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Development and Validation of Indirect Spectrophotometric Methods for Assessing Levofloxacin in Pure and Pharmaceutical Formulations

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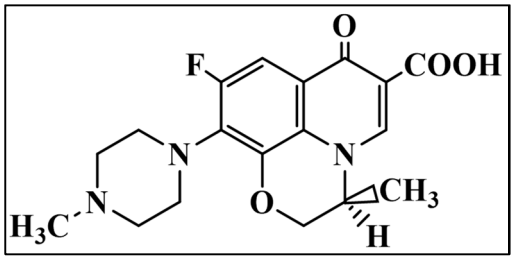
Abstract

Two simple, sensitive, and economical indirect spectrophotometric methods were developed for the determination of levofloxacin (LEVX) in the pure form and pharmaceutical preparations. The first proposed method (A) involved oxidation of LEVX using an excess amount of N-bromosuccinimide (NBS) under acidic 209conditions. Once the reaction was complete, the residual NBS was determined by shortening the color of Orange G (OG) dye, with the absorbance of the remaining dye measured at 476 nm. The second proposed method (B) is also based on the oxidation of LEVX using a fixed excess amount of NBS in an acidic environment. The excess NBS then reacts with p-Anisidine (P-AN), producing a violet-coloured brominated product. The intensity of the resulting colour, measured at 518 nm, is inversely proportional to the amount of LEVX present in the sample. Linear calibration curves were obtained for both methods within the concentration ranges of 0.5 – 10.5 and 1.0 – 15.0 µg.mL^{-1}, respectively, with correlation coefficients of 0.9990 and 0.9992. The molar absorptivity values were 4.17×10^4 and 2.92×10^4 L.mol^{-1}.cm^{-1} for methods A and B, respectively. Whereas, Sandell's sensitivity index values were 0.00867 and 0.0123 µg.cm^{-2}, respectively. The limits of detection (LOD) were 0.014 and 0.46 µg.mL^{-1}, and the limits of quantification (LOQ)were 0.12 and 0.40 µg.mL^{-1} for methods A and B, respectively. The proposed methods were effectively used to estimate LEVX in available dosage forms, and their validity was confirmed through a recovery study using the standard addition method.

Keywords: Levofloxacin, Spectrophotometric, N-Bromosuccinimide, Orange G, P-Anisidine.

Introduction

The IUPAC name of Levofloxacin (LEVX) is(−) -(S)-9-fluoro 2,3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl) -7 -oxo -7H-pyrido[1,2,3-de]-1,4-benzoxazine -6-carboxylic acid. It is a translucent powder with an off-white to yellow color and no smell. It is soluble in water and methanol to a limited extent [1]. Chemical composition of LEVX is shown in the following structure [2].



Levofloxacin($C_{18}H_{20}FN_3O_4$) M.Wt = 361.373 g/mol

One of the second-generation synthetic fluoroquinolones, referred to as respiratory quinolones, is an L-isomer of LEVX [3]. It exhibits a wide range of bactericidal efficacy against gram-positive and gram-negative aerobes [4], and demonstrates a greater tendency to migrate in the direction of gram-positive bacteria and a lesser one toward gram-negative ones [5]. It prevents bacterial DNA gyrase from supercoiling, and the aforementioned mechanisms are what cause the bacteria to die [6]. LEVX is used to treat a variety of conditions, including conjunctivitis, chronic prostatitis, bacterial sinusitis, chronic bronchitis, mastitis, abdominal infections, gastroenteritis, local infections, and acute pyelitis [7]. It is a key medication for treating multidrug-resistant tuberculosis [8]. Given the medical importance and widespread use of LEVX, a number of analytical procedures are available for its determination in dosage forms and biological fluids using different techniques reported in scientific literature, including spectrophotometric [9-17], electrochemical [18-21], and HPLC methods [22-25].

Most of the procedures mentioned require expensive equipment and skilled operations. This research aimed to develop two rapid, accurate, and sensitive spectroscopic methods for the determination of LEVX in pure form and pharmaceutical preparations. These methods rely on the oxidation of LEVX by excess NBS and then estimating the remaining NBS in two different ways using the OG dye and P-AN reagent.

Materials and Methods

Chemicals and reagents

In this study, high-purity materials supplied by Fluka (Burlington, Massachusetts, United States) and LCH Sweden AB (Stockholm, Sweden) Institutions, with a purity of not less than 98% (central supply) were used. Levofloxacin was purchased from SDI (Samarra Pharmaceutical Company, Iraq).

Stock solution of LEVX 1000 µg.mL^{-1}

The stock solution was prepared by dissolving 0.10 g of pure LEVX in a sufficient quantity of water with shaking, then diluted to 100 mL with distilled water in a volumetric flask. The working solution 50 µg.mL^{-1} (1.38×10^{-4} M) was obtained from diluting 5 mL of stock solution to 100 mL of distilled water for method A, and a working solution (100 µg.mL^{-1}) (2.76×10^{-4} M) was prepared by diluting 10 mL of stock solution to 100 mL of distilled water for method B.

Stock solution of N-bromosuccinamide (NBS)1000 µg.mL^{-1}

The stock solution was prepared by dissolving 0.10 g of NBS in distilled water and diluted to 100 mL with distilled water in a volumetric flask. The working solution 400 µg.mL^{-1} used in method A was obtained by diluting 40 mL of stock solution to 100 mL of distilled water, and the working solution 800 µg.mL^{-1} used in method B was obtained by diluting 80 mL of stock solution to 100 mL of distilled water.

Orange G (OG) solution 500 µg.mL^{-1} (1.1×10^{-3} mol.L^{-1})

OG solution was prepared by dissolving 0.05 g of dye in 10 mL of distilled water with shaking and diluting to 100 mL with distilled water using a volumetric flask.

Hydrochloric acid 0.1 mol.L^{-1}

It was prepared by diluting 0.847 mL of concentrated hydrochloric acid (11.8M**)** to 100 mL with distilled water using a volumetric flask.

P-Anisidine (P-AN) solution 800 µg.mL^{-1} (1.1 ×10^{-3} M)

P-AN solution was prepared by dissolving 0.08 g of powder in distilled water and diluting to 100 mL with distilled water using a volumetric flask.

