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Differential Spectrophotometric Determination of Levofloxacin in Bulk and Pharmaceutical Formulations

Sa'ad Toyin Abdullahi[1]*, Faith Oluwanifemi Dada[1], Oluwasegun Ibrahim Eniayewu[1], Olasunkanmi David Bamidele[1], Abdulrazaq Sanusi[1], Fatima Mohammad Madaki[2], Julius Olugbenga Soyinka[3]

[1]Department of Pharmaceutical & Medicinal Chemistry, University of Ilorin, Ilorin, Nigeria
[2]Department of Biochemistry, Federal University of Technology, Minna, Nigeria
[3]Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

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**Corresponding Author:*
Sa'ad Toyin Abdullahi,
Email: abdullahi.st@unilorin.edu.ng
Tel: +2348035883569
Orcid: 0000-0002-6868-8687

ABSTRACT

Background: Levofloxacin, which is pharmaceutically available as a hemihydrate ($C_{18}H_{20}FN_3O_4 \cdot 1/2H_2O$), is the optical S-(-) isomer of ofloxacin. This study proposed and validated a differential ultraviolet spectrophotometric method for determining levofloxacin in bulk and pharmaceutical formulations, based on the principle that levofloxacin exhibits characteristic spectral shifts with changes in the pH of the assay solvents.
Methods: Two solutions of levofloxacin in 0.1N HCl and 0.1N NaOH at the same concentration served as the test and reference solutions (ΔD_1), and as the reference and test solutions (ΔD_2). The amplitude values were calculated and plotted against concentrations.
Results: Beer's law was obeyed, and the calibration curves were found to be linear over the concentration range of 4–20 µg/mL, with an excellent correlation coefficient ($r > 0.9999$) for both scenarios at the observed absorption maxima and minima of 228 and 298 nm for ΔD_1, and 257 and 348 nm for ΔD_2. Accuracy studies showed recoveries close to 100% (100.17–100.35%) with low RSD values (≤1.78%), demonstrating excellent accuracy. Precision was also satisfactory, with intraday precision and robustness RSD values generally below 2%. Sandell's sensitivity (0.0113–0.0166 $\mu g \cdot cm^{-2}$) indicated adequate analytical sensitivity.
Conclusion: The results indicate that the proposed analytical method is accurate, specific, and precise, and can be used for routine analysis of levofloxacin hemihydrate in bulk and pharmaceutical formulations.

INTRODUCTION

Levofloxacin [chemical name: (S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid is a fluoroquinolone antibiotic and the optical S-(-) isomer of racemic ofloxacin, as illustrated in Figure 1.[1] It is significantly more active against both gram-negative and gram-positive bacterial pathogens compared to R-(+)-ofloxacin (i.e., dextrofloxacin). It remains stereochemically stable, without inverting to the inactive isomer following administration.[2-4] It belongs to the third generation of fluoroquinolones, unofficially known as the "respiratory quinolones" due to improved activity, particularly against gram-positive bacteria commonly involved in respiratory infections.[5,6] Levofloxacin hemihydrate, the commercially available formulation, contains 97.6% levofloxacin by weight.[2] Owing to comparable plasma concentrations, its oral and intravenous preparations are clinically

interchangeable.[7] In adults, levofloxacin is indicated for the treatment of a broad spectrum of bacterial infections, including those affecting the respiratory tract, skin and soft tissues, urinary tract, and prostate.[3,8] In recognition of its therapeutic importance, the drug is listed in the 2023 World Health Organization Model List of Essential Medicines.[9]

Levofloxacin ($C_{18}H_{20}FN_3O_4$; molecular mass 361.37 g/mol) is a light-sensitive, pale yellow-white crystalline powder with a bitter taste and a melting point of 225–227 °C.[4,10] It is odorless, exhibits a pH of 7.16 in a 1.0% aqueous solution, and has a pKa of 6.25. The compound is freely soluble in glacial acetic acid, chloroform, dimethylsulphoxide, and dimethylformamide; slightly soluble in ethanol; sparingly soluble in water, acetone, and methanol; and practically insoluble in ether, glycerine, and n-octanol.[4,11] Its aqueous solubility remains essentially constant at approximately 100 pg/mL across the pH range of 0.6–5.8. Above pH 5.8, the solubility rapidly increases to a maximum of about 272 pg/mL at about pH of 6.7.[7]

