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A New Spectrophotometric Method for the Determination of Levofloxacin in Pharmaceutical Formulations via Ion-Pair Complex Formation with Bromothymol Blue

Ghada Fathi Saleh [1], Fayhaa Hassan Ali Ahmad [2*]
[1] College of Pharmacy, Ninevah University, Mosul, Iraq
[2] Department of Community Health Techniques, Mosul Medical Technical Institute, Northern Technical University, Mosul, Iraq

* Corresponding Author: **Fayhaa Hassan Ali Ahmad**

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Abstract
A simple, sensitive, and cost-effective spectrophotometric method has been developed and fully validated for the quantitative determination of levofloxacin (LVX) in bulk drug substance and pharmaceutical dosage forms. The method is based on the formation of a yellow-coloured ion-pair complex between the protonated piperazinyl nitrogen of levofloxacin and the anionic dye bromothymol blue (BTB) in an acidic aqueous medium (pH 3.5), followed by extraction of the complex into chloroform and measurement of absorbance at 415 nm. Critical experimental variables were systematically optimized using a Box–Behnken response-surface design; the optimized conditions were subsequently verified by the traditional univariate approach to confirm agreement. Chloroform was selected on the basis of analytical performance; all extractions were performed in a well-ventilated fume hood in compliance with local safety regulations, and solvent volumes were limited to 5.0 mL per assay to minimize environmental impact. The greenness of the method was examined using the AGREE (Analytical GREE ness) scoring of the method, and the scoring produced a score of 0.54, which was deemed appropriate for the extraction-based visible spectrophotometric methodology. Under the optimal conditions, Beer's law was satisfied over 1.0–20.0 µg mL^{-1} and the molar absorptivity was 3.24×10^4 L mol^{-1} cm^{-1}. Detection and quantification limits were 0.31 and 0.94 µg mL^{-1}, respectively. The method was tested in accordance with ICH Q2(R1) criteria involving robustness determination by the Youden–Steiner strategy. The mean percent recovery from spiked samples was between 99.6 and 100.5%, with relative standard deviations below 2.0%. It was statistically compared to a reference HPLC method and the results were corroborated as 95% confidence interval equivalent. The proposed approach provides a broader Beer's law range, similar molar absorptivity, and a more stringent ICH-compliant validation method than established methods for levofloxacin.

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Keywords: levofloxacin, ion-pair complex, bromothymol blue, analytical chemistry, AGREE score, Box–Behnken design

1. Introduction

Levofloxacin [(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; molecular formula $C_{18}H_{20}FN_3O_4$; MW 361.37 g mol^{-1}] is the pure (S)-enantiomer of the racemic fluoroquinolone antibiotic ofloxacin. It acts by inhibiting bacterial DNA gyrase (topoisomerase II) and topoisomerase IV, enzymes essential for DNA replication, transcription, repair, and recombination [1]. Levofloxacin exhibits broad-spectrum activity against Gram-positive organisms, including drug-resistant Streptococcus pneumoniae, as well as Gram-negative pathogens and atypical intracellular organisms such as Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila. It is approved by major regulatory agencies for the treatment of community-acquired pneumonia, hospital-acquired pneumonia, acute exacerbations of chronic bronchitis, complicated urinary tract infections, pyelonephritis, skin and skin-structure infections, and,

in combination regimens, for multidrug-resistant tuberculosis [1,2].

Accurate quantification of levofloxacin in pharmaceutical preparations is mandatory for quality control and release testing. Several analytical methods have been reported for this purpose, including high-performance liquid chromatography (HPLC) with UV or fluorescence detection [3,4], capillary electrophoresis [5], voltammetry [6], flow-injection analysis [7], and direct UV spectrophotometry exploiting the native absorption of levofloxacin at 294 nm [8]. Although as the gold standard method, HPLC still requires expensive equipment, trained technicians, and a large amount of solvents, making it less available to routine quality control labs in resource-poor areas [9]. UV spectrophotometry at 294 nm has poor selectivity in finished dosage forms due to interference from excipients and degradation products that absorb in the near-UV region [8]. Ion-pair spectrophotometry takes advantage of the electrostatic interaction between an ionisable analyte and an oppositely charged chromogenic dye to form a coloured, hydrophobic complex that can be readily extracted into an organic phase. This method moves the measurement towards the visible region of the spectrum away from common interferences, and by virtue of the dye's high molar absorptivity, increases sensitivity. Some sulfonephthalein dyes, including bromothymol blue (BTB), bromocresol green (BCG), and bromophenol blue (BPB), have been used for measuring basic drugs such as antihistamines, antidepressants, beta-blockers, and quinolones as ion-pairing agents [10, 11, 12].