Acetic acid stock solution 0.1 M

The stock solution was prepared by diluting 0.57 mL of concentrated acetic acid (17.4M) to 100 mL with distilled water using a volumetric flask.

Pharmaceutical solutions

Tablet solution (100 µg.mL^{-1})

10 Levosol therapeutic tablets (each tablet contains 500 mg of LEVX) were weighed carefully (7.0988 g), provided by Pharma Solution LLC – Jordan. Then it was ground and mixed well, and took 0.0142 g, which was equivalent to 0.01g of the pure compound dissolved in 30 mL of distilled water, then filtered and transferred to a 100 mL volumetric flask, and the volume was filled with distilled water up to the mark.

Intravenous solution (100 µg.mL^{-1})

This solution was prepared by withdrawing 2 mL of Levoneer intravenous solution (Each 100 mL vial contains 500 mg of LEVX) supplied by PIONEER Pharmaceutical Industries – Iraq, and transferred to a 100 mL volumetric flask and filled with distilled water up to the mark and then stored in a dark place.

Apparatus

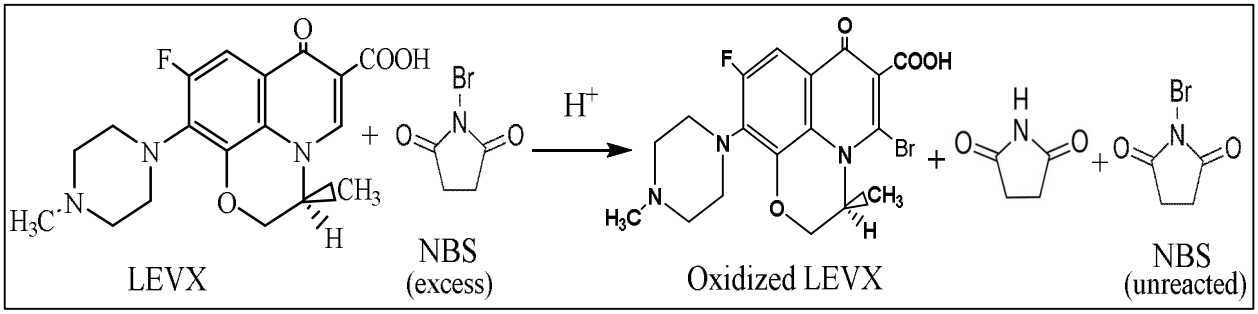
All absorption spectra and the measurements of absorbance were done by using a SHIMADZU – UV1900i double-beam UV-vis spectrophotometer – Japan with 1.0-cm quartz cells. Sensitive balance model Kern & Sohn analytical balance ABS 120-4N – Germany, was used to measure the weight. A BP3001 pH meter (professional) was used to measure the pH value.

Results and Discussion

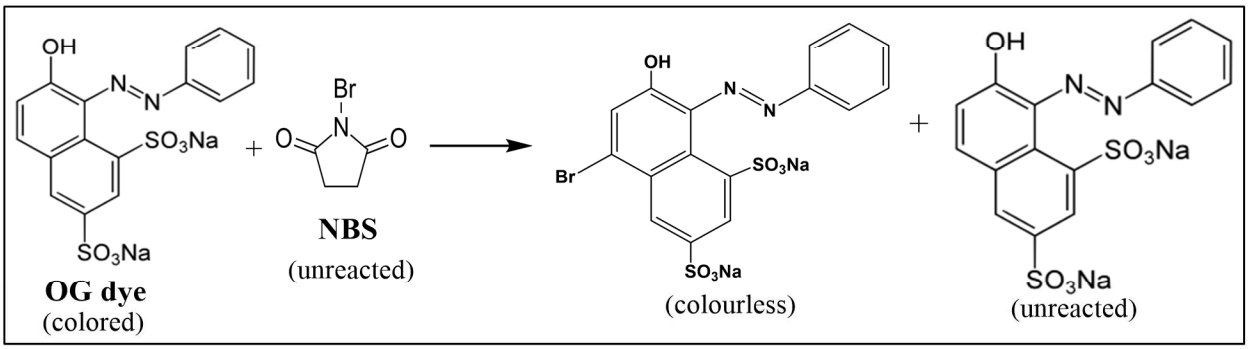
Method A

The principle of the proposed method and the suggested chemical reaction is based on two steps. The first step includes oxidation of LEVX using an excess of the oxidizing agent NBS in acidic (HCl) medium, shown in Scheme 1 [26,27].

Whereas in the second step, a (fixed) amount of OG dye was oxidized by unreacted NBS to a colorless product. Finally, the remaining amount of dye was measured at 476 nm, which is proportional to the concentration of LEVX as shown in Scheme 2.



Scheme 1



Scheme 2

Optimum Reaction Conditions

The following experiments were conducted in 10 mL volumetric flasks with 1 mL of LEVX working solution (50 µg.mL^{-1}) and measuring OG dye absorption at 476 nm.

Amount of OG dye

To obtain the amount of OG dye that can be used in determining LEVX, and which follows Beer's law, increasing amounts of 500 µg.mL^{-1} OG dye solution was added to a series of 10 mL volumetric bottles containing 1 mL of 0.1 M HCl. The volume was supplemented to the mark with distilled water and the absorbance was measured at 476 nm. The standard curve, as shown in Fig. 1, shows that 1.0 mL of 400 µg.mL^{-1} of dye was the best volume that provided high absorbance within the linear relationship.