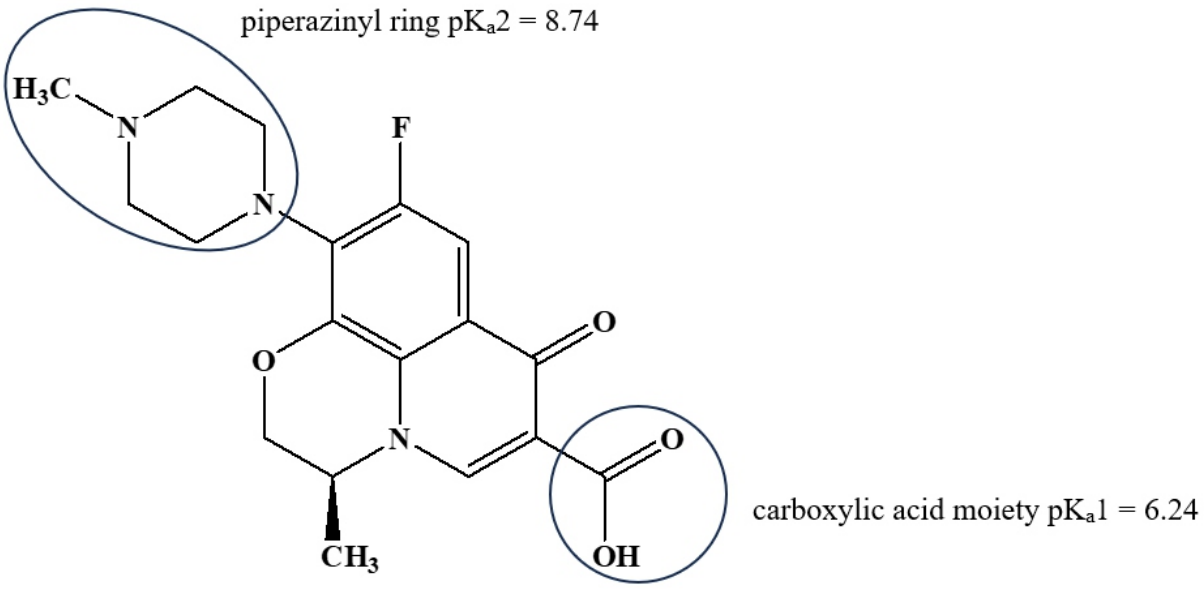

Figure 1: Levofloxacin chemical structure

A review of literature revealed various UV spectrophotometric methods for determining levofloxacin in pharmaceutical formulations, including derivative spectrophotometry.[12-19] A UV spectrophotometric method for levofloxacin hemihydrate using 10% v/v acetonitrile reported detection wavelengths or absorption maxima (λ_{max}) of 288 nm and 297 nm when the spectrum was derivatized to first order.[12] Both methods were found to be linear in the concentration range of 2 - 12 µg/mL (with a correlation coefficient of 0.9999).[12] In another UV spectrophotometric method, using methanol to prepare solutions of the analyte, a λ_{max} of 298 nm was reported, with linearity over a concentration range of 3 - 8 µg/mL.[13] A UV spectrophotometric method for determining levofloxacin at a predetermined λ_{max} of 290 nm with linearity from 0.25 to 12 µg/mL, using 0.1N HCl as the assay solvent, was also reported.[14] However, a similar method using 0.1N HCl as the assay solvent showed maximum absorption at 293 nm and linearity in the concentration range of 2 - 12 µg/mL.[15]

A UV spectrophotometric method to determine levofloxacin content in bulk and pharmaceutical dosage forms using an assay solvent composed of water, methanol, and acetonitrile (9:0.5:0.5) reported a λ_{max} of 292 nm and linearity over a concentration range of 1.0–12.0 µg/mL.[16] Similarly, a UV spectrophotometric method for estimating levofloxacin in bulk and pharmaceutical formulations was developed using 0.1N NaOH as the assay solvent, with the drug's detection λ_{max} at 288 nm and adherence to Beer-Lambert law over 2–12 µg/mL.[17] Additionally, a zero-order derivative UV spectrophotometric method was developed for estimating levofloxacin in bulk and pharmaceutical dosage forms using 0.1N sulphuric acid as the assay solvent, reporting an absorption maximum at 293.6 nm and linearity across an extended concentration range of 5–30 µg/mL.[18] Likewise, first, second, third, and fourth-order derivative UV spectrophotometric methods, employing

peak—zero and peak-peak measurement techniques, were developed for determining levofloxacin individually and in combined formulations with norfloxacin and moxifloxacin.[19]

Differential spectrophotometry is an analytical technique that provides both qualitative and quantitative information. To date, no study has described a differential ultraviolet–visible spectrophotometric method for the determination of levofloxacin. This study proposed a differential ultraviolet spectrophotometric method for the determination of levofloxacin in bulk and pharmaceutical formulations using equal concentrations of the analyte in 0.1N HCl and 0.1N NaOH as the reference and test, and as the test and reference, respectively. The optical and validation parameters obtained with both scenarios at the two different absorption maxima and minima were compared with each other and with those obtained at the detection wavelength of maximum absorption using conventional methods.

MATERIALS & METHODS

Instrumentation

The photometric measurements were carried out using a UV-vis double-beam spectrophotometer [model: GS-UV61(PC); serial No: UQC1212006 by General Scientific Hong Kong Limited], which was equipped with 1 cm matched quartz cells for sample and reference solutions. A calibrated analytical balance (PA214) by Ohaus, USA, was used for weighing. Precision measurements were facilitated by micro pipettes (P20: 2–20 µL) from Switzerland.

Chemicals and Reagents

The levofloxacin analytical reference standard was generously supplied by Unique Pharmaceuticals Limited, Sango-Ota, Ogun State, Nigeria. Sodium hydroxide (NaOH) pellet (500g) and concentrated hydrochloric acid (HCl) (37%) used in the study were of analytical reagent grade without further purification. Available brands of levofloxacin tablets and infusion samples were randomly collected from various community pharmacy outlets and coded to conceal their brand names.

Methodology

Preparation of standard/sample solutions

Calibrated volumetric flasks (50 and 100 mL) and pipettes (1, 5, and 10 mL) were used to prepare stock and working standard solutions. Stock solutions (C1: 500 µg/mL) of levofloxacin standard in 0.1N NaOH and HCl were each prepared in triplicate by accurately weighing and transferring 50 mg of the standard into a 100 mL volumetric flask. They were dissolved and brought up to volume with the appropriate solvent, then allowed to stand for at least 15 minutes. Working standards (C2: 100 µg/mL) were prepared by pipetting 20 mL of the stock solution (C1), transferring it into a 100 mL volumetric flask, and diluting to volume with the appropriate solvent. A standard or test sample solution (C3: 10 µg/mL) was prepared by pipetting 5 mL of the working standard or test solution and diluting to the 50 mL mark in a volumetric flask with the appropriate solvent, as shown in Figure 2.

