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BATCH AND FLOW INJECTION SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF LEVOFLOXACIN IN PHARMACEUTICAL PRODUCTS

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ABSTRACT. Simple and reliable batch and flow injection analysis (FIA) spectrophotometric methods were developed for the determination of levofloxacin (LVF) using bromocresol green (BCG) based on ion-pair complex formation in a neutral medium. The batch method showed linearity over the concentration range of 1.0-25.0 µg/mL with a coefficient of determination (R^2 = 0.9989), limit of detection (LOD = 0.85 µg/mL), and limit of quantification (LOQ = 2.4 µg/mL). The FIA method exhibited linearity over 10.0-50.0 µg/mL with R^2 = 0.9982, LOD = 1.48 µg/mL, and LOQ = 4.48 µg/mL. The relative standard deviation (RSD) was 3.38 for the batch method and 4.43 for the FIA method. The proposed batch method was more sensitive, while the FIA method provided higher sample throughput (90 samples/hour). The methods were successfully applied to the determination of LVF in pharmaceutical formulations with recoveries ranging from 98.2-101.7% (batch) and 99.83-101.58% (FIA). The results were in good agreement with the standard method, demonstrating the accuracy and reliability of the proposed approaches for routine quality control analysis.

KEY WORDS: Levofloxacin, Flow-Injection, Spectrophotometry, Bromocresol green complex

INTRODUCTION

Levofloxacin (LVF) is a broad-spectrum antimicrobial agent that inhibits bacterial DNA gyrase, leading to bacterial lysis. It is the active L-isomer of ofloxacin and effective against certain resistant gram-negative and gram-positive bacteria [1]. LVF, a recent addition, is utilized in the management of multidrug-resistant tuberculosis. It is chemically designated as (S)-9-flouro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido(1,2,3-de)-4,4-benzoxazine-6-carboxylic acid hemihydrate, the chemical formula is $C_{18}H_{20}FN_3O_4$. It is a white to brownish powder with limited solubility in water and methanol, soluble in glacial acetic acid [2].

LVF is efficacious in the treatment of infectious diarrhea induced by *E. coli*, Campylobacter jejuni, and Shigella bacteria. It may also be utilized to address numerous obstetric diseases, including mastitis and breast infection [3]. It is also used in human and veterinary medicine, especially in breeding areas [4].

Numerous analytical techniques have been reported in the literature for the determination of LVF in medication and biological fluids. These include kinetic spectroscopic analysis [5], UV spectrophotometry [6-8], potentiometric method [9,10], cyclic voltammetry [11], high performance liquid chromatography (HPLC) [12-14], infra-red (IR) [15], fluorometry [16-18], potentiometry [17,19], spectrofluorometric techniques [20], capillary electrophoresis [21], low frequency Raman spesctroscopy [22], and solid state spectroscopic techniques [23].

Most spectrophotometric methods reported for the analysis of LVF are based on the formation of ion-pair complexes [27]. In general, these previously reported techniques are often difficult to perform, as they are costly, resource-intensive, and require large quantities of reagents as well as specialized equipment [12]. All previous methods using BCG required buffer solutions and pH adjustments, while other methods required complex and expensive instruments, the addition of various chemicals, and heating steps [25]. Moreover, these procedures are time-consuming,

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making them less suitable for routine analysis in many laboratories. Overall, the mentioned techniques are complex and demand considerable cost, effort, and time. On the other hand, many spectrophotometric methods supported with a flow injection (FIA) system have been effectively enhanced for the assessment of medicines in many pharmaceutical products [26-28].

LVF has been widely determined using chromatographic and spectroscopic techniques, including UV–Vis spectrophotometry, HPLC, capillary electrophoresis, and electrochemical methods, depending on the matrix and required sensitivity. A recent review highlights that UV spectrophotometric methods remain popular due to their simplicity, low cost, and suitability for routine quality control analysis [29].

FIA and spectrophotometric methods are widely used for LVF determination due to their rapidity and simplicity. FIA methods provide high sample throughput and reproducibility, while spectrophotometric methods allow direct or indirect quantification based on derivatization or complex formation reactions. FIA and spectrophotometric methods have been successfully applied for levofloxacin in pharmaceutical formulations and biological samples with good linearity and low detection limits [30].