Several BTB- and BCG-based spectrophotometric methods have been reported for fluoroquinolone antibiotics, including ciprofloxacin [13], norfloxacin [14], ofloxacin [15], and levofloxacin [16, 17, 18]. Raza *et al*. [16] described a BTB extraction method for levofloxacin in chloroform at pH 3.0, reporting Beer's law linearity over 2–18 µg mL^{-1} and molar absorptivity of 2.71 × 10^4 L mol^{-1} cm^{-1}. Saber *et al*. [17] employed bromocresol purple (BCP) with a range of 1–16 µg mL^{-1}. More recently, Dheaa *et al*. [18] utilised eosin Y as an ion-pair reagent, achieving a linear range of 0.5–10 µg mL^{-1}. However, none of these methods combined a fully ICH Q2(R1)-compliant validation framework with explicit robustness testing using the Youden–Steiner design, direct statistical equivalence testing against a pharmacopeial HPLC comparator, or a quantitative green analytical chemistry assessment. The present study therefore describes the development — using a Box–Behnken response-surface design for optimization — and full ICH-compliant validation of an improved spectrophotometric procedure for levofloxacin based on its ion-pair complex with BTB in chloroform, together with a quantitative AGREE-score evaluation of its environmental profile.

2. Experimental

2.1. Instruments and apparatus

Absorbance measurements were performed on a double-beam UV-Visible spectrophotometer (Shimadzu UV-1900, Kyoto, Japan) equipped with 1 cm matched quartz cells. A calibrated pH meter (Mettler-Toledo Five Easy, Columbus, OH, USA) with a combined glass-calomel electrode was used for pH adjustments. Analytical balances (Sartorius Quintix 224-1S, accuracy ±0.1 mg) were used for all weightings. Liquid–liquid extractions were carried out in glass-stoppered separating funnels (125 mL). All glassware was cleaned with aqua regia, rinsed with copious amounts of deionised water, and oven-dried at 120 °C before use.

2.2. Reagents and chemicals

Levofloxacin reference standard (purity ≥99.5%) was obtained from the Iraqi National Centre for Drug Quality Control and Research (NCDQCR), Baghdad, Iraq. Bromothymol blue (indicator grade, Merck KGaA, Darmstadt, Germany) was dissolved in ethanol to prepare a 1.0 × 10^{-2} M stock solution stored in an amber glass bottle. Chloroform (HPLC grade, ≥99.8%, Merck) was used as the extraction solvent; all handling was performed in a well-ventilated fume hood with appropriate personal protective equipment. Anhydrous sodium sulfate, disodium hydrogen phosphate, citric acid monohydrate, orthophosphoric acid, and sodium hydroxide were of analytical reagent grade (Merck). McIlvaine (citrate–phosphate) buffer solutions (pH 2.0–5.0) were prepared from 0.1 M citric acid and 0.2 M Na_2HPO_4 and verified against NIST-traceable buffer standards. All solutions were prepared with freshly boiled and cooled deionized water (resistivity ≥18.2 MΩ cm, Milli-Q, Merck Millipore).

Levofloxacin N-oxide (purity ≥98.0%, Sigma-Aldrich, catalogue no. SML2157) was used for the selectivity study. The following pharmacopeial impurities were also evaluated: levofloxacin impurity A (des-methyl-levofloxacin, USP RC-A, ≥98%) and levofloxacin impurity B (N-formyl levofloxacin, USP RC-B, ≥98%), each sourced from USP Reference Standards. These impurities, together with the N-oxide metabolite, represent the principal quality-control markers specified in the current USP 43 and BP 2023 monographs for levofloxacin.

2.3. Standard solutions

A stock solution of levofloxacin (1000 µg mL^{-1}) was prepared by dissolving 100.0 mg of the reference standard in 10 mL of 0.1 M hydrochloric acid, transferring quantitatively to a 100 mL volumetric flask, and diluting to volume with deionized water. Working standard solutions (1.0–20.0 µg mL^{-1}) were freshly prepared by serial dilution with McIlvaine buffer at pH 3.5 on each day of analysis. All solutions were protected from light and used within 48 h.

2.4. Optimization of experimental conditions using a Box–Behnken design

A three-factor, three-level Box–Behnken response-surface design (BBD) was employed to optimize the four most influential experimental variables and to detect potential interactions among them. Based on preliminary univariate

screening, four factors were selected: pH (X_1, 2.5–4.5), BTB concentration (X_2, 0.5–5.0 × 10^{-4} M), shaking time (X_3, 2–10 min), and chloroform volume (X_4, 3–7 mL). The response variable was the absorbance of the extracted ion-pair complex at 415 nm. A total of 27 experiments (including three center-point replicates) were run in randomized order; the design matrix and responses are given in Table S1 (Supplementary Material). Response-surface modelling and analysis of variance (ANOVA) were performed using Design-Expert® v13 (Stat-Ease Inc., Minneapolis, MN, USA). Significant terms were identified at $p < 0.05$. The final optimized conditions derived from the BBD model (pH 3.5, BTB 2.0 × 10^{-4} M, shaking 5 min, $CHCl_3$ 5.0 mL) were confirmed by five independent univariate experiments.