Qualitative Analysis

Spectrophotometric identification tests for both reference standards and test samples were carried out in 0.1N HCl and 0.1N NaOH, with spectra recorded between 400–200 nm. The levofloxacin spectra of the test samples were compared with the reference standard to verify that absorption maxima in the normal spectrum, as well as maxima and minima in the pH-induced difference spectrum (ΔD), appeared at identical detection wavelengths. Molar absorptivity values at these wavelengths—λmax in the normal spectrum and maxima/minima in the difference spectrum—were then calculated and compared, confirming that differences between standard and test samples did not exceed ±2%.[20]

Quantitative Analysis

Generation of a standard calibration curve

Suitable volumes/aliquots (2, 4, 6, 8, and 10 mL) of the 100 µg/mL working standard solutions were taken in 50 mL volumetric flasks and made up to volume with the appropriate solvent to prepare a series of standard solutions (4, 8, 12, 16, and 20 µg/mL). Each concentration level was prepared in triplicate, and its absorbance was measured immediately against blanks using conventional spectrophotometry, and by comparing levofloxacin in 0.1N NaOH in the reference cell with levofloxacin in 0.1N HCl in the sample cell, and vice versa for differential spectrophotometry. The concentration of each solution was determined from the corresponding calibration curve.

Validation

Method validation parameters, including linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision, and accuracy, were performed according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines.[21]

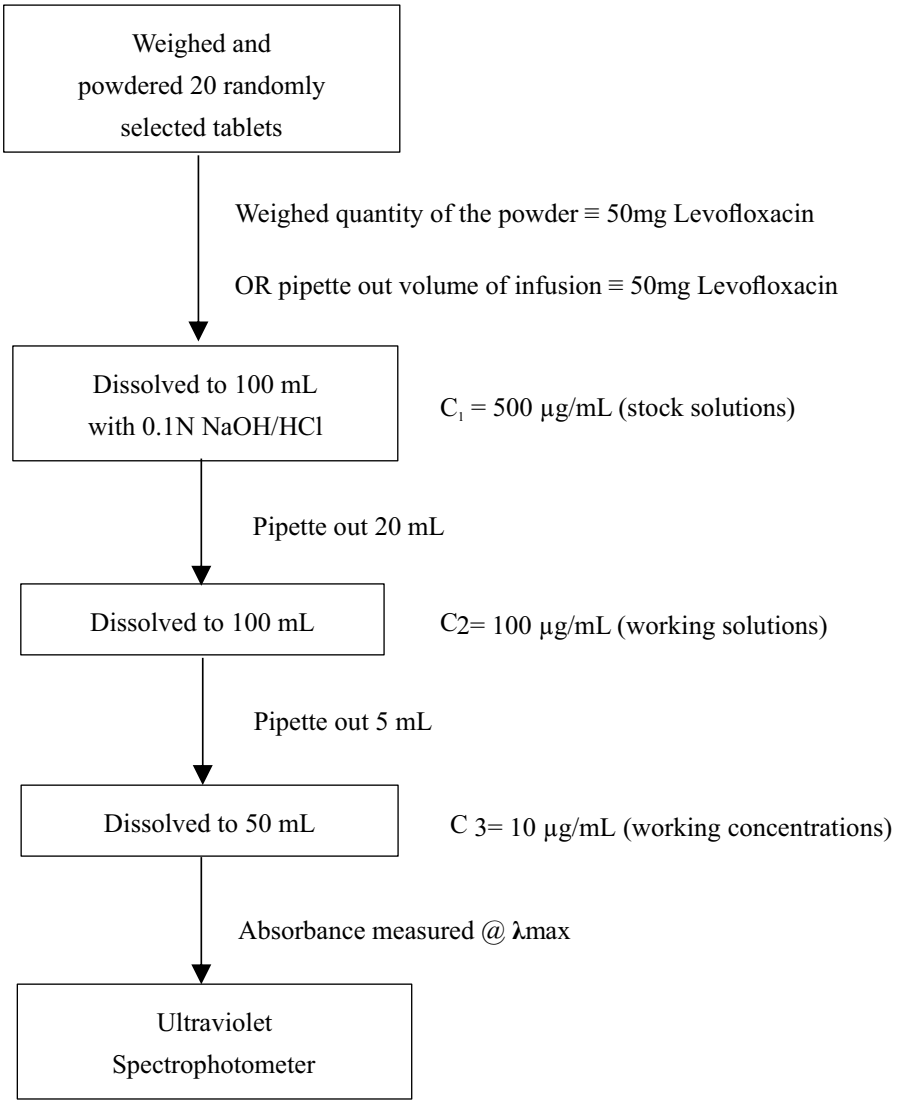

Figure 2: Summary of the study design for preparation of the stock, working, and sample levofloxacin standard or test solutions

Linearity: Calibration curves were created by plotting absorbance or amplitude against the corresponding concentration using linear regression analysis.

Limit of detection (LOD): is the lowest possible concentration at which the method can detect but not quantify the analyte within the matrix with a certain level of confidence. This was calculated based on the standard deviation of the y-intercept and the slope of the calibration curve using the equation:

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

Limit of quantification (LOQ): represents the lowest concentration of the analyte that can be reliably quantified by the method. Similarly, this was calculated based on the standard deviation of the y-intercept and the slope of the calibration curve using the equation: [21]

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

Precision: The intraday and interday precision studies were carried out by estimating the working concentration of levofloxacin (i.e., 10 µg/mL) at three different intervals (i.e., 0, 6, and 12 hours) on the same day to demonstrate

repeatability; and within a day (24 hours) to demonstrate inter-day precision. The results were reported in terms of relative standard deviation (i.e., the standard deviation expressed as a percentage of the mean).