LVF, like other fluoroquinolones, can form stable ion-pair complexes with acidic sulfonephthalein dyes such as bromocresol green (BCG), bromophenol blue (BPB), and other similar dyes under acidic conditions. These complexes are often extractable into organic solvents and show strong absorbance in the visible region, making them suitable for spectrophotometric quantification. Ion-pair spectrophotometric methods using BCG are widely reported for basic drugs. The mechanism is based on electrostatic interaction between protonated drug species and anionic dye, forming a stable colored complex suitable for spectrophotometric analysis [31].
This study aimed to develop and validate simple, reliable, and cost-effective spectrophotometric methods-Batch and FIA for the quantitative determination of LVF in pure and pharmaceutical formulations. The methods are designed to offer high sensitivity, accuracy, and precision while eliminating the need for buffers, heating, or solvent extraction.

EXPERIMENTAL

Chemicals and reagents

A standard BCG reagent was obtained from Sigma-Aldrich (97% purity, molar mass = 698.01 g/mol). A standard LVF reference material was obtained from Awamedica (Kurdistan, Iraq) with a purity of 99%.

Instruments and apparatus

The UV-1900i Shimadzu double-beam UV-Vis spectrophotometer (Tokyo, Japan) was used for all spectrum measurements, employing 10 mm matched quartz cells. For the FIA system, a Jenway 6300 visible spectrophotometer with a 10 mm path length quartz flow cell was used. A multichannel peristaltic pump (Watson-Marlow 502S) was employed. A two-channel recorder (LKB-BROMMA 2210) was also used. In addition, A six-way injection valve with interchangeable sample-volume loops was used for sample injection in the FIA technique. A pH meter (Adwa AD12, Romania) was also used.

Preparation of solutions

BCG solution: A 4 x 10^{-4} M BCG solution was prepared by dissolving 0.136 g of the pure compound in a quantity of distilled water, then the volume was completed to 500 mL in a calibrated volumetric flask.

Stock solution of LVF hemihydrate: LVF stock solution (500 µg/mL) was prepared by dissolving 0.250 g of LVF hemihydrate (provided by Awamedica Company for drugs) in a quantity of distilled water, then completed to 500 mL. A working standard solution (100 µg/mL) was prepared from the stock solution using distilled water.

Preparation of sample solutions

Ten tablets of LVF from each manufacturer were accurately weighed and finely powdered using a mortar and pestle. An accurately weighed portion of the powder equivalent to one tablet (500 mg of LVF) was transferred into a suitable vessel and dissolved in distilled water with sonication to ensure complete dissolution. The solution was filtered to remove any insoluble excipients. The filtrate and washings were quantitatively transferred into a 500 mL volumetric flask and diluted to volume with distilled water to obtain a stock solution with a theoretical concentration of 1000 µg/mL.

A working solution of 100 µg/mL was prepared by transferring 25 mL of the stock solution into a 250 mL volumetric flask and diluting to the mark with distilled water. Further dilutions were prepared from the working solution using distilled water to obtain concentrations within the calibration range of 10-50 µg/mL. For example, a 10 µg/mL solution was prepared by transferring 1 mL of the 100 µg/mL working solution into a 10 mL volumetric flask and diluting to volume with distilled water. All solutions were freshly prepared before analysis.

Recommended procedure

Batch method

The batch procedure was carried out by transferring aliquots of LVF corresponding to 10.0-250.0 µg into a series of 10 mL volumetric flasks, giving final concentrations of 1.0-25.0 µg/mL. BCG solution (0.8 mL, 4×10^{-4} M) was added to each flask, and the solutions were diluted to volume with distilled water. Absorbance was measured at 616 nm against a reagent blank prepared in the same manner without LVF. Because the reaction gave a stable and reproducible response in distilled water, no buffer was used.

Flow injection method

The FIA manifold used for LVF determination is shown in Figure 1. One channel of the multichannel peristaltic pump was used to propel BCG solution (3.0×10^{-4} M) through 1.0 mm i.d. Teflon tubing at 0.7 mL min^{-1}. A 350 µL aliquot of LVF standard or sample solution was introduced through the injection valve into the reagent stream. No reaction coil was used, and the transient peak height was measured at 616 nm.

