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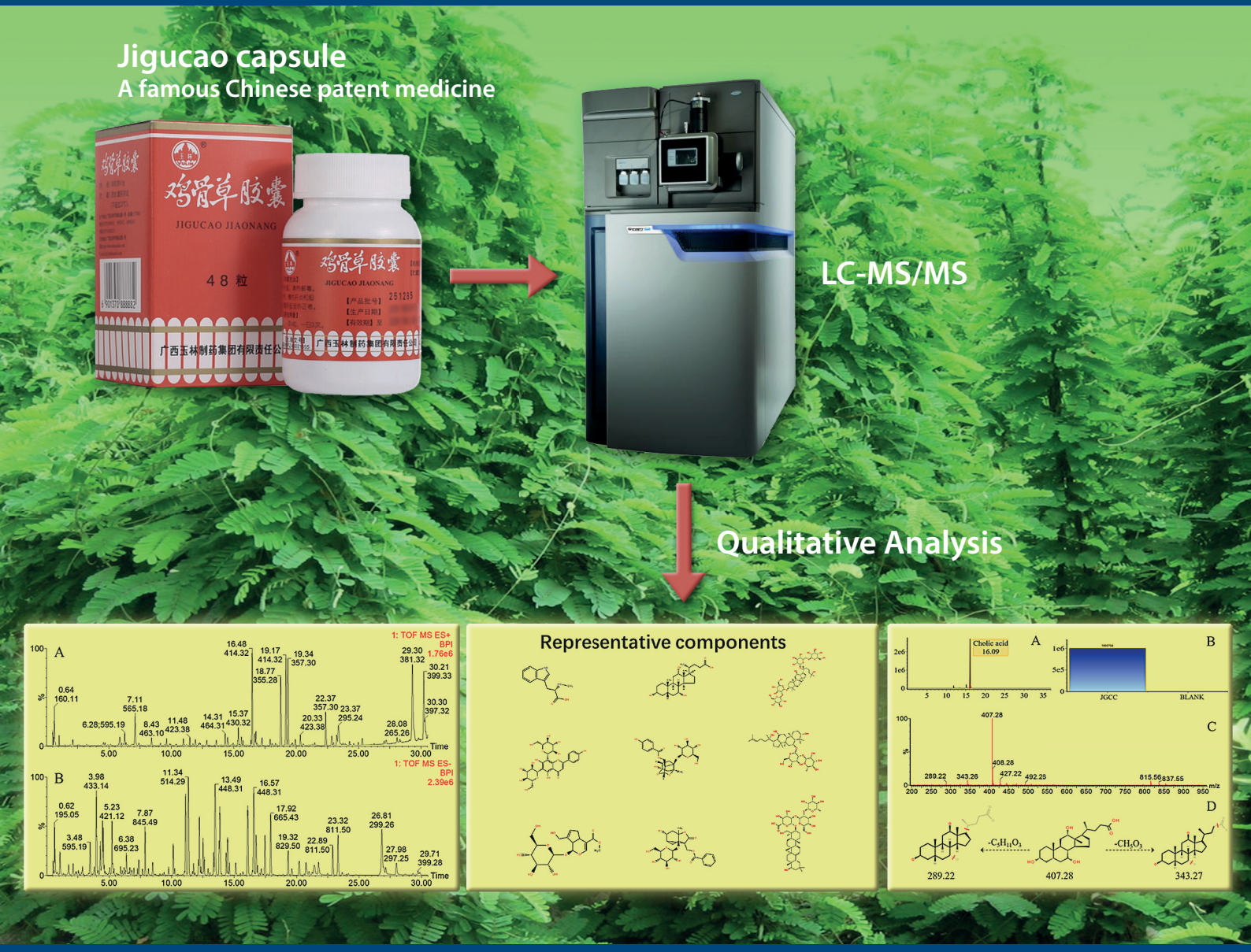