Accuracy (% Recovery): This measures the accuracy of the analytical method and is expressed as the percentage of the true value (termed as % recovery). The study was conducted using tablet and infusion samples containing levofloxacin along with common excipients. It was evaluated by the standard addition method at three different concentration levels of 80%, 100%, and 120%, with replicate analysis (n=3).[21] The accurately measured absorbance/concentration of 25 mL solutions of previously analysed tablet/infusion samples was spiked with 20 µL of the stock standard solutions, thoroughly mixed by vigorous shaking, and the absorbance re-measured. The amount of added analyte concentrations was calculated, and the total amount of levofloxacin was determined using the corresponding regression equations. The percentage recoveries at each level were calculated using the formula:

$$\text{Recovery} = \frac{Observed\ concentration}{Calculated\ concentration} x\ 100$$

Assay of Levofloxacin Content of Tablet and Infusion Formulations

The developed and validated differential spectrophotometric method was successfully used to quantify levofloxacin in tablet and infusion dosage forms using two different scenarios. Seven commercial brands of levofloxacin formulations (five for tablets and two for infusions) were randomly obtained from the market and analysed. Twenty tablets from each of the five brands were weighed together and triturated into a fine powder. The powder (or volume of infusion) equivalent to 50 mg of levofloxacin was calculated, weighed (or pipetted), and transferred to a 100 mL measuring flask in triplicate using 0.1N HCl/NaOH to dissolve and fill to the mark. The resulting mixtures were allowed to stand for 15 minutes at room temperature, then thoroughly shaken to ensure complete dissolution of the analyte. The resulting 500 µg/mL stock solutions (C1) were filtered through Whatman filter paper Grade 42 (125 mm). Exactly 20 mL of each of the triplicate solutions (C1) was transferred into another 100 mL flask and diluted to volume with the appropriate solvent to prepare a 100 µg/mL working solution (C2). Precisely 5 mL of this working solution (C2) was pipetted into a 50 mL volumetric flask, and the final volume was adjusted to mark to produce a 10 µg/mL sample (C3), as shown in Figure 2. The absorption spectra were scanned over the range of 200–400 nm, and absorbance values were recorded. The levofloxacin concentrations were calculated from the regression equations derived from the corresponding calibration curves.

Statistical Analysis

Recovery results are expressed as mean values with relative standard deviations (%RSD) based on triplicate measurements, while precision is reported as mean with range. For assay testing, levofloxacin tablets and infusions are required to contain between 90.0% and 110.0% of the labelled drug content, corresponding to an allowable variation of 20% between the highest and lowest values. Statistical comparisons of brand potencies were conducted using the Paired Sample T-test, with significance defined at $P < 0.05$. All analyses were performed using Excel 2021 and SPSS version 27.

RESULTS

Descriptive Characteristics of Analyzed Samples

The descriptive features of all seven brands analysed are shown in Table 1. All the study brands are registered with the National Agency for Food and Drug Administration and Control (NAFDAC) and have a shelf life of about three years. Five of the brands are tablet forms manufactured in India, while the remaining two infusions originate from Pakistan and Nigeria.

Identification Tests

The spectra of all test samples from the seven brands closely matched the conventional UV spectrum of the levofloxacin reference standard, showing λmax at 293 nm in 0.1 N HCl and 288 nm in 0.1 N NaOH. Similarly, the absorption maxima and minima observed in the pH-induced difference spectra (ΔD1: 298 and 228 nm; ΔD2: 257 and 348 nm) were consistent with those of the standard. Comparison of the calculated molar absorptivity values of all levofloxacin test samples (LVX-1 to LVX-7) indicates that they closely matched those of the reference standard, with differences consistently below 1% across normal spectra (ε288, ε293) and pH-induced difference spectra (εD1, εD2). These results fall well within the USP acceptance limit of ±2%, confirming the identity of the test samples as levofloxacin (Table 2).

Table 1: Descriptive characteristics of sampled brands of Levofloxacin-containing formulations

Sample code	Batch number	NAFDAC number	Manufactured country	Manufactured date	Expiry date	Formulation strength
LVX-1	TESH0025	A4-5980	India	09/2023	08/2026	750mg tablet
LVX-2	E305	A4-100169	India	03/2023	02/2026	750mg tablet
LVX-3	N-2670	A4-3216	India	12/2021	11/2024	750mg tablet
LVX-4	LF301	C4-1910	India	06/2023	05/2026	500mg tablet
LVX-5	AF54301	A4-1186	India	10/2023	09/2026	500mg tablet
LVX-6	B.464PA1	A4-9539	Pakistan	03/2022	03/2025	500mg/100mL Infusion
LVX-7	2152001	B4-1968	Nigeria	02/2022	01/2025	500mg/100mL Infusion

Abbreviation: NAFDAC - National Agency for Food and Drug Administration and Control

Table 2: Identification test by comparison of the calculated absorptivity values of the test samples with the reference standard.