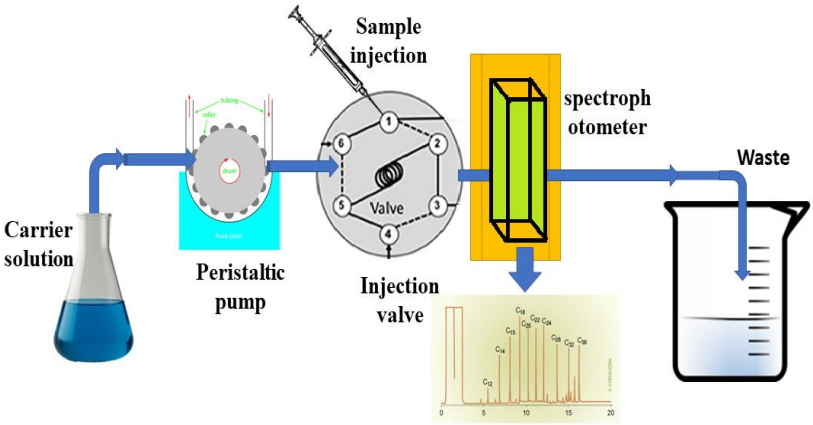


Figure 1. Schematic diagram of the FIA manifold used for the determination of LVF.

Statistical analysis

The statistical analysis was performed using the t-test and F-test through the SPSS program to compare the results obtained from the batch and FIA methods with standard methods [32].

RESULTS AND DISCUSSION

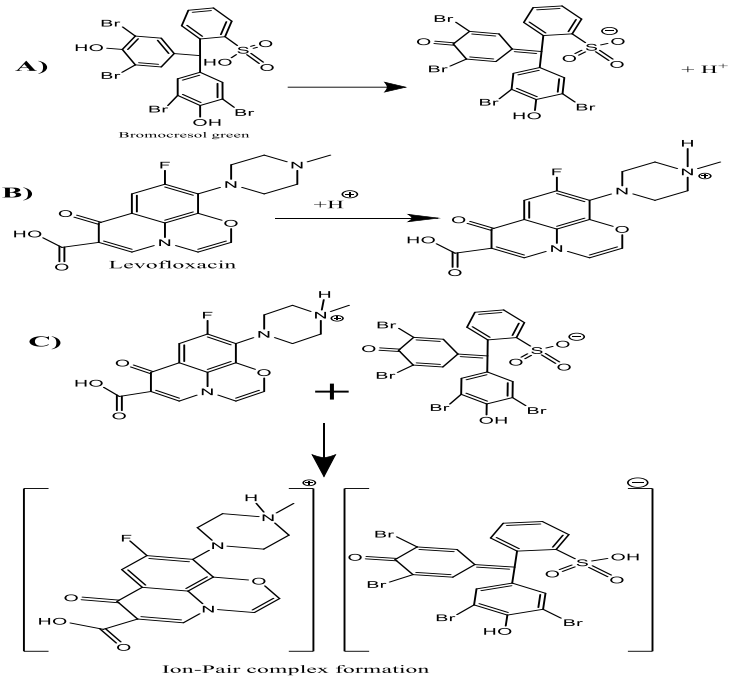
Ion pair spectrophotometry refers to analytical methods based on the optical properties of the ion-pair. The ion pair, also known as ionic association or ionic association complexes, they are pairs of oppositely charged ions held together by Coulomb attraction without the formation of a covalent bond. When the interaction between oppositely charged ions is strictly electrostatic, no new electronic bands appear in the absorption spectrum [33].

The ion-pair complexation in Scheme 1 illustrates the mechanism between LVF and BCG. In neutral medium, LVF is protonated at its nitrogen site, forming a positively charged species. Meanwhile, BCG, a sulfonated dye, exists as an anionic form. The electrostatic attraction between BCG and LVF solutions forms a stable ion-pair complex through both ionic and possible hydrogen-bonding interactions. The intensity of color increased when the reaction occurred between BCG and LVF [34], as shown in the absorption spectrum (Scheme 1).

At pH 7, BCG is mainly found in its deprotonated dianionic form, which gives it a blue-green color. Since the dye has a pKa of about 4.7, values above this lead to deprotonation. Thus, while BCG is yellow in acidic conditions, it appears blue-green at neutral pH [35].

At pH values below 6, LVF mainly exists in its fully protonated form. As the pH rises into the range of 6 to 9, it gradually loses a proton and shifts into its dominant intermediate form. When the environment becomes more alkaline (above pH = 9), this form further transforms into the free ligand [36].