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RESEARCH ARTICLE

A novel liquid chromatography-fluorescence method for the determination of delafloxacin in human plasma

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Delafloxacin is a novel fluoroquinolone antibiotic that was approved by the European Medicine Agency to treat bacterial infections of the skin and underlying tissues, and community-acquired pneumonia. Despite being in the market since 2019 in the European Union, there is no published liquid chromatography-fluorescence method for delafloxacin quantification in biological samples. A novel, rapid, and sensitive high-performance liquid chromatographic method was developed to determine delafloxacin in human plasma using its native fluorescence. Plasma delafloxacin concentrations were determined by reverse-phase chromatography with fluorescence detection at 405/450 nm of excitation/emission wavelengths. Delafloxacin was separated on a Kromasil C18 column 250 × 4.6 mm id, 5 μm using isocratic elution. The mobile phase was a mixture of 0.05% trifluoroacetic acid/acetonitrile (52/48). Retention times were 5.4 and 11.6 min for delafloxacin and valsartan (internal standard), respectively. Regression calibration curves were linear over the range of 0.1–2.5 μg/mL. The lower limit of detection was 0.05 μg/mL, and the lower limit of quantification was 0.1 μg/mL. Accuracy and precision were always <11%, and the limit of quantification was <16%. Mean recovery was 98.3%. This method can be applied to determine delafloxacin in human plasma and could be useful to perform pharmacokinetic studies.

KEYWORDS
delafloxacin, fluorescence detection, high-performance liquid chromatography, human plasma

1 | INTRODUCTION

Delafloxacin (DF) is a novel non-zwitterionic fluoroquinolone (Figure 1) which has now been licensed by the European Medicine Agency in the European Union. This antibiotic is used for the treatment of acute bacterial skin and skin structure infections and community-acquired pneumonia [1]. This fluoroquinolone exhibits advantageous properties compared to other members of the group as a broad spectrum of activity including methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, ability to penetrate staphylococcal biofilms, enhanced antibacterial potency in acidic environments, stability, and overall tolerability in clinical trials;

Article Related Abbreviations: CC, calibration curve; CV, coefficient of variation; DF, delafloxacin; FDA, Food and Drug Administration; FL, fluorescence detection; QC, quality control

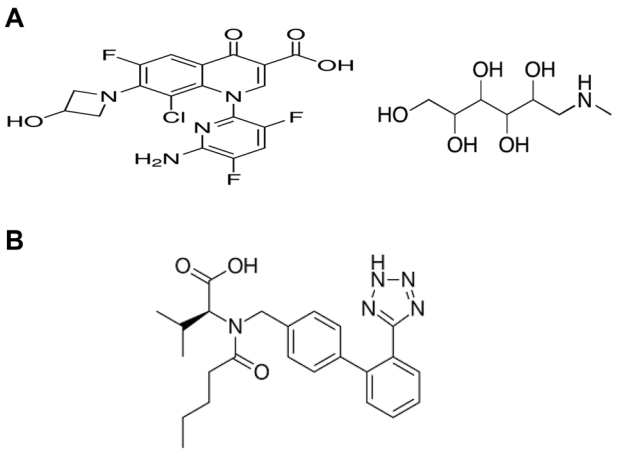

FIGURE 1 Chemical structure of delafloxacin meglumine (A) and internal standard valsartan (B)

given DF's favorable pharmacological properties, potentially therapeutic, and clinical applications need to be further explored [2].

The pK_a values for certain fluoroquinolones ranged from 5.7 to 8.3 [3–5], consequently, these compounds present the ability to act as zwitterions (a no-net-charge molecule with two ionized groups) at physiological pH (Figure 2A), like ciprofloxacin (Figure 2B). However, DF is a non-zwitterionic compound and at physiological pH, the molecule is charged (Figure 2C). The chemical structure of DF differs from other members of the group (Figure 2D) especially in the absence of a basic group at the C7 position which causes its weak acid nature (pK_a = 5.4). As a result, DF in inflammatory (acidic) environments can easily pass through membranes into the bacterial cell. At physiological pH, inside the cell, DF is deprotonated, being the ionic form that enhances its potency [6–8]. This reason could justify the excellent in vivo activity shown in confined infections, usually acidic, such as abscesses and empyema, or acidic anatomical areas such as urine, vagina, and stomach [9].