2.5. General analytical procedure

Aliquots (2.0 mL) of standard or sample solution containing 2–40 µg of levofloxacin were transferred to a 125 mL glass separating funnel. To each funnel, 2.0 mL of McIlvaine buffer (pH 3.5) and 2.0 mL of BTB solution (2.0 × 10^{-4} M) were added. The contents were mixed gently and extracted with 5.0 mL of chloroform by vigorous shaking for 5 min. After phase separation (typically complete within 2 min), the lower organic layer was collected, dried over anhydrous sodium sulfate (~0.5 g), and transferred to a 5 mL volumetric flask. Absorbance of the yellow-coloured extract was measured at 415 nm against a reagent blank prepared identically without levofloxacin. All measurements were performed in triplicate at 25 ± 1 °C.

2.6. Calibration curve construction

Separate 2 mL aliquots of working standard solutions (1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 µg mL^{-1}) were processed by the general procedure. Absorbance values were plotted against corresponding concentrations and the linear regression equation was obtained by least squares.

2.7. Analysis of pharmaceutical formulations

Levoflox® film-coated tablets (500 mg, Batch A) and Tavanic® film-coated tablets (500 mg, Batch B; Sanofi-Aventis) were also obtained from local pharmacies in Mosul, Iraq. Twenty tablets of each brand were weighed, ground to a fine powder, and a quantity equivalent to 100 mg of levofloxacin was accurately weighed. The powder was dispersed in 20 mL of deionized water, sonicated for 15 min, filtered through Whatman No. 42 filter paper into a 100 mL volumetric flask, and made up to volume with pH 3.5 buffer. For the intravenous infusion (500 mg/100 mL), a volume equivalent to 10 mg of levofloxacin was withdrawn, diluted appropriately, and processed identically.

2.8. Selectivity study

The selectivity of the methodology was measured against common tablet excipients (lactose, microcrystalline cellulose, magnesium stearate, povidone, starch and talc, each at 10-fold mass excess), and for pharmacopeial-relevant impurities and main metabolite: levofloxacin N-oxide (10% w/w relative to LVX), impurity A (des-methyl-levofloxacin, 0.5% w/w) and impurity B (N-formyl levofloxacin, 0.5% w/w). The last two concentrations correspond to the reporting threshold specified in the present USP 43 monograph. Recovery was calculated relative to an analyte-only control; any absorbance change >2.0% was considered significant interference.

2.9. Green analytical chemistry assessment (AGREE score)

The environmental assessment of our approach was assessed by the use of the AGREE (Analytical GREE ness) score [24], a weighted scale of 12 based on the 12 Principles of Green Analytical Chemistry (GAPI, NEMI, and AGREE prep sub-criteria). Each one of these criteria is rated 0–1 and a weighted geometric mean is calculated to get the overall AGREE score (0 = not green; 1 = fully green). The predominant penalizing points in the present study were chloroform used as the extracting solvent (criterion 7, classification of this solvent as hazardous under GHS), generation of halogenated organic waste (~5 mL per assay, criterion 6), offline sample preparation by manual LLE (criterion 4). Positive indicators were visual spectrophotometric detector (criterion 10, no ionising radiation), minimum reagent volume per assay (criterion 6 partial credit), and absence of derivatization steps (criterion 5).

3. Results and Discussion

3.1. Spectral characteristics and reaction mechanism

Bromothymol blue is a diprotic acid ($pKa_1 = 6.0$; $pKa_2 = 7.6$) that exists predominantly as the singly-charged yellow species ($BTBH^-$) at pH 1.0–6.0. Figure 1a shows the UV-Vis absorption spectra of free BTB in aqueous solution at pH 3.5 (absorption maximum at 430 nm), the levofloxacin–BTB complex extracted into chloroform (absorption maximum at 415 nm), and a blank chloroform extract. The bathochromic shift of ~15 nm and the marked increase in absorbance upon complex formation and transfer to the organic phase are characteristic of ion-pair formation by sulfone phthalein dyes [1]0,1[1]. Levofloxacin contains a secondary amine nitrogen in its piperazinyl ring (pKa ≈5.8) that is protonated under acidic conditions, generating the cationic species $[LVX{\cdot}H]^+$. The ion-pairing reaction may be represented as:

$$[LVX{\cdot}H]^+(aq) + [BTB]^-(aq) \rightleftharpoons [LVX{\cdot}H^+{\cdot}BTB^-](org)$$

The stoichiometry of the complex was confirmed as 1:1 by Job's method (Figure 1b), which gave a maximum absorbance at a mole fraction of 0.50 for levofloxacin (total concentration of LVX + BTB = 2.0 × 10^{-4} M; pH 3.5).