In the proposed method, no buffer solution was required because the reaction between LVF and BCG proceeded efficiently and reproducibly in a neutral medium under the optimized experimental conditions. It was observed that the system provided stable and maximum analytical response without the need for buffering, and the use of a buffer did not significantly improve the signal. Therefore, to simplify the procedure, reduce reagent consumption, and avoid possible interference from buffer components, the reaction was carried out in the absence of a buffer system.



Scheme 1. Showing a] deprotonation of BCG, b] protonation of LVF, and c] the reaction between LVF and BCG.

Preliminary test for the batch method

For the preliminary spectral test, an aliquot of the LVF working solution was transferred to a 10 mL volumetric flask to give a final LVF concentration of 10.0 µg/mL. BCG solution (1.0 mL, 4×10^{-4} M) was added; dilute to volume with distilled water, mix thoroughly, and measure against a reagent blank using a 1.0 cm quartz cell. The LVF–BCG mixture showed a higher absorbance than the reagent blank, with the analytical maximum at 616 nm (Figure 2A). No appreciable shift in the wavelength maximum was observed.

Effect of the pH

The effect of pH was examined using ammonium acetate, potassium citrate, phosphate, and Tris–HCl buffer systems over their applicable pH ranges. These buffered media did not improve the analytical response compared with distilled water.

The pH of the reaction mixture prepared with distilled water was experimentally measured as 6.8. Under these conditions, the LVF–BCG system produced a stable and reproducible absorbance. Because buffering did not increase the response or improve analytical performance, distilled water was selected as the reaction medium for the subsequent experiments.

Effect of concentration of the reagent

The effect of BCG volume on the analytical response is shown in Figure 2B. Absorbance increased as the BCG volume was raised to 0.8 mL and then decreased slightly at higher volumes. Therefore, 0.8 mL of 4×10^{-4} M BCG was selected for the batch procedure.

Effect of the reaction time

The reaction between LVF and BCG remains stable for up to 24 hours after mixing, indicating that the ion-pair complex formation is immediate and does not change with time. Therefore, fixed-time measurements can be used without affecting the results.

Mole ratio

The stoichiometry of the complex formation between LVF and BCG was determined using the mole ratio method. A stock solution of LVF (10 µg/mL) was prepared by dissolving 1.00 mg of LVF in 100.00 mL of distilled water. A fixed aliquot of 1.00 mL of this solution was transferred into a series of 10 mL volumetric flasks, providing a constant amount of LVF in all measurements. The concentration of BCG (0.0004 M) was varied by adding different volumes (0.00-5.00 mL) to each flask while keeping the total volume constant (10 mL). The solutions were mixed thoroughly and allowed to equilibrate before measurement. The absorbance of the formed complex was recorded at the selected wavelength against a reagent blank. The mole ratio was evaluated by plotting absorbance versus the molar ratio of BCG to LVF, where the stoichiometric composition was determined from the point of maximum absorbance.

The moles of LVF were kept constant in all flasks at 2.7×10^{-7} mol. The moles of BCG were gradually increased from 0 to 2.0×10^{-6} mol. The maximum absorbance was obtained when the molar ratio of BCG to LVF approached unity, confirming that the formed complex has a 1:1 stoichiometry, as illustrated in Figure 2D.

Final absorption spectra

The absorption spectra of the LVF–BCG system showed a maximum analytical response at 616 nm; therefore, this wavelength was selected for the batch and FIA measurements.

Calibration curve for the batch spectrophotometric method

The batch calibration curve was linear over 1.0-25.0 µg/mL, with a coefficient of determination of $R^2 = 0.9989$ (Figure 2C). The molar absorptivity and Sandell's sensitivity were 1.08×10^4 M^{-1} cm^{-1} and 3.4×10^{-5} µg cm^{-2}, respectively.