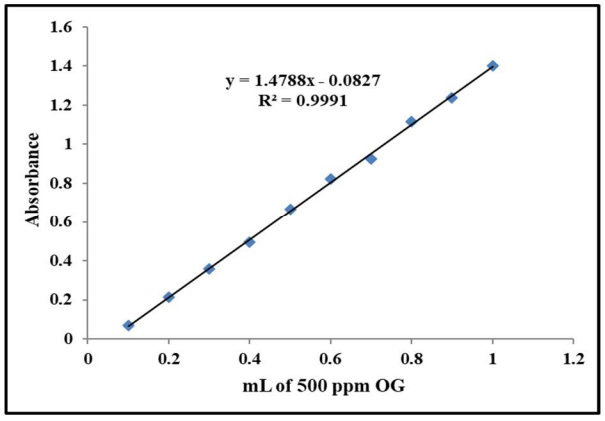


Figure 1. **Selection of the best amount of OG dye**

Selection of an oxidant agent

To find the best oxidizing agent, 1 mL of available oxidizing agents that decolorized OG dye (N-bromosuccinamid, Sodium periodate, Potassium iodate, and Potassium dichromate) with conc. 400 µg.mL^{-1} of each one into a 10 mL volumetric flask, which contains 500 µg OG dye and 1 mL of 0.1 M HCl, then the volume was completed to the mark with distilled water and the absorbance was measured at 476 nm. Fig. 2 shows that NBS provides the best results, so it was chosen in the subsequent experiments.

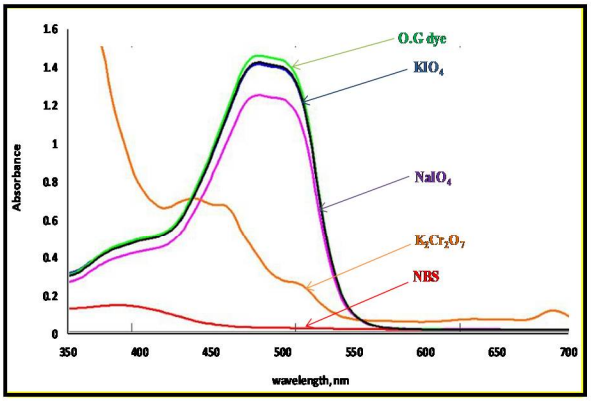


Figure 2. **Overlaid UV-visible spectrum of various oxidizing agents**

Amount of oxidant agent

The amount of NBS was studied by varying the volume of NBS solution (400 µg.mL^{-1}) while other parameters were kept constant in a series of 10 mL volumetric

flasks. It was found that 0.8 mL of 400 µg.mL^{-1} NBS solution was sufficient to completely ablate 500 µg of OG dye, so this amount was kept constant in subsequent experiments.

Effect of acidity

This study was carried out using different types of strong and weak acids and monitored the extent of their effect on the oxidation of LEVX and palace the color of the dye. By adding 1.0 mL of these acids at a concentration of 0.1 M, the results obtained showed that hydrochloric acid gave the best absorption value. Therefore, this acid was used in subsequent experiments.

Effect of the volume of hydrochloric acid

The volume of hydrochloric acid (0.2 to 1.2 mL) needed to complete the oxidation process and bleach the color of the dye was studied and the results are shown in Table 1. It was observed that using 1.0 mL of 0.1M hydrochloric acid was the optimal volume, so it was adopted in subsequent experiments.

Table 1. **Effect of the volume of hydrochloric acid on absorbance.**

mL of 0.1M HCl	Absorbance/µg.mL^{-1} of LEVX				R^2
	1	2	5	10	
0.2	0.068	0.136	0.237	0.339	.95670
0.5	0.088	0.169	0.291	0.473	0.9867
0.8	0.095	0.193	0.385	0.698	0.9969
1.0	0.104	0.213	0.421	0.828	0.9981
1.2	0.101	0.206	0.402	0.809	0.9975

Effect of oxidation time

In this study, the time required to oxidize 50 µg of LEVX was evaluated by waiting for different periods after adding the oxidizing agent with hydrochloric acid and before adding the dye, as well as waiting for different periods after adding the dye and before diluting with distilled water to the mark, to complete the dye oxidation process. The obtained results showed that the time required to oxidize the LEVX was 3 min, and the time required to complete the oxidation of the dye was 5 min.

Effect of temperature and stability

The stability of the absorption value of the reaction product was studied at different temperatures, i.e., 5, 25, and 40 ◦C. It was found that the absorption was constant for 40 min at room temperature (25±2 ◦C).

Effect of surfactants

This study was conducted to determine the effect of adding different types of surfactants (neutral, negative, and positive) on dye absorption by adding different volumes of these agents to the reaction components, and the results are listed in Table 2. From observing the results, it was found that adding surfactants did not give positive results, but rather decreased the absorption values of the colored product, so surfactant use in subsequent experiments was excluded.

Table 2. **Effect of surfactants on absorbance.**

Surfactants	Absorbance/mL of surfactants				
	Without	0.5	1	2	3
TritonX-100 (1%)	0.524	0.518	0.510	0.507	0.508
SDS (1×10^{-3} M)		0.511	0.520	0.519	0.516
CPC (1×10^{-3} M)		Turbid	Turbid	Turbid	Turbid
CTAB (1×10^{-3} M)		Turbid	Turbid	Turbid	Turbid

Table 3. **Effect of sequence of additions on absorption of the colored product.**

Sequence order	Reaction component *	Absorbance
Primary I	H+OX+L+OG	0.525
II	L+H+OX+OG	0.539
III	L+OX+H+OG	0.522
IV	OX+L+H+OG	0.421
V	L+OX+OG+H	0.285

* Hydrochloric acid (H), NBS (OX), Levofloxacin (L), Orange G dye (OG)

Effect of organic solvents

The effect of using a number of organic solvents for dilution on dye adsorption was studied under the optimum conditions previously determined. The results in Fig. 3

show that both water and ethanol gave excellent results. In this study, water was chosen as the solvent for the proposed method because it is readily available, safe, and inexpensive.

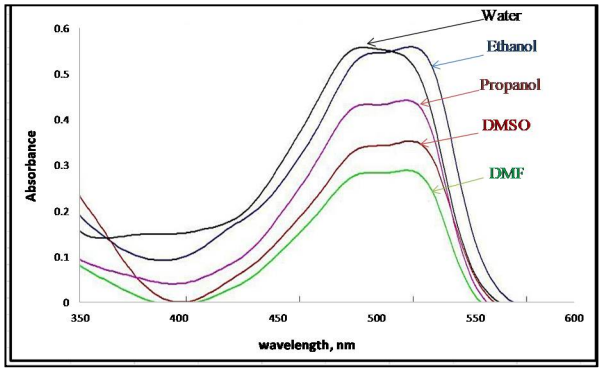


Figure 3. **Effect of solvents on dye absorption**

Final absorption spectrum

Under optimal conditions for the suggested method, 50 and 100 µg of LEVX were used in a 10 mL volumetric flask, then the rest of the ingredients were added according to the proposed method and the volume was supplemented with distilled water to the mark. The absorption spectrum of the resulting solutions was taken against the blank solution that gave the highest absorption value at the wavelength of 476 nm. It was noted that the blank solution gave the lowest absorption at the same wavelength (Fig. 4).

