forms in a static quenching mechanism. Dynamic quenching, on the other hand, occurs as a result of interactions between excited fluorescent species and quencher species, which are aided by collisions (Abd Ali, et al., 2019). The study findings reveal that the process of fluorescence quenching may be quantitatively examined using the Stern-Volmer equation (Park, et al., 2009; Duan, et al., 2017; Gökoğlu, Kıpçak and Seferoğlu, 2014; Yang et al., 2018).

$$F_0/F = 1 + K_{SV}\ C_{que}$$

The words F and F_0 relate to the intensity of the fluorescence signals generated by L-Tryptophan when employed as a fluorescence reagent, both when the quencher (C_{que}) is present and when it is not. The Stern-Volmer equation is used to

TABLE I
DATA GATHERED FOR THE SUGGESTED FLUOROMETRIC MEASUREMENT OF LEVOFLOXACIN UTILIZING L-TRYPTOPHAN AS THE FLUORESCENT REAGENT IN ORDER TO ENSURE PRECISION AND ACCURACY

Contained value (μg/mL)	Found value (μg/mL)	SD	RSD%	Error[1]%
0.3	0.31	1.42	0.15	3.33
8.0	8.21	1.63	0.35	2.62
18.0	17.68	−1.58	0.79	−1.70

SD: Standard deviation, RSD %: Relative standard deviation. [1]Mean value derived from five separate measurements

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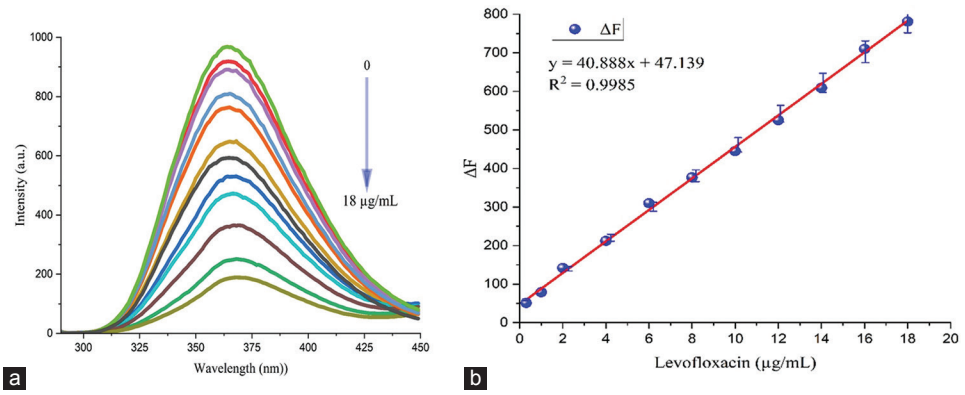

Fig. 8. (a) The influence of various Levofloxacin concentrations (0.3, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, and 18.0 µg/mL) on the fluorescence intensity of the L-Tryptophan reagent at pH 9.0 was examined (b) Calibration graph for the fluorometric quantitative measurement of levofloxacin utilizing L-Tryptophan as the fluorescent reagent.

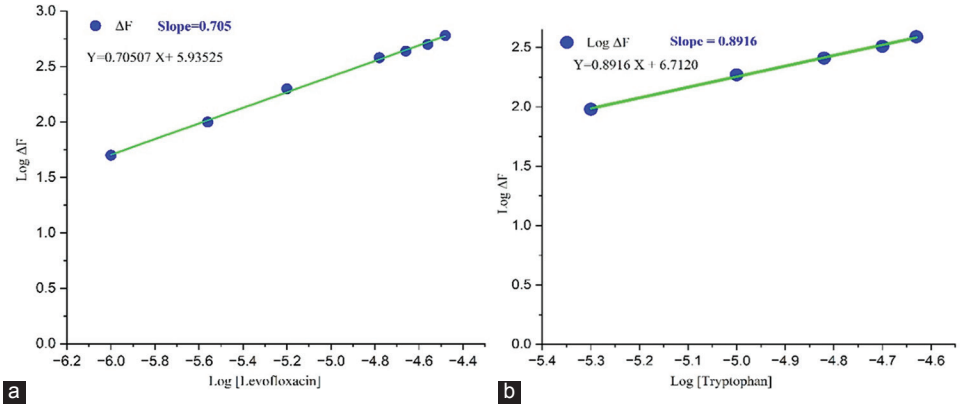

Fig. 9. Illustrates the stoichiometric ratio of the fluorometric reaction involving the L-Tryptophan reagent and levofloxacin (LEVO) (a). Graphing log (LEVO) against log ΔF (b), Graphing log (L-Tryptophan) against log ΔF.