Levofloxacin	ε288(% Diff)	ε293 (% Diff)	εD1(% Diff)	εD2(% Diff)
LVX	25,718	32,062	28,887	21,831
LVX -1	25,644 (-0.29)	31,875 (-0.58)	28,865 (-0.08)	21,728 (-0.47)
LVX -2	25,659 (-0.23)	31,880 (-0.57)	28,867 (-0.07)	21,731 (-0.46)
LVX -3	25,653 (-0.25)	31,904 (-0.49)	28,868 (-0.07)	21,741 (-0.41)
LVX -4	25,664 (-0.21)	31,902 (-0.50)	28,869 (-0.06)	21,741 (-0.41)
LXX-5	25,644 (-0.29)	31,875 (-0.58)	28,865 (-0.08)	21,727 (-0.48)
LVX -6	25,648 (-0.27)	31,893 (-0.53)	28,866 (-0.07)	21,734 (-0.45)
LVX -7	25,655 (-0.25)	31,880 (-0.57)	28,867 (-0.07)	21,729 (-0.47)

Abbreviation: A:- Absorbance; ε:-molar absorptivity; Diff:- Difference; LVX: Reference

Linearity and Range

Calibration curves were constructed using levofloxacin analytical reference standard solutions by plotting absorbance versus concentration. Linearity was observed over a concentration range of 4–20 µg/mL using both the conventional and differential methods. The regression equations showed a correlation coefficient (r) of 0.9999 for both methods in 0.1N HCl and 0.1N NaOH, and exceeded 0.9999 for the differential method [scenarios ΔD_1: 298, 228 nm, and ΔD_2: 257, 348 nm], indicating excellent linearity. The % RSD of the amplitude in triplicate analyses was less than 2.0% (Table 3 and 4). The concentrations obtained from the calibration plots demonstrated remarkable recovery [range (%) of 0.44 (ΔD_1) and 0.57 (ΔD_2)] for the differential spectrophotometry, compared to [range (%) of 1.70 (at 288 nm) and 1.17 (at 293 nm)] for conventional spectrophotometry. Calibration curves of levofloxacin standards for both the normal and differential methods are shown in Figure 3 (A and B) respectively.

Precision [RSD (%)]

Precision measures the level of agreement among individual test results when the method is applied repeatedly to triplicate samples of a homogeneous sample. Intraday precision, demonstrating the repeatability of the methods, shows smaller % RSD ranges (0.22 at ΔD_1 and 0.47 at ΔD_2) for the differential method compared to larger ranges (0.60 at 288 nm and 1.09 at 293 nm) for the conventional method, as summarized in Table 5. Similarly, interday precision also shows smaller % RSD ranges (0.32

at ΔD_1 and 0.62 at ΔD_2) for the differential method versus larger ranges (0.62 at 288 nm and 0.63 at 293 nm) for the conventional methods, as shown in Table 5. The robustness of the differential spectrophotometric methods is confirmed by the smaller % RSD range (0.08 at ΔD_1 and 0.14 at ΔD_2) resulting from slight variations (±1) in the absorption maxima and minima, which compares favourably with the % RSD range (0.04 at 288 nm and 0.17 at 293 nm) for the normal spectrophotometric methods, as presented in Table 5.

Table 3: Calibration curve plots of maximum absorption (λ_{max}) at different aliquots for the conventional ultraviolet method

Conc (µg/mL)	Absorbance [RSD (%)]	Observed conc. (µg/mL)	Recovery (%)	Absorbance [RSD (%)]	Observed conc. (µg/mL)	Recovery (%)
	λmax of 288 nm			λmax of 293 nm		
4	0.295 (0.7 06)	4.04	101.06	0.371 (1.7 68)	3.98	99.41
8	0.570 (0.90 0)	8.00	99.97	0.712 (1.79 3)	7.99	99.91
12	0.845 (0.31 3)	11.94	99.49	1.051 (0. 798)	11.99	99.91
16	1.121 (0.3 09)	15.90	99.36	1.400 (1.7 15)	16.09	100.58
20	1.412 (1.60 0)	20.08	100.39	1.724 (0. 067)	19.92	99.58

Abbreviations: Conc:- concentration; RSD:- relative standard deviation

Table 4: Calibration curve plots of maximum absorption (λ_{max}) at different aliquots for the differential ultraviolet method

Conc (µg/mL)	Absorbance [RSD (%)]	Observed conc. (µg/mL)	Recovery (%)	Absorbance [RSD (%)]	Observed conc. (µg/mL)	Recovery (%)
	ΔD1 (298, 228) nm			ΔD2 (257, 348) nm		
4	0.333 (1.0 66)	4.01	100.17	0.253 (1.23 4)	4.01	100.34
8	0.641 (1.396)	8.00	99.95	0.485 (1.6 88)	8.00	100.00
12	0.948 (0.5 49)	11.97	99.73	0.716 (0.4 09)	11.97	99.77
16	1.260 (1.0 45)	16.01	100.04	0.953 (1.23 4)	16.04	100.26
20	1.568 (0.724)	19.99	99.95	1.184 (0.57 2)	20.00	100.01