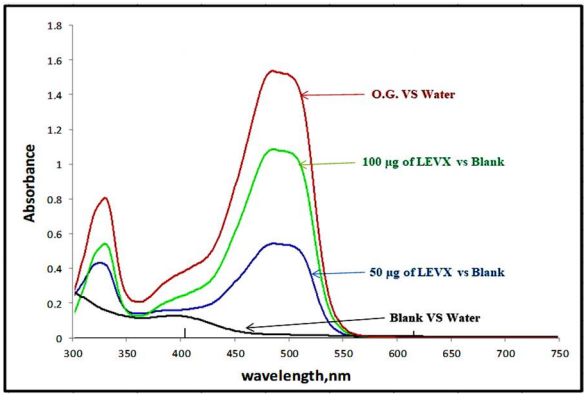


Figure 4. **Absorption spectrum of two concentrations of LEVX according to the proposed method**

Approved working method and standard curve

A standard curve for the determination of LEVX was prepared by adding increasing volumes of LEVX solution (50 µg.mL^{-1}) to a series of 10 mL volumetric vials to cover the range of concentration (0.1-20 µg.mL^{-1}) under optimized parameters. Fig. 5 represents the standard curve for the determination of LEVX that follows Beer's law in the concentration range (0.5 - 10.5 µg.mL^{-1}). The higher concentrations gave a negative deviation. The molar absorption-coefficient value was equal to 4.17× 10^4 L.mol^{-1}.cm^{-1}and the Sandell's index value was 0.00867 µg.cm^{-2}.

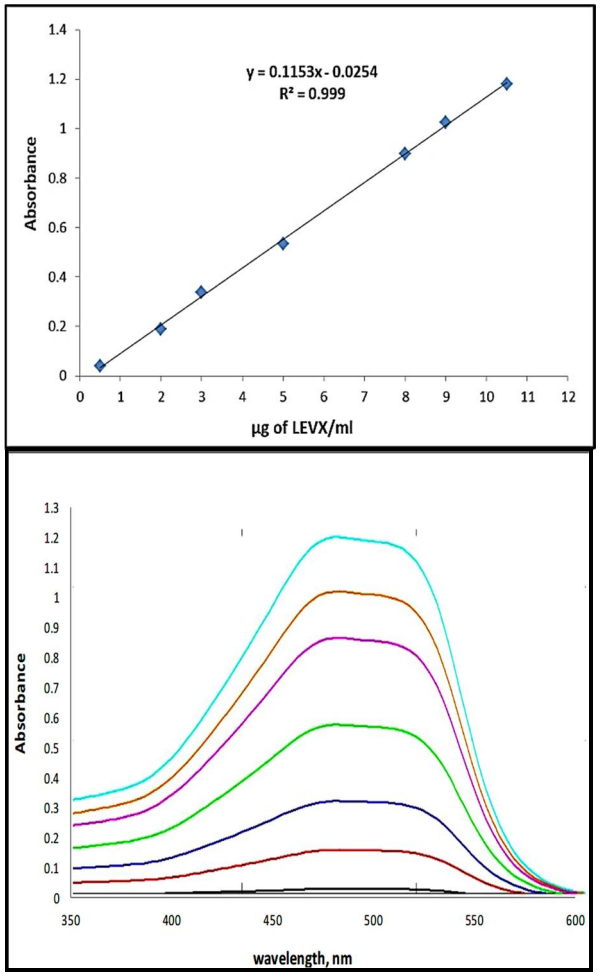


Figure 5. **Standard curve for the determination of LEVX (method A)**

Accuracy and Precision

The accuracy and precision of the proposed method were verified using two different concentrations of LEVX (3 and 6 $\mu g.mL^{-1}$) by preparing five samples of each. The recovery values were ranged between 98.16 - 98.33%, with relative errors between -1.84–1.67%, and relative standard deviations ranging from 0.845 - 1.208%. These results indicate that the developed method has good accuracy and agreement, and thus it can be adopted for the estimation of LEVX in pharmaceutical preparations.

Effect of Additives

To evaluate the selectivity of the method and its potential application to various pharmaceutical preparations, the effect of some substances used as additives (Starch, Glucose, Arabic gum, Sucrose, and Lactose) on absorption was studied. This was carried out by adding different quantities (20, 40, 100, 200 µg) of each additive to 1 mL of LEVX solution (50 $\mu g.mL^{-1}$), following the developed procedure. It was noted that these additives had no effect on the recovery rate of LEVX, indicating the method's efficiency, selectivity and suitability for pharmaceutical applications.

Application of Method A

The proposed method for the estimation of LEVX in pharmaceutical preparations was applied using two different concentrations (4 and 8 $\mu g.mL^{-1}$) from two different preparations (tablets and intravenous solution) from different origins. The accuracy and compatibility of the method were examined by calculating the error. The relative standard deviation and the recovery percentage shown in Table 4 prove the success of the developed method for LEVX determination in pharmaceutical preparations.

Table 4. **Results of application of method A on pharmaceuticals.**

Pharmaceutical preparations of LEVX	Present amount of LEVX $\mu g.mL^{-1}$	Measured amount of LEVX $\mu g.mL^{-1}$	Recovery %	RE %*	RSD %*	t-test
Levosol tablets 500mg/Tab. Pharma solution -Jordan	4	3.87	96.75	-3.25	0.681	2.08
	8	7.79	97.37	-2.63	1.092	1.94
Levoneer, infusion solution 500mg/100 mL PIONEER -IRAQ	4	3.91	97.75	-2.25	0.884	2.39
	8	7.94	99.25	-0.75	0.904	2.41

*Average of five determinations

Evaluation of the selectivity of the proposed method

The standard addition method [28] was applied to assess the selectivity of the proposed method for determining LEVX in its pharmaceutical preparations (therapeutic tablets and intravenous solutions). Two different concentrations (3 and 6 µg.mL^{-1}) of each of the androgenic preparations were tested.