Only a few methods have been reported in the literature for DF quantification. Several matrices such as rat plasma, rabbit aqueous humor samples [10], and human plasma [11–16] have been investigated using UHPLC-MS and HPLC coupled with triple-quadrupole MS, respectively. However, both methods require expensive equipment not commonly available in most clinical laboratories or hospitals, need a laborious sample preparation, and highly competent and robustly trained staff. Moreover, the lack of standardization and occasional analytical problems require rigorous validation [17,18].

For that reason, there is a need to achieve a chromatographic method with enough sensitivity and trueness to be applied in routine analyses. Other authors have quantified DF using UV-spectrometry, green RP high-performance TLC, and normal-phase high-performance TLC methods [19,20]. Even though, the LOQ is higher than the methods before mentioned. HPLC coupled with a fluorescence detector (HPLC-FL) is widely used in laboratories due to its high sensitivity, sometimes similar to HPLC-MS, and it is an excellent alternative if the compounds of interest have native fluorescence [3]. In this case, DF shows intrinsic fluorescence at $\lambda_{exitation}$ = 405 nm and $\lambda_{emission}$ = 450 nm wavelength [21,22]. Because of this, the objective of the present study is to propose a simple, isocratic, and rapid HPLC-FL to quantify DF in human plasma samples.

2 | MATERIALS AND METHODS

2.1 | Chemicals, solvents, and reagents

DF meglumine (99.53%) from targetmol, valsartan (98%), internal standard (IS), from TCI (Tokyo Chemical Industry), and other drugs were tested to choose IS and for specificity. They were obtained from Cymit Química (Barcelona, Spain). All solvents were HPLC analytical grade. ACN, methanol, formic acid (98–100%), TFA (99%), and water were purchased to Merck Life Science (Madrid, Spain). Ethyl acetate was supplied by PanReac AppliChem (Barcelona, Spain).

2.2 | Instruments

The HPLC system was equipped with a model RF-10AXL FL, a SIL-10ADvp autosampler, a CTO-10ASvp column oven, an LC-10ADvp pump, and a DGU-14A degasser (Shimadzu, Japan). Chromatographic separation was achieved using a Kromasil C18 column 250 x 4.6 mm id, 5 µm with a tracer Excel ODS guard column C18, 10 x 3.2 mm from Teknokroma (Barcelona, Spain). The mobile phase consisted of 0.05% TFA in water-ACN (52:48). Isocratic elution was used at a flow rate of 1.0 mL/min. The injection volume was 100 µL. The FL was made at a $\lambda_{exitation}$ = 405 nm and $\lambda_{emission}$ = 450 at 20°C. The retention times for DF and IS in plasma were 5.4 and 11.6 min, respectively. Finally, the chromatogram's total run time was 13 min.

2.3 | Standard solutions

Stock solutions of DF and IS were prepared at a concentration of 100 µg/mL in methanol. Working solutions of DF at 2.5, 5, 10, 25, 50 µg/mL were prepared by the corresponding dilution of the stock solutions with methanol. Working solutions of DF and IS were freshly prepared daily.