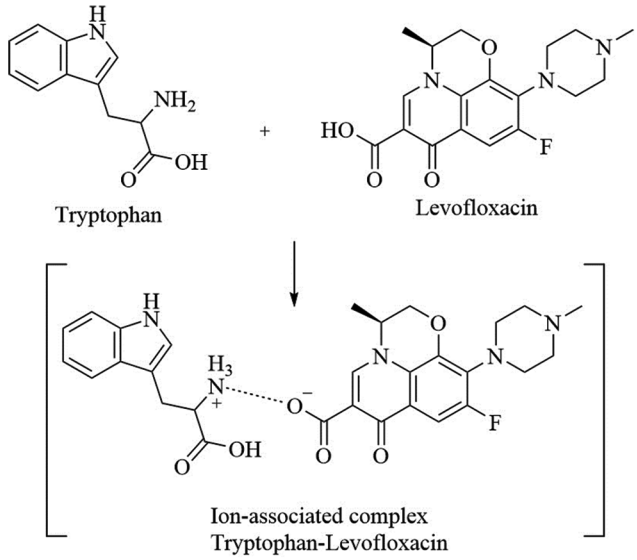

Fig. 10. The formulation of an L-Tryptophan-levofloxacin ion-associated combination.

study fluorescence quenching and to distinguish between static and dynamic quenching at various temperatures. At this stage, the Stern-Volmer quenching constant (K_{SV}) is utilized (Duan, et al., 2017). The equation was used to examine fluorescence quenching at two different temperatures (298.0 and 308.0 K) (Yang, et al., 2018). Fig. 11 shows Stern-Volmer graphs of F_0/F versus C_{que} (LEVO concentration) at two temperatures. A static quenching process is characterized by a reduction in the Stern-Volmer quenching constant (K_{SV}) with increasing temperature, while an increase indicates a dynamic quenching process. The data indicates a linear connection between F_0/F and C_{que}, suggesting a mathematical link (Fig. 11 at both temperatures). This suggests that the findings were made mostly through quenching (Park, et al., 2009; Duan, et al., 2017). As temperature rises, the K_{SV} value decreases, suggesting static quenching as the primary cause of fluorescence quenching.

K. Analysis using the Fluorometric Method

In a structured analysis, the fluorometric technique provides a method for quantifying LEVO in pharmaceutical tablets, utilizing L-tryptophan as a fluorescent reagent. To evaluate the reliability of this approach, the results are compared with those obtained from a standard HPLC method provided by the KMCA in Erbil, Iraqi Kurdistan. As shown in Table II, the findings demonstrate a strong degree of agreement between the proposed fluorometric method and

TABLE II
THE EXAMINATION OF COMMERCIAL PHARMACEUTICAL TABLETS CONTAINING LEVOFLOXACIN WAS CARRIED OUT UTILIZING THE SUGGESTED FLUOROMETRIC METHOD WITH L-TRYPTOPHAN REAGENT IN CONJUNCTION WITH THE STANDARD HPLC METHOD

Pharmaceutical tablets	Observed Values (mg)	Proposed Procedure (mg)	HPLC Procedure (mg)	Recovery %	Error[1]%
Brand 1	500	493.5	506.0	97.5	-2.47
Brand 2	500	491.0	492.0	99.8	-0.20
Brand 3	500	498.5	506.5	98.4	-1.58
Brand 4	500	508.5	520.5	97.7	-2.30
Brand 5	500	488.5	471.5	103.6	3.61
Brand 6	500	501.0	491.5	101.9	1.93