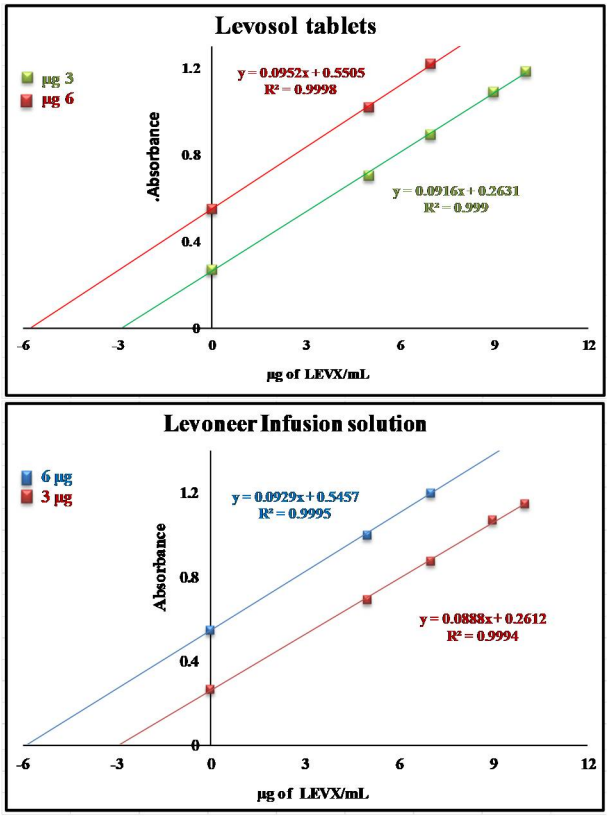


Figure 6. **Standard addition curves according to method A**

The results, shown in Fig. 6 and Table 5, demonstrate strong agreement between the standard addition method and the proposed method for the determination of LEVX in pharmaceutical preparations.

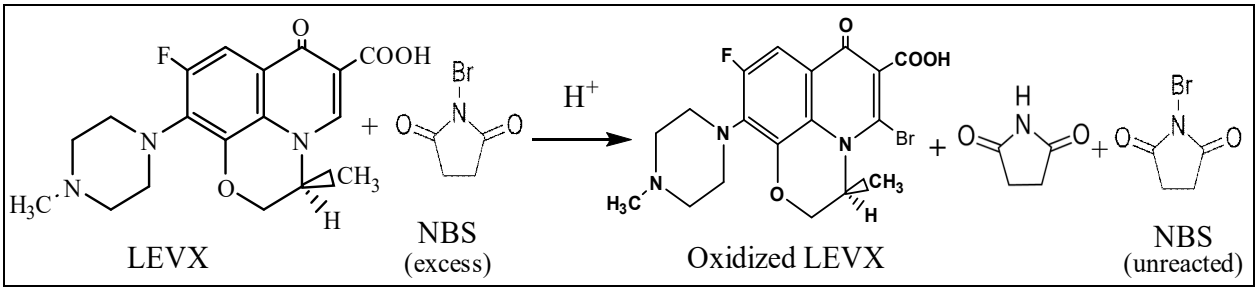
Table 5. **Results of the standard additions method for method A.**

Pharmaceutical preparation	Taken amount of LEVX µg.mL^{-1}	Measured amount of LEVX µg.mL^{-1}	Recovery %
Levosol tablets 500 mg/Tab. Pharma solution-Jordan	3	2.87	95.66
	6	5.78	96.33
Levoneer, infusion solution 500 mg/100 mL PIONEER-IRAQ	3	2.94	98.0
	6	5.87	97.83

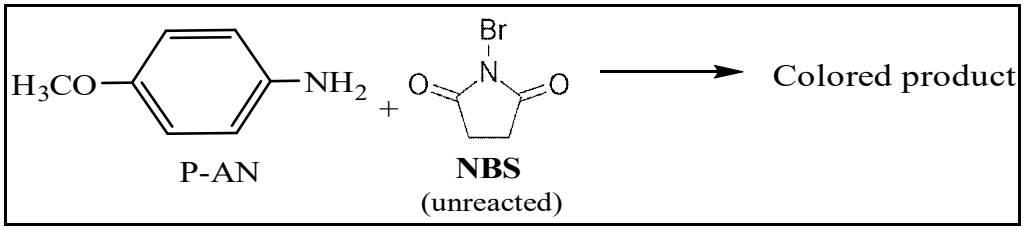
Method B

The principle of the proposed method and the suggested chemical reaction is based on two steps. The first step includes oxidation of LEVX using an excess amount of the oxidizing agent NBS in acetic acid medium as in Scheme 3 [27].

In a second step, the unreacted NBS was reacted with P-AN to produce a colored solution, which is inversely proportional to the amount of LEVX present in the sample. Finally, the absorbance of the colored solution was measured at 518 nm as in Scheme 4.



Scheme 3



Scheme 4

Optimum reaction conditions

The following experiments were conducted in a 20 mL volumetric flask with 2 mL of LEVX working solution 50 µg.mL^{-1} and measured the absorbance of colored product at 518 nm by reversing the positions of the cells in the device to obtain a positive absorbance value that is directly proportional to the amount of LEVX in the sample.

Effect of acidic medium, concentration and volume

In this study, 1.0 mL of each of the available acids was used at a concentration of 0.1 M (Acetic acid, Phosphoric acid, Sulphuric acid, Nitric acid, and Hydrochloric acid) that provided the pH values at 4.12, 3.81, 2.33, 2.47, and 2.56, respectively. The experimental results showed that acetic acid was the best acid because it showed the highest absorption value. Later, the effect of acetic acid concentration was checked from 0.005 – 0.05 M with a volume of 1 mL for each concentration. The experimental absorption values showed that 0.01 M showed the highest value of absorption at 518 nm. After that, different volumes (0.5, 1, 1.5, 2, and 2.5 mL) of optimized concentration 0.01 M were evaluated and found that 1.0 mL of acetic acid was optimal, so it was relied upon for subsequent experiments.