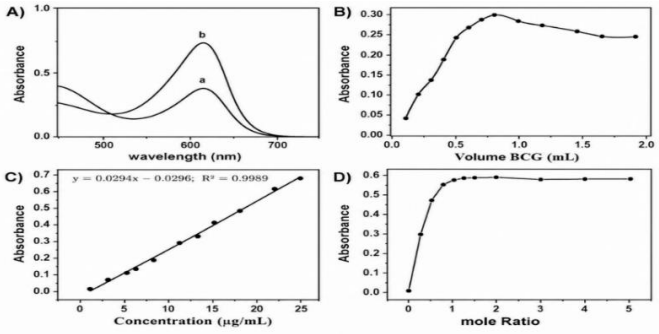


Figure 2. Batch-method optimization and calibration: (A) absorption spectra of (a) BCG against distilled water and (b) the LVF–BCG ion-pair system measured against the BCG reagent blank; (B) effect of BCG volume on the absorbance of 10.0 µg/mL LVF; (C) calibration curve for LVF over 1.0-25.0 µg/mL; and (D) mole-ratio plot used to establish the LVF: BCG stoichiometry.

Stoichiometric relationship

The mole-ratio study indicated a 1:1 LVF: BCG stoichiometric relationship (Figure 2D), consistent with association between oppositely charged LVF and BCG species under the selected conditions. This result supports the proposed ion-pair composition used for the spectrophotometric determination.

Preliminary test of the flow injection system

The FIA manifold used in this study is shown in Figure 1. For the preliminary trial, one channel of the multichannel peristaltic pump was used to propel 4×10^{-4} M BCG at an initial flow rate of 1.0 mL min^{-1}. A 30 µg/mL LVF solution was injected without a reaction coil, and the peak height was monitored at 616 nm using the flow cell. These initial conditions were subsequently optimized.

Optimization of the FIA system

The FIA conditions were optimized by varying the flow rate, sample-loop volume, reaction-coil configuration, and BCG concentration while monitoring peak height and signal reproducibility.

Effect of flow rate

The carrier flow rate influences both residence time and dispersion of the injected sample zone. As shown in Figure 3A, the peak height increased up to 0.7 mL min^{-1} and decreased at higher flow rates. A flow rate of 0.7 mL min^{-1} was therefore selected because it provided the maximum analytical response under the tested conditions.

Effect of reaction coil

Reaction-coil lengths were examined to determine whether additional mixing time improved the FIA response. No increase in peak height was obtained with a reaction coil; instead, the highest response was observed when the injected zone passed directly to the detector. Therefore, no reaction coil was used in the optimized manifold.

Effect of sample volume

The injected sample volume was varied from 150 to 400 µL by changing the sample-loop volume while the other FIA parameters were kept constant. Peak height increased with sample volume and approached a plateau at 350-400 µL (Figure 3B). A 350 µL loop was selected to obtain a high response while limiting unnecessary sample consumption.

Effect of BCG concentration

The influence of BCG concentration on peak height is shown in Figure 3C. The response increased to a maximum at approximately 3.0×10^{-4} M and then decreased as the reagent concentration was increased further. Accordingly, 3.0×10^{-4} M BCG was selected for the optimized FIA procedure.

Calibration curve of the FIA method for the determination of LVF

A calibration curve was constructed for LVF over 10.0-50.0 µg/mL under the optimized FIA conditions: a flow rate of 0.7 mL min^{-1}, a 350 µL sample loop, 3.0×10^{-4} M BCG, no reaction

coil, and detection at 616 nm. Peak height was plotted against LVF concentration, giving a linear response with R^2 = 0.9982 (Figure 3D). The regression equation and R^2 value are displayed in Figure 3D. The analytical characteristics of the batch and FIA procedures are summarized in Table 1. The lower concentration range of the batch method indicates greater sensitivity, whereas the FIA procedure provides higher sample throughput.

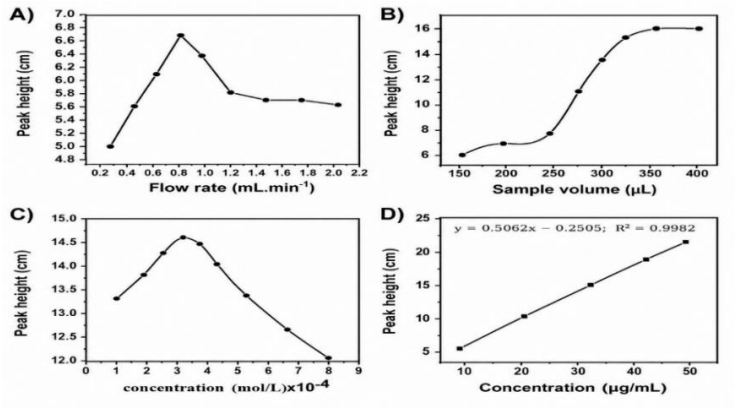


Figure 3. Optimization and calibration of the FIA method: (A) effect of flow rate on the LVF–BCG peak height, with 0.7 mL min^{-1} selected; (B) effect of injected sample volume, with 350 µL selected; (C) effect of BCG concentration, with an optimum at approximately 3.0 × 10^{-4} M; and (D) FIA calibration curve for LVF over 10.0–50.0 µg/mL. Peak height was measured at 616 nm.