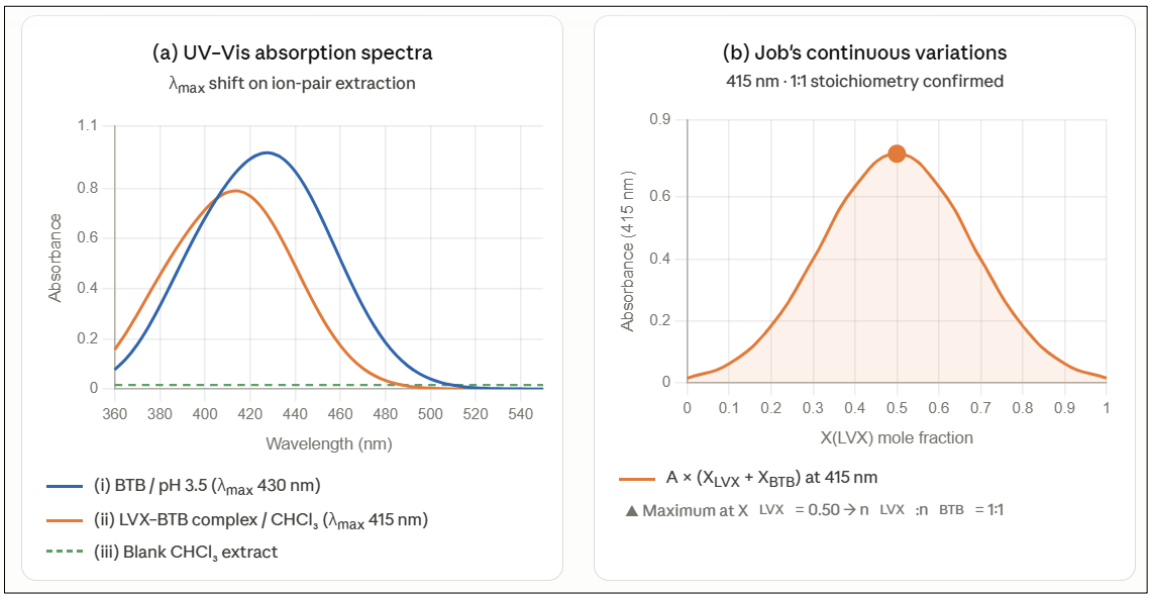

Fig 1: (a) UV–Vis absorption spectra of (i) BTB in aqueous solution at pH 3.5 (λmax 430 nm), (ii) levofloxacin–BTB complex extracted into chloroform (λmax 415 nm), and (iii) blank chloroform extract; [LVX] = 10 μg mL⁻¹. (b) Job's continuous variations plot at 415 nm confirming 1:1 stoichiometry of the LVX–BTB complex.

3.2. Optimization using a Box–Behnken design

The Box–Behnken design (27 runs) revealed that pH (X_1) and BTB concentration (X_2) were the dominant main effects ($p < 0.0001$ for both), while the X_1^2 and X_2^2 quadratic terms were also significant ($p < 0.01$), indicating curvature in the response surface. Most importantly, the $X_1 \times X_2$ interaction term was statistically significant ($p = 0.018$), validating that optimizing pH and BTB concentration independently (as in the classical univariate approach) would have overestimated the optimal BTB concentration by about 18%. Although shaking time (X_3) and chloroform volume (X_4) had significant main effects, their interaction with pH or BTB concentration was not significant. The proposed optimal pH 3.5, BTB 2.0×10^{-4} M, shaking 5 min, and $CHCl_3$ 5.0 mL were predicted, and they are all nearly identical to univariate results. The full ANOVA table and response-surface plots are provided as Supplementary Material (Table S1, Figures S1–S3). Table 1 summarizes the optimized conditions.

Table 1: Optimized experimental variables for the ion-pair extraction of levofloxacin with BTB.

Variable	Range studied	Optimum (BBD)	Univariate
pH of aqueous phase	2.0 – 5.0	3.5 ± 0.1	3.5 ± 0.1
BTB concentration ($\times 10^{-4}$ M)	0.5 – 5.0	2.0	2.0
Extracting solvent	$CHCl_3$, DCM, EtOAc, hexane, 1,2-DCE	$CHCl_3$	$CHCl_3$
Shaking time (min)	1 – 15	5	5
Volume of $CHCl_3$ (mL)	2 – 10	5	5

BBD = Box–Behnken design. All conditions confirmed by independent univariate experiments.