Abbreviations: Conc:- concentration; RSD:- relative standard deviation

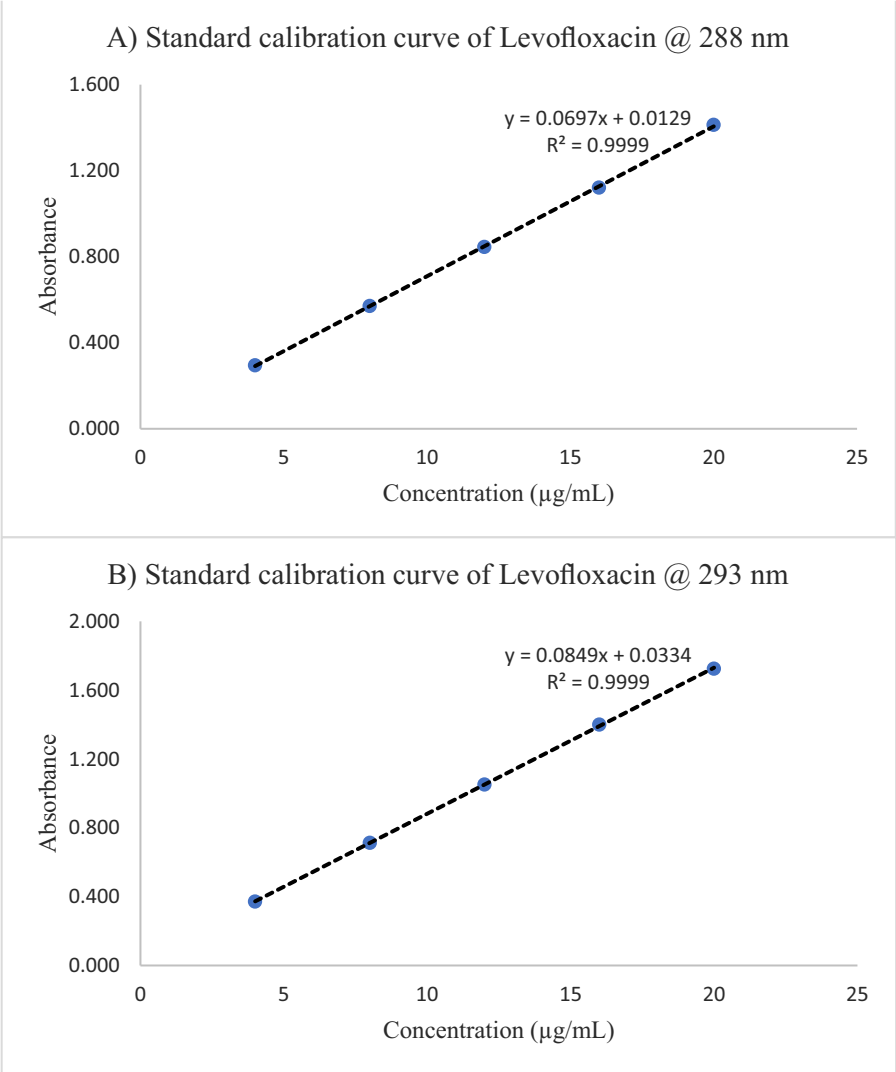

Figure 3: Linearity graphs of levofloxacin reference standard for A) in 0.1N HCl as a sample and B) in 0.1N NaOH as a sample using differential spectrophotometry.

Accuracy [Recovery (%)]

Accuracy assesses how specific the method is when excipients are present under the spectrophotometric conditions used to analyse levofloxacin tablets and infusion formulations. It reflects how close the test results are to the actual true value. The values obtained are within ± 2%, indicating good accuracy of the method for both the tablet and infusion formulations, i.e., average recovery (%) of [ΔD_1: 100.38 versus 100.13%; ΔD_2: 100.29 versus 100.05%] for the two scenarios of differential spectrophotometry, compared to [at 288: 100.47 versus 100.22%; at 293 nm: 100.31 versus 100.05%] for conventional spectrophotometry, as shown in Table 6.

Other Validation and Optical Parameters

A summary of the optical characteristics of the levofloxacin analytical reference standard, including absorption maxima and minima, specific absorbance, molar absorptivity, and Sandell's sensitivities for the normal and differential methods, is presented in Table 8. The differential spectrophotometric method is more sensitive in scenario 1, with levofloxacin in 0.1N HCl as the sample against 0.1N NaOH as the blank, compared to scenario 2, which has 0.1N NaOH as the sample against 0.1N HCl as the blank: [Sandell's sensitivity ($\mu g.cm^{-2}$) of ΔD_1: 0.0125 versus ΔD_2: 0.0166]. It was, however, much more sensitive at the detection λ_{max} of 293 nm with the analyte in 0.1N HCl against the blank, compared to 288 nm in 0.1N NaOH against the blank using the conventional spectrophotometric method. Other sensitivity parameters, including molar absorptivity, as well as LOD and LOQ, similarly aligned well with the Sandell sensitivity values (Table 7).

Table 5: Methods' Precision

Methods wavelength (nm)	Time (h) / λ_{max} (nm)	Concentration (µg/mL)	RSD (%)	Recovery (%)
Repeatability				
288	0	9.95	0.32	99.49
	6	9.98	0.67	99.84
	12	10.04	0.82	100.44
293	0	9.99	0.82	99.91
	6	9.91	1.14	99.06
	12	9.97	1.91	99.65
ΔD_1 (228, 298)	0	9.81	0.00	98.10
	6	9.95	0.85	99.51
	12	10.01	0.93	100.13
ΔD_2 (257, 348)	0	9.95	0.32	99.49
	6	9.98	0.67	99.84
	12	10.04	0.82	100.44
Interday precision				
288	0	9.84	1.31	98.37
	24	9.92	0.69	99.21
293	0	9.91	1.42	99.06
	24	9.84	0.79	98.41
ΔD_1 (228, 298)	0	10.09	0.89	100.91
	24	9.93	1.21	99.32
ΔD_2 (257, 348)	0	9.84	1.31	98.37
	24	9.92	0.69	99.21
Robustness ($\lambda_{max} \pm 1$)				
288	287	10.00	0.86	100.04
	288	10.04	0.82	100.44
	289	10.02	0.83	100.20
293	292	9.92	1.80	99.20
	293	9.97	1.91	99.65
	294	9.93	1.74	99.26
ΔD_1 (228, 298)	-1	11.95	0.87	99.58
	0	12.00	0.90	100.01
	1	11.96	0.82	99.69
ΔD_2 (257, 348)	-1	9.97	1.23	99.74
	0	10.01	1.33	100.12
	1	9.97	1.19	99.72