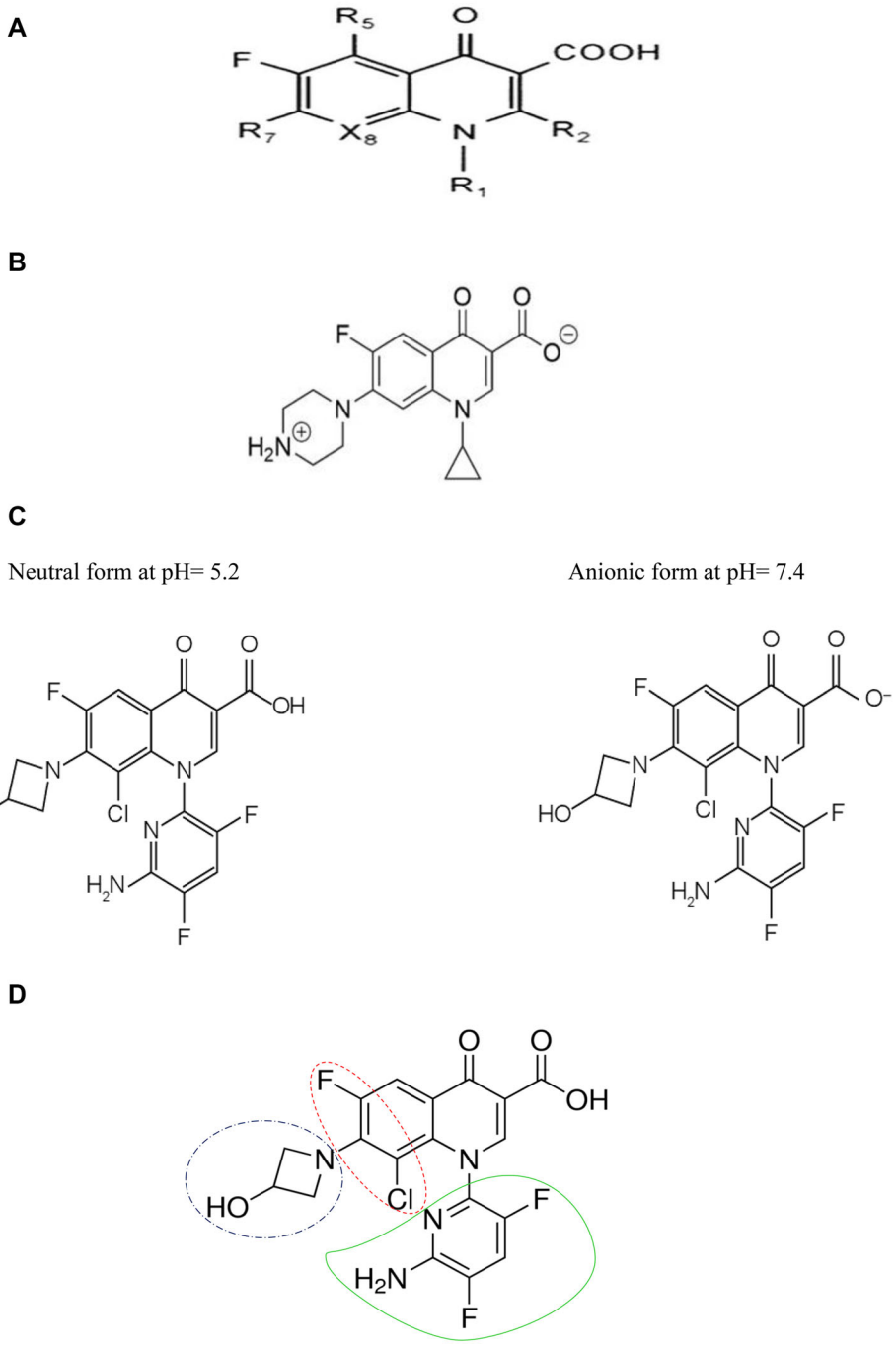

FIGURE 2 Basic structure of fluoroquinolone (A) and zwitterionic ciprofloxacin (B). Chemical structure of delafloxacin in acidic medium and physiological medium (C). Basic structure of delafloxacin (D)

2.4 | Preparation of calibration curves and quality controls

Calibration curve (CC) and quality control (QC) samples were prepared with blank human plasma (500 µL) spiked with an appropriate amount of DF working solution. Plasma drug-free samples were obtained from the blood of donors supplied by Murcia Hemodonation Center (Spain). After mixing, these samples were spiked with 20 µL of IS (10 µg/mL). For CCs, final concentrations of DF were 0.1, 0.25, 0.5, 0.75, 1, 1.5, and 2 µg/mL. Four concentrations were prepared for QCs at 0.1, 0.5, 1, and 2 µg/mL.