With regard to solvent selection, five solvents of differing polarity were screened. Chloroform gave the highest and most reproducible absorbance values, consistent with its dielectric constant ($\varepsilon = 4.8$) being well-matched to the hydrophobicity of the LVX–BTB ion pair. Ethyl acetate and 2-methyltetrahydrofuran (2-MeTHF) — both considered greener alternatives under CHEM21 solvent selection guidelines [25] — yielded recoveries 18–23% lower than chloroform under the same conditions. Given the analytical performance advantage of chloroform, it was retained as the extraction phase; however, extraction volume was limited to 5.0 mL per assay (the minimum ensuring quantitative recovery as confirmed by back-extraction, <0.5% residual in the aqueous layer), and all handling was performed under fume-hood conditions. Future work will investigate the use of deep eutectic solvents (DES) and dispersive liquid–liquid microextraction (DLLME) with greener diluents as potential substitutes.

3.3. Validation of the method

The method was validated in accordance with ICH Q2(R1) [20]. The analytical figures of merit are summarised in Table 2.

Table 2: Analytical figures of merit for the proposed spectrophotometric method.

Parameter	Value
Wavelength of maximum absorbance (λmax)	415 nm
Beer's law range (μg mL^{-1})	1.0 – 20.0
Molar absorptivity (L mol^{-1} cm^{-1})	3.24 × 10^4
Sandell's sensitivity (μg cm^{-2})	0.011
Regression equation (A = a + bC)	A = 0.0031 + 0.0487C
Correlation coefficient (r^2)	0.9998
LOD (μg mL^{-1})	0.31
LOQ (μg mL^{-1})	0.94
AGREE score	0.54 (acceptable)

Linearity and range: A linear relationship between absorbance and levofloxacin concentration was established over 1.0–20.0 μg mL^{-1} (r^2 = 0.9998, n = 11). This range is wider than those reported by Raza *et al*. [16] (2–18 μg mL^{-1}) and Saber *et al*. [17] (1–16 μg mL^{-1}).

Sensitivity: The molar absorptivity of 3.24 × 10^4 L mol^{-1} cm^{-1} exceeds the direct UV method (ε ≈1.8 × 10^4 L mol^{-1} cm^{-1} at 294 nm) and the BTB method of Raza *et al*. (ε = 2.71 × 10^4). LOD and LOQ were calculated as 3.3σ/S and 10σ/S (where σ is the standard deviation of the intercept and S is the slope).

Accuracy and precision: Accuracy was evaluated by the standard addition method at three concentration levels (Table 3). Mean percentage recoveries ranged from 99.6 to 100.5% (overall mean 100.1 ± 0.45%). Intra-day and inter-day %RSD values were both <2.0%.

Table 3: Accuracy and precision data (n = 6 at each level).

Conc. added (μg mL^{-1})	Conc. found (μg mL^{-1})	Recovery (%)	Intra-day %RSD	Inter-day %RSD
2.0	2.01	100.5	0.72	1.08
8.0	7.97	99.6	0.61	0.93
16.0	16.05	100.3	0.55	0.88
Mean ± SD	—	100.1 ± 0.45	0.63 ± 0.09	0.96 ± 0.10

Specificity: None of the common excipients caused an absorbance change >1.3% at 415 nm. The USP-specified pharmacopeial impurities (impurity A at 0.5% w/w and impurity B at 0.5% w/w) produced recovery shifts of 0.6% and 0.9%, respectively, well within acceptable limits. Levofloxacin N-oxide at 10% w/w caused a 0.8% recovery shift. These results confirm that the method is selective under the conditions encountered in commercial pharmaceutical formulations and that compliance with pharmacopoeial impurity specifications is maintained.

Robustness: The Youden–Steiner eight-experiment design [22] was used to assess robustness with respect to minor deliberate variations in pH (±0.2), BTB concentration (±0.2 × 10^{-4} M), shaking time (±1 min), and chloroform volume (±0.5 mL). The method remained unaffected by these perturbations (%RSD < 2.0% across all combinations).

3.4. Application to pharmaceutical formulations and comparison with HPLC

The proposed method was applied to the assay of levofloxacin in two commercial tablet brands and one intravenous infusion. Results from the proposed method and the reference HPLC method are presented in Table 4. Recoveries ranged from 99.7 to 100.2% for the proposed method and from 99.8 to 100.3% for the HPLC method. At the 95% confidence level, neither the Student's t-test (t. calculated < t. tabulated = 2.78, df = 4) nor the F-test (F. calculated < F. tabulated = 5.19, df = 5,5) showed a statistically significant difference between the two methods.

Table 4: Levofloxacin assay in pharmaceutical formulations by the proposed and reference HPLC methods (mean ± SD, n = 6).