Abbreviations: RSD: relative standard deviation; λ_{max}: wavelength of maximum absorbance

Assay of Levofloxacin Tablet and Infusion Dosage Forms

The levofloxacin contents of the seven commercial brands, as determined by the two scenarios of the proposed differential spectrophotometric method [ΔD_1 (298, 228) nm versus ΔD_2 (257, 348) nm], were in good agreement with the label claims and well within the USP permissible range of [3.21% versus 2.03%, respectively]. The comparison between the two methods showed that the active levofloxacin contents were not significantly different, as indicated by the 95% confidence intervals of the difference spanning zero (Table 8).

Table 6: % Recovery of added amount to tablet and infusion samples

Method detection wavelength (nm)	Before addition Concentration (µg/mL)			After addition Concentration (µg/mL)			Recovery (%)
	Predicted	Observed	RSD (%)	Calculated	Observed	RSD (%)	
Tablet formulation							
288	8	7.83	2.60	8.23	8.28	2.04	100.66
	10	9.84	2.12	10.24	10.27	2.12	100.30
	12	11.86	1.99	12.26	12.31	1.63	100.44
293	8	7.42	1.08	7.82	7.84	1.44	100.31
	10	9.35	1.52	9.75	9.78	1.36	100.33
	12	11.31	1.20	11.71	11.75	1.06	100.31
ΔD_1 (228, 298)	8	7.60	1.47	8.00	8.04	1.57	100.47
	10	9.57	1.75	9.97	10.00	0.57	100.31
	12	11.56	1.52	11.96	12.00	1.16	100.37
ΔD_2 (257, 348)	8	7.55	1.20	7.95	7.98	1.51	100.41
	10	9.51	1.70	9.91	9.94	0.54	100.24
	12	11.51	1.68	11.91	11.93	1.03	100.22
Infusion formulation							
288	8	8.16	1.96	8.56	8.60	2.71	100.36
	10	10.17	2.97	10.57	10.58	0.46	100.15
	12	12.16	2.68	12.56	12.58	1.74	100.17
293	8	7.91	4.14	8.31	8.20	1.74	98.64
	10	9.95	0.34	10.35	10.44	0.23	100.88
	12	11.93	0.92	12.33	12.41	1.00	100.64
ΔD_1 (228, 298)	8	8.03	2.94	8.43	8.38	1.62	99.42
	10	10.05	1.37	10.45	10.51	1.22	100.55
	12	12.04	0.96	12.44	12.49	0.94	100.42
ΔD_2 (257, 348)	8	8.01	3.36	8.41	8.32	1.47	98.99
	10	10.02	0.67	10.42	10.45	0.05	100.37
	12	12.01	0.38	12.41	12.50	0.31	100.79

Abbreviations: RSD: relative standard deviation

Table 7: Comparison of the optical characteristics and validation parameters of the methods using the conventional and pH-induced difference UV.

Parameter (unit)	Value (RSD)/range/equation			
Detection wavelength [(λ_{max}) nm]	288	293	ΔD_1 (298, 228)	ΔD_2 (257, 348)
Linearity range (µg/mL)	4.0 - 20.0			
Correlation coefficient (r)	0.9999	0.9999	1.0	1.0
Regression equation	*y = 0.0697x + 0.0129*	*y = 0.0849x + 0.0334*	*y = 0.0773x + 0.0231*	*y = 0.0582x + 0.0194*
Limit of detection (µg/mL)	0.2929	0.4198	0.1030	0.2901
Limit of quantification (µg/mL)	0.8874	1.2722	0.3122	0.8790
Specific absorptivity (dL/gm/cm)	712	887	799	604
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	25,718	32,062	28,887	21,831
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.0141	0.0113	0.0125	0.0166
Accuracy [Recovery (RSD) %]	100.35 (1.78)	100.18 (1.14)	100.26 (1.18)	100.17 (0.82)
Precision [RSD (range) %]				
Repeatability	0.60 (0.50)	1.29 (1.09)	0.95 (0.08)	0.60 (0.50)
Interday precision	1.00 (0.62)	1.11 (0.63)	1.05 (0.32)	1.00 (0.62)
Robustness	0.84 (0.04)	1.82 (0.17)	0.87 (0.08)	1.25 (0.14)

Abbreviations: RSD: - relative standard deviation; λ_{max} - wavelength of maximum absorption

Table 8: Assay of the sampled brands of levofloxacin tablets and infusion

Brand code	Amount found (mg) ΔD_1 (298, 228) nm	RSD (%)	Content (%)	Amount found (mg) ΔD_2 (257, 348) nm	RSD (%)	Content (%)	Remarks*	Difference (95% CI)	P-value
LVX-1	761.38	0.96	101.52	755.28	0.50	100.70	passed	6.10 (-10.86, 23.06)	0.262
LVX-2	748.77	0.48	99.84	752.28	0.27	100.30	passed	-3.51 (-8.76, 1.74)	0.103
LVX-3	744.89	1.23	99.32	740.68	1.07	98.76	passed	4.21 (-0.02, 8.44)	0.050
LVX-4	491.53	0.72	98.31	494.36	1.05	98.87	passed	-2.83 (-7.21, 1.54)	0.108
LVX-5	507.37	0.59	101.47	503.95	0.66	100.79	passed	3.42 (-4.64, 7.30)	0.063
LVX-6	501.55	0.35	100.31	499.08	0.10	99.82	passed	2.47 (-2.35, 7.28)	0.158
LVX-7	500.91	1.21	100.18	502.38	1.32	100.48	passed	-1.47 (-11.64, 8.70)	0.597