2.5 | Sample preparation

First, 500 µL of human plasma were spiked with 20 µL of IS (10 µg/mL) and 50 µL of formic acid in water (50:50) was

added. After mixing, the sample was sonicated for 5 min in an ultrasonic bath at 20°C. Then, 1 mL of ethyl acetate was added and this solution was homogenized in a vortex for 1 min. After that, the sample was centrifuged for 10 min at 15 000 g. The organic layer was extracted, transferred to another polypropylene tube, and evaporated to dryness at 45°C in a SpeedVac Vacuum Concentrator (Fisher Scientific, Madrid, Spain). The residue was reconstituted with 200 μL of the mobile phase, and 100 μL was injected into the HPLC system.

3 | METHOD VALIDATION

Method validation for human plasma was performed according to Food and Drug Administration (FDA) guidelines for bioanalytical method validation [23]. The following parameters were evaluated: LOD, LOQ, sensitivity, precision, accuracy, recovery, selectivity, carry-over, and stability.

3.1 | Linearity, LODs, and LOQs

The linearity of the established chromatographic method was evaluated by analyzing seven calibration standards of DF in human plasma. Linear regression was obtained by plotting the ratio peak-area compound/IS versus known nominal concentrations of DF. Five replicates of each level were assayed. The lower LOD of DF was established as the concentration that provides an S/N $\geq$ 3. The lower limit of quantification was accepted as the lowest concentration on the CC that can be determined with adequate precision (coefficient of variation [CV] $\leq$ 20%).

3.2 | Intra- and inter-day accuracy and precision

The accuracy and precision of this method were obtained by measuring five spiked human plasma samples with IS at four different concentrations: LOQ QC, low QC, medium QC, and high QC (0.1, 0.5, 1, and 2 μg/mL). Intra-day precision and accuracy were calculated on single-day replicates at each concentration level. Inter-day precision and accuracy were evaluated over three different days. The accuracy was calculated with the following bias expression: [(measured concentration – nominal concentration)/nominal concentration] × 100. The accuracy results should be ±15% of nominal concentrations, except for LOQ where ±20% is allowed. For precision values, results should be ≤15% for CV and ≤20% for LOQ.

3.3 | Recovery

Five replicates were evaluated at each concentration level: low, medium, and high QCs. The extraction recovery was determined by comparing the mean peak area of the analyte obtained after plasma extraction versus the mean peak area of extracted blank plasma samples spiked with the analyte at the appropriate concentration.

3.4 | Selectivity, specificity, and carry-over

The selectivity of this method was studied by analyzing six samples of drug-free plasma to demonstrate no interference at the retention times of DF and the IS. To evaluate the specificity of the method, a set of different drugs were evaluated focusing on the retention times of DF and IS. Several drugs were checked: lidocaine, morphine, fentanyl, paracetamol, metamizole, heparin, insulin, ondansetron, ranitidine, propofol, and vasoactive drugs, such as noradrenaline and dobutamine. Finally, carry-over effects were excluded by analyzing human blank samples ($n = 6$) directly after injection of a set of samples containing a high concentration of DF. The carry-over effects should not exceed 20% of LOQ.

3.5 | Stability

The stability of DF in human plasma was performed by using five replicates of two QCs concentrations and two stock solutions (low and high) at different temperatures and storage conditions. The short-term stability of QC was determined by storing samples at room temperature (25°C) and at 4°C, both for 24 h. The long-term stability was evaluated at 15 and 30 days under −40°C storage conditions. Freeze-thaw stability (5 cycles at −40°C) was also analyzed. Besides, stock solutions were stored at −40°C for 7, 15, and 30 days, and stability results were obtained. The acceptance limits for all stability parameters were within ±15% (±20% for LOQ) of nominal concentrations.