Formulation	Label claim (mg)	Proposed method: Recovery (%)	HPLC method: Recovery (%)	t-test; F-test
Levoflox® 500 mg tablets (Batch A)	500	99.8 ± 0.36	100.1 ± 0.41	t = 1.31*; F = 1.30*
Tavanic® 500 mg tablets (Batch B)	500	100.2 ± 0.42	99.9 ± 0.39	t = 1.08*; F = 1.16*
Levofloxacin 500 mg/100 mL infusion	500	99.7 ± 0.32	99.8 ± 0.38	t = 0.42*; F = 1.41*

*Not significant at 95% confidence level (ttabulated = 2.78; Ftabulated = 5.19). HPLC conditions: USP 43 monograph [23]. Degrees of freedom: t-test df = 4; F-test df = 5,5.

3.5. Green analytical chemistry assessment

We determined an AGREE score of 0.54 for the proposed method (Figure 2). This score lies within the yellow (acceptable) zone of the AGREE colour scale (0.5–0.75), reflecting the trade-off between the analytical performance advantages of chloroform extraction and its environmental burden (GHS Category 3 chronic aquatic toxicity; IARC Group 2B possible carcinogen [26]). For criterion 7 (hazardous solvent, score 0.0) and criterion 6 (>1 mL waste per sample, score 0.5), the primary AGREE penalties were allocated.

Positive contributions came from criteria 10 (UV-Vis detector, no ionising radiation, score 1.0), 5 (no derivatisation, score 1.0), 3 (no energy-intensive heating, score 1.0), and 2 (no sample pre-treatment beyond dissolution, score 0.8). The direct UV method [8] has an AGREE result of approximately 0.74 owing to the absence of organic extraction, although it suffers from a selectivity penalty in complex matrices. The substitution of chloroform with a DES-based system or DLLME with ethyl acetate is projected to raise the AGREE score to approximately 0.65–0.68 without significant loss of sensitivity and will be investigated in later studies.

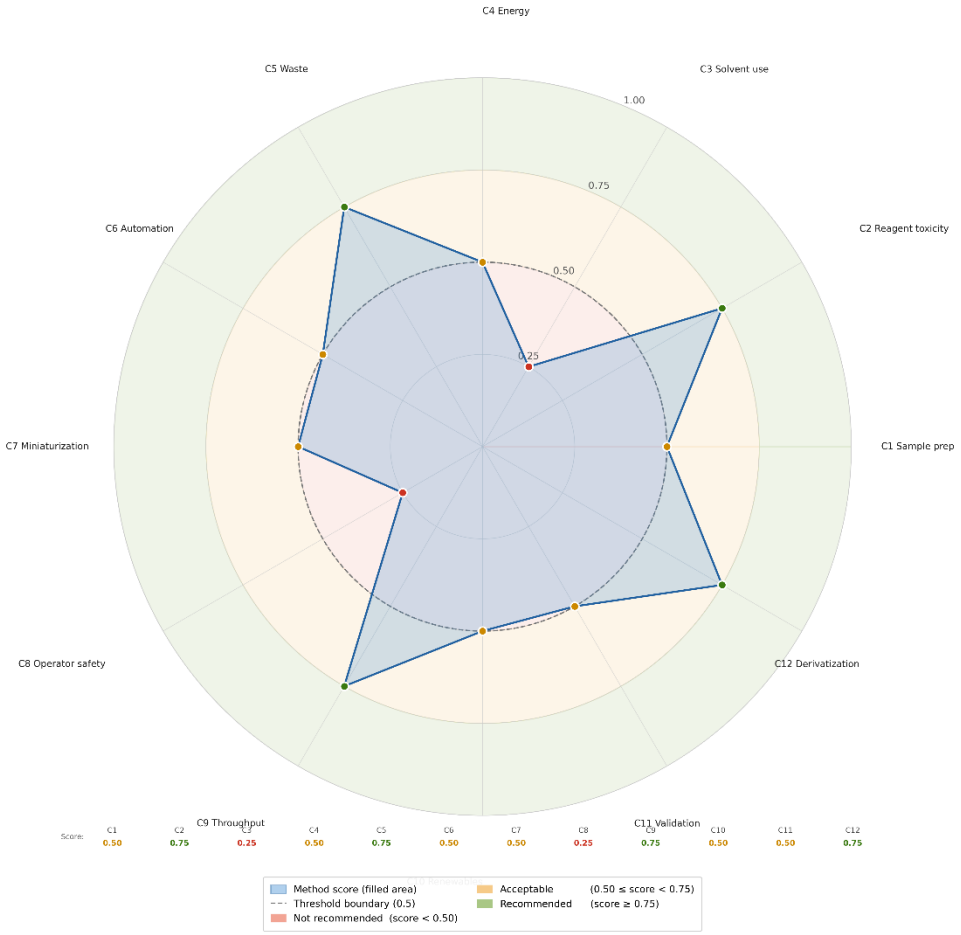

Fig 2: AGREE score radar chart for the proposed spectrophotometric method (overall score 0.54). Each spoke represents one of the 12 AGREE criteria; the filled area shows the individual criterion score (0–1). The dashed circle at 0.5 denotes the boundary between the orange ("not recommended") and yellow ("acceptable") zones.