Selection of the oxidizing agent

The goal of studying the oxidizing agents was to obtain the best colored product. A number of available oxidizing agents were used, namely NBS, N-chlorosuccinimide (NCS), potassium periodate (KIO_4), potassium iodate (KIO_3), and potassium dichromate ($K_2Cr_2O_7$). Added 1 mL of each at a concentration of 500 µg.mL^{-1} to 20 mL volumetric bottles containing 1 mL of 0.01 M acetic acid and 1 mL of LEVX solution (50 µg.mL^{-1}), then waited for 5 min and added 3 mL of P-AN at a concentration of 500 µg.mL^{-1} and left the solution for 5 min. The spectrum curves were measured against the blank solutions, and the results showed that the NBS was a better oxidizing agent with higher absorbance at 518 nm, so it was chosen in subsequent experiments, as shown in Fig.7.

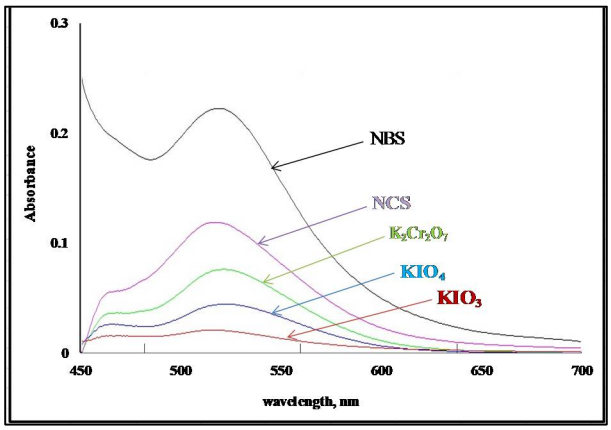


Figure 7. **Overlaid UV-visible spectrum of various oxidizing agents**

Effect of concentration and amount of NBS

The effect of different concentrations of NBS solution (200 – 900 µg.mL^{-1})was studied, and the results showed that the best conc. was 800 µg.mL^{-1} to get the best

absorbance value. Then different volumes (1-5 mL) of the selected concentration were checked, and the results showed that 3 mL of NBS gives the best value of absorbance, so it was adopted within the working method.

Effect of concentration and amount of P-AN reagent

In this experiment, 3 mL of different concentrations of P-AN (400 – 1000 $\mu g.mL^{-1}$) were used against 3 mL of 800 $\mu g.mL^{-1}$ NBS and the results showed that the best concentration was 800 $\mu g.mL^{-1}$. After that tested different volumes (1-7 mL)of this concentration under the same conditions, and the results showed that 5 mL of 800 $\mu g.mL^{-1}$ P-AN was suitable to give good absorbance variations for the reaction result.

Effect of time on the oxidation reaction

To obtain complete oxidation of the LEVX, wait for different periods after adding the oxidizing agent (NBS) and before adding the addition of P-AN. The appropriate time was also fixed to complete the reaction of the remaining NBS with P-AN. By waiting for periods of time after adding the reagent, Table 6 shows that 3 min was the best time to completely oxidize LEVX, and 1 min was the appropriate time to complete the reaction between NBS and P-AN.

Table 6. **Effect of time on oxidation reaction and product formation.**

Standing time before addition of P-Anisidine, min.	Absorbance/Standing time after addition P-AN and before dilution, min.				
	Immediately	1	2	3	4
Immediately	0.301	0.314	0.313	0.312	0.313
1	0.321	0.325	0.324	0.323	0.323
2	0.352	0.356	0.354	0.355	0.354
3	0.354	**0.368**	0.367	0.368	0.366
4	0.348	0.355	0.354	0.352	0.353
5	0.344	0.348	0.351	0.352	0.350

Effect of temperature and the stability of the product

The effect of temperature on the absorption value of the colored product and its stability duration was studied at different temperatures (10, 25, and 40 °C). The results showed that the absorption values remained constant for at least 30 min at room temperature (25±2 °C), and this period was sufficient to perform the necessary measurements.

Effect of surfactants on the absorption of the colored product

Different types of surfactants were used: SDS, CPC, CTAB, and Triton X-100 at different concentrations (Table 7), to evaluate their effect on the absorbance values of the colored product. The results showed that the addition of surfactants had no positive effect on the absorbance values, and some even resulted in a cloudy solution, so they were excluded from subsequent experiments.

Table 7. **Effect of surfactants on the absorption of the colored product.**

Surfactants	Absorbance/mL of surfactant				
	without	0.5	1	2	3
TritonX-100 (1%)	**0.368**	0.358	0.350	0.347	0.348
SDS (1×10^{-3} M)		0.361	0.360	0.359	0.356
CPC (1×10^{-3} M)		Turbid	Turbid	Turbid	Turbid
CTAB (1×10^{-3} M)		Turbid	Turbid	Turbid	Turbid

Effect of the addition sequence

Several experiments with different addition sequences were performed to obtain the best absorption value. The results shown in Table 8 confirm that reaction components of the first addition sequence (I) order gave the highest absorption value; therefore, it was chosen for subsequent experiments.

Table 8. **Effect of addition sequence on the absorption of the colored product.**

Sequence order	Reaction component *	Absorbance
I	**H + LE + OX + R**	**0.369**
II	H + OX + LE + R	0.356
III	LE + OX + H + R	0.352
IV	LE + OX + R + H	0.094

***LEVX (LE), Acetic acid (H), NBS (OX), P-AN®**

Effect of added excipients

Six types of non-pharmaceutical additives that we expect to be added when manufacturing pharmaceutical preparations (starch, gum Arabic, glucose, lactose, sorbitol, and sucrose) were studied under optimal conditions by adding different amounts of these additives and components of the proposed method. The results confirmed that the presence of these compounds with LEVX does not affect the efficiency and selectivity of the proposed method when applied to its pharmaceutical preparations.

Final absorption spectrum

The proposed method was applied under the previously determined optimal conditions to two concentrations (5 and 10 $\mu g.mL^{-1}$) of LEVX. Fig. 8 shows the final absorption spectrum of the sample compared to the blank solution, and also of the blank solution compared to distilled water.