Table 1. Optical characteristics of methods for the determination of LVF.

Parameters	Batch method	FIA method
Wavelength λmax nm	616	616
Regression equation	y = 0.0294x - 0.0296	y = 0.5062x - 0.2505
Linear range (µg/mL)	1.0-25.0	10.0-50.0
LOD (µg/mL)	0.85	1.48
LOQ (µg/mL)	2.4	4.48
Molar absorptivity (M^{-1} cm^{-1})	1.08 x10^4	
R^2	0.9989	0.9982
Sandell sensitivity (µg/cm^2)	3.4 x10^{-5}	

The molar absorptivity (ε) was calculated only for the batch method because this procedure is based on direct absorbance measurements and can be expressed using the Beer-Lambert relationship. In the FIA method, the analytical response is recorded as transient peak height and is also affected by sample-zone dispersion and manifold hydrodynamics; therefore, a conventional molar absorptivity value was not calculated for FIA.

Validity of the methods

Accuracy and precision were evaluated under the optimized conditions using five replicate measurements of standard LVF solutions. Relative standard deviation (RSD) and relative error (%, E_{rel}) were calculated at the tested concentration levels. The results are summarized in Table 2.

Table 2. Accuracy and precision results for both methods for the determination of LVF.

Method	LVF present (µg/mL)	Found (µg/mL)	RSD	% Erel
Batch method	1	1.03	3.33	3.40
	6	5.77	3.13	-3.80
	25	24.70	0.144	-1.20
FIA method	10	10.37	2.50	3.69
	30	31.26	1.88	4.20
	50	48.03	0.46	-3.90

All values are expressed as mean ± standard deviation (n = 5).

Method selectivity was evaluated by examining common pharmaceutical excipients, including citric acid, cellulose, KCl, sucrose, lactose, NaCl, and glucose. Each substance was tested individually, and the maximum tolerated concentration and corresponding percentage error were determined. The results are summarized in Table 3.

The lower tolerance levels obtained for cellulose and citric acid in the FIA procedure indicate greater susceptibility to these matrix components under flow conditions than in the batch procedure. This behavior can be associated with the non-steady-state FIA signal and the dispersion of the injected sample zone during transport through the manifold. Nevertheless, within the tested pharmaceutical samples, these excipients did not prevent acceptable LVF determination under the optimized conditions.

Table 3. Effect of interfering species on the proposed batch spectrophotometric and FIA methods for LVF determination.

Method	Interfering method	Maximum concentration (µg/mL)	%, Error
Batch method	Citric acid	0.0625	1.40
	cellulose	1.0	2.97
	KCl	200.0	-0.28
	Sucrose	300.0	-1.54
	Lactose	400.0	1.35
	NaCl	400.0	4.05
	Glucose	500.0	2.70
FIA method	cellulose	0.50	-3.60
	Citric acid	0.750	1.80
	KCl	1.0	2.40
	NaCl	50.0	-3.01
	Lactose	200.0	-2.40
	Glucose	350.0	-3.10
	Sucrose	400.0	-3.60

Application of the method

The proposed batch and FIA methods were applied to pharmaceutical products containing LVF hemihydrate as the active ingredient. The assay results obtained with the proposed methods and the UV reference method are summarized in Table 4.

Table 4. Application of the proposed batch and standard FIA methods to the determination of LVF in pharmaceutical products.