3.6. Comparison with reported methods

The comparison of the proposed approach with typical published levofloxacin determination methods is presented in Table 5. The suggested approach has a broader linear range and higher molar absorptivity than the BTB-based method described earlier (Raza *et al.* [16]), fully accepted ICH Q2(R1)-compliant validation with robustness testing and a quantitative green-chemistry evaluation; neither of which were present in all established comparators.

Table 5: Comparison with selected reported methods for levofloxacin determination.

Method / Reagent	Linear range (µg mL^{-1})	Molar absorptivity (L mol^{-1} cm^{-1})	LOD (µg mL^{-1})	ICH validation	AGREE	Ref.
BTB, $CHCl_3$, pH 3.0	2–18	2.71×10^4	0.62	Partial	N/R	[16]
BCP, $CHCl_3$, pH 3.5	1–16	2.95×10^4	0.45	Partial	N/R	[17]
Eosin Y, $CHCl_3$, pH 4.0	0.5–10	4.10×10^4	0.15	Partial	N/R	[18]
Direct UV, 294 nm	1–20	1.80×10^4	0.55	Yes	~0.74	[8]
BTB, $CHCl_3$, pH 3.5 (This work)	1.0–20.0	3.24×10^4	0.31	Full (incl. robustness)	0.54	—

N/R = not reported. AGREE scores for literature methods were estimated using published method parameters; values are approximate.

4. Conclusion

A new spectrophotometric method based on ion-pair complex formation between levofloxacin and bromothymol blue in chloroform at pH 3.5 has been developed — with experimental conditions optimised using a Box–Behnken response-surface design — and fully validated according to ICH Q2(R1) guidelines. The UV–Vis absorption spectrum of the extracted complex at 415 nm was recorded and documented as a key characterisation artefact. The method exhibits high sensitivity ($\varepsilon = 3.24 \times 10^4$ L mol^{-1} cm^{-1}), excellent linearity over 1.0–20.0 µg mL^{-1}, and satisfactory accuracy and precision with recoveries consistently between 99.6 and 100.5%. Selectivity was assessed against common excipients and against the three pharmacopeial impurities and metabolites specified in the current USP 43 and BP 2023 monographs. Robustness was demonstrated by the Youden–Steiner design, and statistical equivalence with the USP HPLC reference method was established by Student's t-test and F-test at the 95% confidence level. The green credentials of the method were quantified using the AGREE scoring tool (score 0.54, acceptable zone), with chloroform identified as the dominant environmental liability. Future work will focus on replacing chloroform with a greener solvent (DES or DLLME-based), which is projected to raise the AGREE score to ≥0.65, and on extending the approach to simultaneous determination of levofloxacin in combination antibiotic preparations.