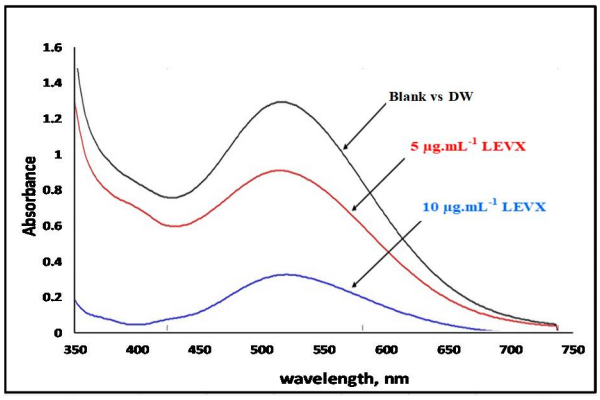


Figure 8. **Final absorption spectrum of two concentrations of LEVX according to method B**

Adopted procedure and calibration curve

To several 20 mL volumetric flasks containing 1.0 mL of 0.01 M acetic acid, different volumes of 100 $\mu g.mL^{-1}$ LEVX standard solution was added within the concentration range of 0.5-20 $\mu g.mL^{-1}$. The calibration graph was linear in the concentration range of 1.0 - 15 $\mu g.mL^{-1}$. A negative deviation from Beer's law was noted at concentrations more than 15 $\mu g.mL^{-1}$, as is evident in Fig. 9. The molar absorption and Sandell's index values were calculated and equal to 2.92×10^4 $L.mol^{-1}.cm^{-1}$ and 0.0123 $g.cm^{-2}$, respectively.

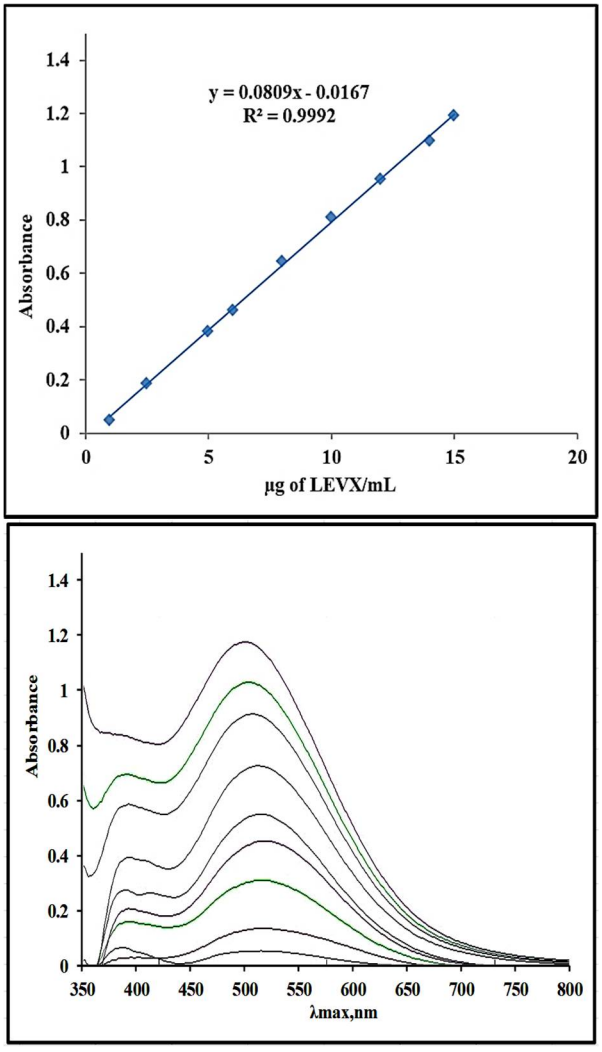


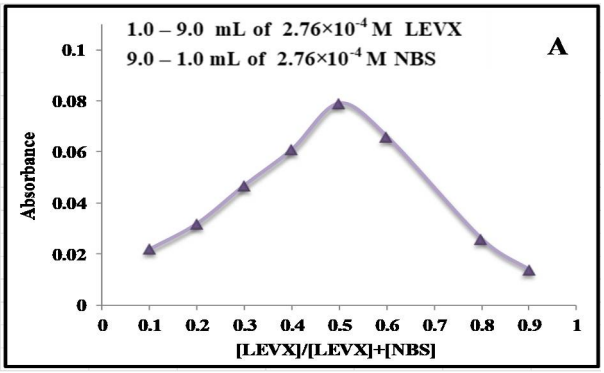
Figure 9. **Standard curve for determination of LEVX (method B)**

Accuracy and precision

Using two different concentrations (5 and 10 µg.mL) of LEVX, the accuracy and precision of the proposed method were verified by preparing five samples of each concentration. The recovery percentage values ranged between 99.6 - 100.2%, with relative errors between -0.4 - 0.2%, and the relative standard deviations ranging from 0.427−0.635 %. These results indicate that the method has acceptable accuracy and reliability.

The nature of the reaction ratio

Under the optimal conditions for the method and to determine the reaction ratio between LEVX and the oxidizing agent NBS, the continuous changes method (Job's method) and the molar ratio method were applied [29] using equal concentrations of both LEVX and NBS at (2.76×10^{-4} M). The results shown in Fig. 10 indicate that the reaction ratio was 1:1 for LEVX and NBS.



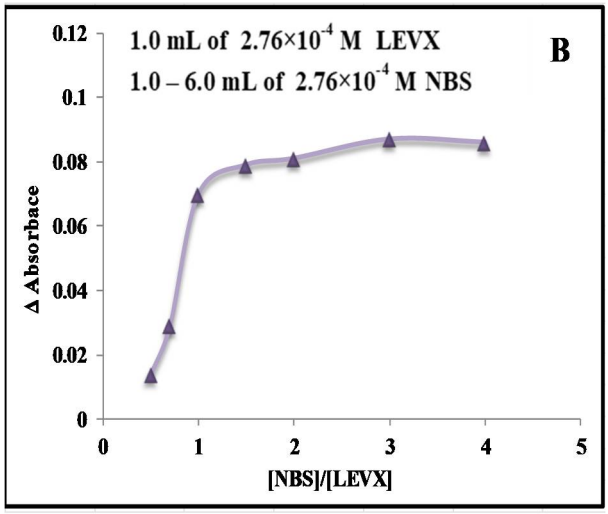


Figure 10. **(A) Continuous variation and (B) Mole ratio plots for LEVX**

Application of method B

The proposed method was applied to estimate LEVX in available therapeutic preparations (tablets and intravenous solutions from different origins) using two different concentrations (5 and 10 µg.mL^{-1}). The relative standard deviation and recovery (%) results listed in Table 9 show that the proposed method is successful in estimating LEVX in pharmaceutical preparations with an acceptable recovery.