Abbreviations: * Acceptance Criteria: 90.0 - 110.0%; RSD:- relative standard deviation;

DISCUSSION

This study developed differential UV spectrophotometric methods for assaying levofloxacin using two identical solutions in 0.1N HCl and 0.1N NaOH at the same concentration, alternately serving as test and reference (ΔD1 and ΔD2). Results were compared with conventional UV methods, where analyte solutions in 0.1N HCl or 0.1N NaOH were measured against blanks. The differential spectra showed absorption maxima and minima at 298/228 nm for ΔD1 and 257/348 nm for ΔD2, consistent with the reference spectrum of levofloxacin. These differential peaks provide reliable identification parameters depending on the chosen reference medium. Additionally, levofloxacin exhibited absorption maxima at 288 nm in NaOH and 293 nm in HCl, aligning with established reference spectra. The study thus confirms the well-documented detection wavelengths used in conventional spectrophotometric determination of levofloxacin in both media.[15,17,22]

Differential spectrophotometry enhances measurement selectivity and accuracy by calculating absorbance differences between two equimolar solutions of an analyte in distinct chemical forms.[23,24] This approach eliminates matrix interference from excipients, resolves spectral overlap from impurities or degradation products, and avoids the need for time-consuming extraction or separation steps, enabling straightforward routine analysis of levofloxacin in pharmaceutical dosage forms.

Levofloxacin is a chiral fluorinated carboxyquinolone with two ionizable groups: a carboxylic acid (pKa1 = 6.24) and a basic piperazinyl moiety (pKa2 = 8.74), as shown in Figure 1.[25,26] Above pH 6.24, the carboxyl group is deprotonated, while below pH 8.74 the nitrogen remains protonated, giving the molecule an ionic charge across varying pH conditions.[4] This results in pH-dependent spectral shifts—288 nm in 0.1N NaOH and 293 nm in 0.1N HCl—when measured by conventional spectrophotometry. Such sensitivity enhances selectivity in differential spectrophotometry, where the difference-absorbance (the full-wave amplitude) also provides a reliable basis for quantitative analysis.[23,24]

Comparison of optical and validation parameters showed that differential spectrophotometry is more sensitive when levofloxacin in 0.1N HCl is used as the sample (ΔD1: 298, 228 nm) with an equal-concentration NaOH solution as the blank, than in the reverse setup (ΔD2: 257, 348 nm). This is supported by Sandell's sensitivity (0.0125 vs. 0.0166 $\mu g \cdot cm^{-2}$), lower LOD (0.1030 vs. 0.2901 µg/mL) and LOQ (0.3122 vs. 0.8790 µg/mL), and higher molar absorptivity (28,887 vs. 21,831 $L \cdot mol^{-1} \cdot cm^{-1}$) for ΔD1 compared to ΔD_2, respectively (Table 7). Despite these differences in sensitivity, both methods demonstrated comparable selectivity, accuracy, and precision, as reflected in recovery (100.26% vs. 100.17%) and RSD values (0.96 vs. 0.95%).

Comparison of differential spectrophotometry (ΔD1: 298, 228 nm) with conventional spectrophotometry at 293 nm in 0.1N HCl revealed that the differential method was slightly less sensitive (Sandell's sensitivity: 0.0125 vs. 0.0113 $\mu g \cdot cm^{-2}$; molar absorptivity: 28,887 vs. 32,062 $L \cdot mol^{-1} \cdot cm^{-1}$). However, it offered markedly lower detection limits (LOD: 0.1030 vs. 0.4198 µg/mL; LOQ: 0.3122 vs. 1.2722 µg/mL). Accuracy was comparable, with recoveries of 100.26% and 100.18%, but differential spectrophotometry demonstrated superior precision (RSD: 0.96 vs. 1.41%) and reduced variability (RSD range: 0.16 vs. 0.63%).

The primary aim of differential spectrophotometry is to improve selectivity by eliminating interference from foreign absorptions, which requires identical treatment of both sample and reference solutions—such as ensuring accurate cell path lengths and using the same pipette for dilutions to minimize background absorption.[22,23] A key limitation of this study is the absence of levofloxacin co-formulated products on the market, preventing their evaluation with the proposed method. Moreover, the reduced sensitivity of differential spectrophotometry compared to conventional spectrophotometry in 0.1N HCl at 293 nm indicates that it is unsuitable for trace analysis of levofloxacin in biological fluids, particularly when benchmarked against high-performance liquid chromatography.

CONCLUSION

The proposed method is simple, accurate, and highly selective for quantifying levofloxacin in both bulk drug and pharmaceutical formulations. Its selectivity enables reliable determination of levofloxacin content in tablets and infusions, even in the presence of excipients or degradation products, thereby supporting its application in routine quality control analysis.

Declarations

Ethics approval and consent to participate: Not applicable.
Consent for publication: Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

STA conceptualized the study, and FOD conducted the experiments. STA and OIE analysed the data. AS and OIE contributed to supervising the project. STA and FOD drafted the manuscript, while AS and ODM designed the data curation. FMM and JOS revised the manuscript. All authors read and approved the final version.

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