Method	Pharmaceutical products	Content declared (mg)	Found (mg)		%, R
			Proposed method	Standard method	
Batch Method	Aristo Tab-Spain	500	497.70	499.88	99.56
	Levomark infusion-India	500	500.76	497.20	100.7
	Valmox Tab-Georgia	500	504.95	496.20	101.7
	Valmox Aversi	500	497.70	495.88	100.36
	Levopyx	500	496.20	505.5	98.2
FIA method	Aristo Tab-Spain	500	500.00	499.88	100.02
	Levomark infusion-India	500	504.04	497.20	101.37
	Valmox Tab-Georgia	500	495.40	496.20	99.83
	Valmox Aversi	500	500.00	495.88	101.24
	Levopyx	500	513.50	505.5	101.58

The performance of the proposed methods was compared with the UV reference method [3]. Student's t-test and Fisher's F-test were applied at the 95% confidence level, and the statistical results are presented in Table 5. The calculated values indicated no statistically significant difference between the proposed and reference methods under the conditions used.

Small differences were observed between the proposed and reference results for individual pharmaceutical products. For example, the FIA result for Levopyx was 513.50 mg compared with 505.5 mg by the reference method, corresponding to a difference of approximately 1.58%. Such differences may reflect normal experimental variation, matrix effects, and dispersion characteristics of the FIA system. Interpretation of statistical equivalence should be based on the calculated t- and F-values relative to the appropriate critical values at the stated confidence level.

Table 5. Statistical comparison of the proposed methods with the reference method using student's t-test and fisher's F-test.

Statistical parameters	Batch method	FIA method
t-test	0.176	2.08
f-test	1.31	2.9

Chemiluminescence-based methods provide very high sensitivity due to the detection of emitted light generated during chemical reactions, allowing determination of analytes at very low concentration levels. However, these methods generally require specialized instrumentation and optimized reaction conditions compared with conventional spectrophotometric approaches [35].

Comparison of the proposed methods with other reported methods

The analytical performance of the proposed batch and FIA spectrophotometric methods was compared with previously reported methods for LVF determination, including UV spectrophotometric, spectrofluorometric, and flow-injection chemiluminescence approaches. The comparison was based on analytical parameters including detection limit, linear range, precision, sample applicability, and analytical throughput. These parameters provide an overview of sensitivity, operational simplicity, and suitability for routine pharmaceutical analysis.

Table 6. Comparison of the proposed batch and FIA spectrophotometric methods with previously reported methods for the determination of LVF.

Parameters	Batch method	FIA method	Rf_1, UV Spectrophotometric [3]	Rf_2, Spectrofluorometric [20]	Rf_3, FI Chemiluminescence [35]
LOD (µg/mL)	0.85	1.48	NR	0.100	$7x10^{-9}$
Linear Range (µg/mL)	1.0-25.0	10.0-50.0	0.25 to 12.0	0.3 - 18.0	0.01-0.10
RSD	3.38%	4.43%	7%	0.79%	2%
Sample	Tablets, syrup, pure powder, injection, and Infusion	Tablets, pure powder, syrup, and Infusion	Granules and commercial tablet brands	Tablets	tablets and injections
Application	Pharmaceutical dosage forms	Pharmaceutical dosage forms	tablet dosage formulations	Pharmaceutical preparation	Routine quantitative determination
Samples/hour	Not applicable	90	N R	N R	60

Note: Rf_1: Reference method based on UV spectrophotometric determination of levofloxacin; Rf_2: Reference method based on spectrofluorometric determination of levofloxacin using L-tryptophan as a fluorescent probe; and Rf_3: Reference method based on flow-injection chemiluminescence determination of levofloxacin; NR: Not reported.

CONCLUSION

A simple, sensitive, and reliable batch and FIA spectrophotometric method was successfully developed for the determination of LVF in pharmaceutical formulations. The proposed methods exhibited good linearity within the studied concentration ranges, with high correlation coefficients, indicating excellent analytical performance. The methods were validated in terms of accuracy, precision, sensitivity, and reproducibility, yielding satisfactory results. Low limits of detection and quantification demonstrated the suitability of the methods for trace analysis. The developed procedures were successfully applied to the determination of LVF in pharmaceutical products without significant interference from common excipients. Compared with previously reported methods, the proposed methods offer advantages such as simplicity, rapid analysis, cost-effectiveness, and reduced reagent consumption. Therefore, they can be recommended for routine quality control and pharmaceutical analysis of LVF. Although the proposed methods showed satisfactory precision, accuracy, and selectivity toward common pharmaceutical excipients, further validation studies in accordance with ICH Q2 (R1), including intermediate precision and specificity toward structurally related fluoroquinolones, would provide additional information regarding the broader applicability of the methods and will be considered in future work.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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