[1]Mean value calculated from five measurements

TABLE III
COMPARISON OF THE ANALYTICAL PERFORMANCE METRICS OF THE PRESENT RESEARCH WITH OTHER RELEVANT TECHNIQUES

Reagent	Methods	LOD (μg/mL)	Linear ranges (μg/mL)	References
Terbium (III) ions were used to oxidize a subset of fluoroquinolones using potassium bromate	Spectrofluorometric	0.11	0.3–13	Kaczmarek, Staninski and Stodoln, 2021
Graphene oxide composites coated with silver nanoparticles (GO@AgNPs)	Voltammetric	0.012	0.0154–788.38	Chinnaraj, et al., 2021
HPLC method with fluorescence detection	HPLC-fluorescence	6.3×10^{-4}	0.0025–0.5	Toker, Kızılçay and Sagirli, 2021
The carbon paste electrode (CPE) is produced from a film created via the electropolymerization of α-cyclodextrin (CPEα-CD).	Spectrofluorometric	0.21	1–90	Hashmi, et al., 2021
Graphene quantum dots, magnetite (Fe3O4) nanoparticles, and porous graphene form a selective molecularly imprinted polymer for levofloxacin detection	Spectrofluorometric	0.3×10^{-3}	0.00010–0.0250	Chansud, Longnapa and Bunkoed, 2021
This method is grounded on the fact that levofloxacin can still quench the intrinsic fluorescence signal of L-Tryptophan and they can be combined to form a stable complex by introducing ions as a bridge.	Spectrofluorometric	0.100	0.3–18	Present work

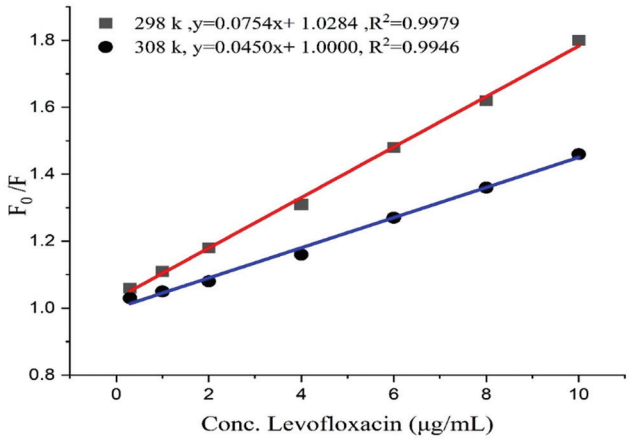

Fig. 11. Exhibits the fluorescence quenching process via a Stern-Volmer plot at temperatures of 298.0 K and 308.0 K.

the HPLC reference method. The recovery percentages for all brands range from 97.5% to 103.6%, indicating the proposed method's accuracy and reliability in detecting LEVO content close to the labeled 500 mg. The error percentage values also remain within an acceptable range, further supporting the method's precision. For instance, Brand 2 shows a nearly negligible error of −0.20%, while Brand 5 exhibits a slightly higher positive error of 3.61%, yet still within acceptable pharmaceutical analytical limits. These results confirm that the fluorometric method is suitable for routine analysis of LEVO in commercial formulations.

L. Comparison with other Reported Methods

Other methods for measuring LEVO have been compared to the present one. Table III compares the LEVO LOD and linear ranges obtained in this study with those reported in previously published methods. Although the LOD of the proposed spectrofluorometric method (0.100 μg/mL) is only slightly lower than that of some other fluorometric techniques, such as the method using terbium (III) ions (0.110 μg/mL) (Kaczmarek, Staninski and Stodolnym, 2021), this improvement reflects the enhanced sensitivity achieved through method optimization. Importantly, the suggested technique eliminates the need for sample pre-extraction, a limitation in some analytical procedures with lower LODs. In addition, the proposed method offers several advantages over older techniques, such as HPLC and voltammetry, including simplicity, higher sensitivity, shorter analysis time, and lower cost. These features make the present method highly suitable for routine analysis of LEVO in pharmaceutical formulations.

IV. CONCLUSION

The present study conducted a quantitative investigation of levofloxacin using L-Tryptophan as a fluorescence-quencher agent. The method demonstrated an excellent linear fit (R^2 = 0.9985) and a minimum detectable concentration of 0.100 μg/mL over a range of 0.3–18 μg/mL. This method is used for accurate and reliable measurement of LEVO in pharmaceutical compositions. Offering significant advantages owing to its

straightforwardness and the ability to immediately quantify the ion-associated complex. This eliminates the need for further processing or extraction methods. This method demonstrates good specificity, as common pharmaceutical excipients found in tablet formulations – such as starch, lactose, stearic acid, magnesium stearate, cellulose, and silicon dioxide – produce negligible interference, typically causing signal changes of <3%. This underscores the reliability of L-tryptophan as an innovative fluorescence quenching reagent with promising implications for drug analysis.

V. Acknowledgment

We thank the directors of the chemistry departments of Koya University of Iraq for giving us permission to carry out our research study in their laboratories.

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