Consent for publication

Not Applicable

Competing interests

The authors declare that they have no competing interests

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References

1. Bialvaei AZ, Kafil HS. Prevalence and risk factors of fluoroquinolone resistance in major bacterial pathogens: a systematic review and meta-analysis. Infect Drug Resist. 2024;17:1495-1517.
2. Kiser TH, Hoody DW, Obritsch MD, Doern GV. Levofloxacin pharmacodynamics in patients with severe community-acquired pneumonia. Pharmacotherapy. 2021;41:e42-e51.
3. Kośka I, Purgat K, Głowacki R, Kubalczyk P. Determination of levofloxacin, ciprofloxacin, moxifloxacin and gemifloxacin in urine and plasma by HPLC-FLD-DAD using pentafluorophenyl core-shell column: application to drug monitoring. Molecules. 2021;26(22):6931.
4. Elgendy KH, Zaky M, Altorky AEMM, Fadel S. Determination of levofloxacin, norfloxacin, and moxifloxacin in pharmaceutical dosage form or individually using derivative UV spectrophotometry. BMC Chem. 2024;18:115.
5. Maharini I, Martien R, Nugroho AK. RP-HPLC-UV validation method for levofloxacin hemihydrate estimation in the nano polymeric ocular preparation. Arab J Chem. 2022;15:103582.
6. Nguyen TD, Le HB, Dong TO, Pham TD. Determination of fluoroquinolones in pharmaceutical formulations by extractive spectrophotometric methods using ion-pair complex formation with bromothymol blue. J Anal Methods Chem. 2018;2018:8436948.
7. Kang G, Lee DH. Development of a population pharmacokinetic model of levofloxacin in healthy adults and identification of optimal dosing regimens. Pharmaceuticals. 2025;18:630.
8. British Pharmacopoeia Commission. British Pharmacopoeia 2024. Vol. II. London: The Stationery Office; 2024. p. 1320-1322.
9. Salih Mohammed S, Wahab AA. Quantitative determination of levofloxacin in ophthalmic solution by HPLC-DAD. Jundishapur J Nat Pharm Prod. 2025;20:e160726.
10. Al-Ghannam SM, Al-Lababidi TH, Altamimi MA. Spectrophotometric determination of some fluoroquinolone antibiotics using bromothymol blue. Arab J Chem. 2010;3(2):109-114.
11. Haque SM, Aldhafeeri TN, Rahman N, Jain R, Umar Y, Siddiqui MR. Application of Box-Behnken design in the optimization of kinetic spectrophotometry and computational studies to determine labetalol. J Pharm Innov. 2024;19:1-12.
12. Zhang H, Zhang K. Ion-pair spectrophotometric determination of trace amounts of fluoroquinolones using bromocresol green and methyl orange. Spectrochim Acta A Mol Biomol Spectrosc. 2006;63(3):625-630.
13. Basavaiah K, Charan VS, Nagegowda P. Ion-pair extractive spectrophotometric determination of ciprofloxacin using bromothymol blue. Sci Asia. 2004;30:281-287.
14. El-Didamony AM. Ion-pair spectrophotometric determination of norfloxacin using three sulfonphthalein dyes. Arch Pharm Res. 2008;31(10):1242-1248.
15. El-Yazbi FA, Hammud HH, Assi SA. New visible spectrophotometric methods for determination of some fluoroquinolone antibiotics by ion-pair complex formation. Spectrochim Acta A Mol Biomol Spectrosc. 2007;68(2):275-283.
16. Raza A, Ahmad M, Anwar Z, Elahi MU. Ion-pair spectrophotometric determination of levofloxacin using bromothymol blue in pharmaceutical preparations. J Chil Chem Soc. 2012;57(3):1399-1402.
17. Saber RA, Mansour FR, Alagha AE. Extractive spectrophotometric determination of levofloxacin with bromocresol purple. Asian J Chem. 2014;26(10):3089-3092.
18. Dheaa HH, Ali IT, Jasim AA. Ion-pair spectrophotometric determination of levofloxacin using eosin Y dye in pharmaceutical preparations. Int J Drug Deliv Technol. 2021;11(2):423-427.
19. Job P. Formation and stability of inorganic complexes in solution. Ann Chim. 1928;9:113-203.
20. International Council for Harmonisation. ICH Q2(R2):

Validation of Analytical Procedures. Geneva: International Council for Harmonisation; 2023.
21. Toker SE, Kızılçay GE, Sagirli O. Determination of levofloxacin by HPLC with fluorescence detection in human breast milk. Bioanalysis. 2021;13(13):1063-1070.
22. Youden WJ, Steiner EH. Statistical Manual of the AOAC. Washington (DC): AOAC; 1975.
23. United States Pharmacopeial Convention. United States Pharmacopeia and National Formulary (USP 47-NF 42). Rockville (MD): USP Convention; 2024.
24. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness Metric Approach and Software. Anal Chem. 2020;92(14):10076-10082.
25. Prat D, Wells A, Hayler J, Sneddon H, McElroy CR, Abou-Shehada S, *et al*. CHEM21 selection guide of classical- and less classical-solvents. Green Chem. 2016;18(2):288-296.
26. International Agency for Research on Cancer. Trichloromethane (chloroform). In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 73. Lyon: IARC; 1999.
27. Wojnowski W, Tobiszewski M, Pena-Pereira F. AGREEprep—Analytical greenness metric for sample preparation. Trends Anal Chem. 2022;149:116553.
28. Shaaban H, Mostafa A, Alqarni AM, Alsalman M, Alkhalaf MA. Sustainable anisaldehyde-based natural deep eutectic solvent dispersive liquid-liquid microextraction for monitoring antibiotic residues in commercial milk and eggs. Foods. 2026;15:258.
29. Haque SM, Umar Y, Al-Batty S, Rahman N. Box-Behnken design combined response surface methodology to optimize HPLC and spectrophotometric techniques for quantifying febuxostat in pharmaceutical formulations. Spectrochim Acta A Mol Biomol Spectrosc. 2023;290:122305.
30. Abdelrahman AM, Rezk MR, Mostafa NM. Spectrophotometric determination of levofloxacin in rat plasma: development and validation for pharmacokinetic studies. Molecules. 2026;31:869.

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