Table 9. **Results of an application of method B on pharmaceuticals.**

Pharmaceutical preparations of LEVX	Present amount of LEVX µg.mL^{-1}	Found the amount of LEVX µg.mL^{-1}	Recovery %*	RE %*	RSD %*	t-test
Levosoltablets 500mg/Tab. Pharma solution -Jordan	5	4.89	97.8	-3.25	1.243	1.17
	10	9.83	98.3	-1.7	0.952	1.83
Levoneer, infusion solution 500mg/100 mL PIONEER -IRAQ	5	5.02	100.4	0.4	0.498	2.04
	10	9.95	99.5	-0.5	0.328	2.51

*Average of five determinations

Evaluation of the proposed method by standard addition

To prove the selectivity of the proposed method for determining LEVX in its pharmaceutical preparations (tablets and infusion), the standard addition method was applied [28].

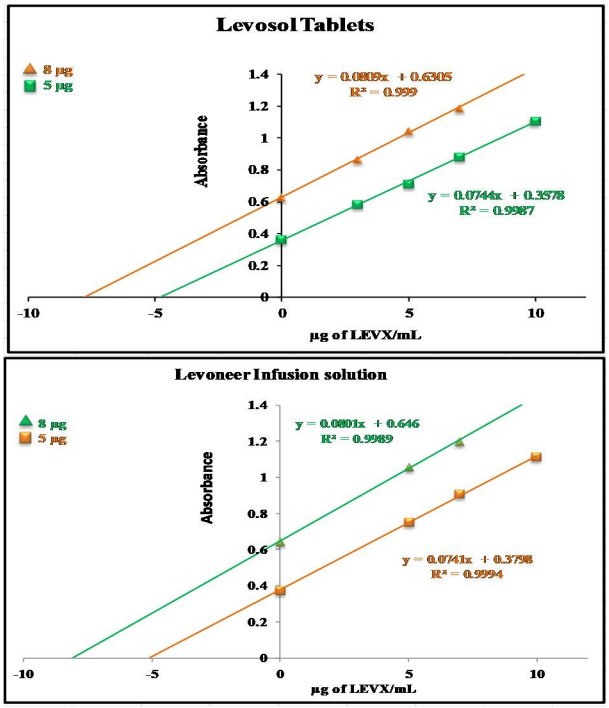


Figure 11. **Standard addition curves according to method B**

Two different concentrations (5 and 8 $\mu g.mL^{-1}$) of each of the preparations were tested. The results in Fig.11 and Table 10 indicate that the standard addition method is acceptably compatible with the proposed method, and there are no interactions with the additives (non-pharmaceuticals) in pharmaceutical preparations.

Table 10. **Results of the standard additions method for method B.**

Pharmaceutical preparation	Taken amount of LEVX $\mu g.mL^{-1}$	Measured amount of LEVX $\mu g.mL^{-1}$	Recovery %
Levosol tablets 500 mg/Tab. Pharma solution-Jordan	5	4.81	96.2
	8	7.79	97.37
Levoneer, infusion solution 500 mg/100 mL PIONEER-IRAQ	5	5.12	102.51
	8	8.06	100.81

Comparison of proposed methods

The accuracy and sensitivity of the proposed methods evaluated through various analytical parameters and their application in determining LEVX were compared with established spectrophotometric methods reported in the literature. As shown in Table 11, the results indicate that the proposed methods offer greater sensitivity than the previously reported methods.

Table 11. **Comparison of the proposed methods with other spectroscopic methods.**

Parameter	Present		Literature	
	Method (A)	Method (B)	Method [17]	Method [30]
Type of reaction	Oxidation–reduction	Oxidation–reduction	Ion-pair formation	Chelating complex
Oxidant agent	NBS	NBS	-	-
Reagent	OG dye	P-AN	Bromothymol blue	Al (III)
λ_{max}(nm)	476	518	420	420
Medium of reaction	Acidic	Acidic	Acidic	Neutral
Beer's law range($\mu g.mL^{-1}$)	0.5 – 10.5	1 – 15	0.5 – 25	5 – 25
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	4.17×10^4	2.9×10^4	2.07×10^4	11.11×10^4
Sandell'ssensitivity ($\mu g.cm^{-2}$)	0.00867	0.0123	0.048	0.0033
Recovery (%)	96.75 -99.25	97.8 – 100.4	98.8–102.7	100.83 -101.65
RSD%	< 1.5	<1.3	<2.03	<4.95
LOD ($\mu g.mL^{-1}$)	0.014	0.12	0.101	0.009
LOQ ($\mu g.mL^{-1}$)	0.046	0.40	0.303	0.25
R^2	0.9992	0.9992	0.9970	0.9993
Application	Tablets and intravenous solution	Tablets and intravenous solution	Tablets and infusion	Infusion

Conclusion

Two indirect spectrophotometric methods were developed for the assay of LEVX in pharmaceutical formulations, each method characterized by its simplicity, fast, inexpensive, and sensitivity. The first method (A) relies on the oxidation of LEVX using excess NBS in acidic medium. The unreacted NBS was then estimated using a fixed amount of OG dye, and the remaining dye's absorbance was measured at 476 nm. In method B, LEVX reacts of with an excess NBS in an acetic acid medium. The unreacted NBS subsequently reacted with P-AN, produce a colored solution (inversely proportional to the concentration of LEVS) whose absorbance was measured at 518 nm. Both methods proved to be easy and effective for analyzing pharmaceutical formulations, and their results were compatible with the standard method of addition.

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Conflict of Interest

The authors have no conflict of interest.

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