4 | RESULTS AND DISCUSSION

4.1 | HPLC method development and optimization

This is the first verified and reliable report about DF quantification in human plasma using an HPLC-FL method. Before tackling that point in detail, it is necessary to note

that DF is a novel fluoroquinolone recently approved by FDA, thus few analytical methods have been published in biological matrices (Table 1) mainly using HPLC-MS/MS systems. Regarding the mobile phase, the use of the acidic mobile phase was the best option instead of buffer solutions. Advantages of this mobile phase include a faster and simple preparation and a reduced tendency to precipitate and clog inside the chromatographic system. It is important to mention that, in green chemistry, a new trend when developing novel chromatographic methods is to avoid buffers [24–26]. Gradient and isocratic elutions were also investigated to find the best IS using different fluorescent drugs (difloxacin, marbofloxacin, norfloxacin, enrofloxacin, ciprofloxacin, phenformin, pregnenolone, alfaxalone, and candesartan). In accordance, valsartan was chosen as IS and an isocratic elution was selected to obtain short running times and a linear baseline.

Several usual C18 columns were checked: Brisa LC^2 C18 column 250 × 4.6 mm id, 5 µm, (Teknokroma, Barcelona, Spain), ODS C18 column 250 x 4.6 mm id, 5 µm (Análisis Vínicos, Tomelloso, Spain), Symmetry RP-Shield C18 column 150 × 4.6 mm id, 5 µm (Waters, Barcelona, Spain). The best resolution was obtained with Kromasil C18 column 250 × 4.6 mm id, 5 µm.

For protein precipitation, the use of ACN has been widely applied for the extraction of different antibiotics from various biological samples [27]. Different ratios have been proposed to achieve this goal, for example, a solution of plasma:ACN (1:2 ratio) was sufficient to remove 99.4% of proteins [28]. Based on our experience in fluoroquinolone quantification, different preliminary experiments were carried out to obtain the best recovery. As DF is a neutral molecule at pH = 5.2, if pH is not correct, it would be impossible to extract DF using liquid-liquid extraction [5,29,30]. DF was treated as other fluoroquinolone compounds adding ACN to precipitate proteins, but results were not satisfactory. For that reason, an liquid-liquid extraction technique with formic acid was used to extract DF. Several quantities of formic acid 50% (25, 50, 75 µL) were tested to reach the adequate pH. The results are shown in Figure 3. After this, different solvents (Figure 4) were also checked to obtain the best recovery. The best results were obtained by adding 50 µL of formic acid (50%) and ethyl acetate as extracting reagent. The final conditions were described in Section 2.

4.2 | Validation

4.2.1 | Linearity, LODs, and LOQs

Peaks corresponding to DF and IS were obtained at 5.4 and 11.6 min for human plasma (Figure 5). Calibration

TABLE 1 Summary of HPLC methods for quantitation of delafloxacin in biological matrices

Sample matrix	Extraction tecnique	Mobile Phase	Detection	Analysis time (minutes)	Linearity range (µg/mL)	LOQ (µg/mL)	Precision (%)		Accuracy (%)		Recovery (%)	References
							Inter-day	Intra-day	Inter-day	Intra-day		
Rat plasma	LLE	10 mM ammonium acetate /ACN (0.1%formic acid)	UHPLC-MS/MS	2	0.0035–5	0.0035	≤ 10.5	˂ 9.48 %	−2.4- (−10.3)	3.5 - 13.2	82.3	[10]
Rabbit aqueous humour	Protein precipitation (PP)	10 mM ammonium acetate /ACN (0.1% formic acid)	UHPLC-MS/MS	2	0.014–10	0.014	≤ 10.0	11.3%	0.8– (−13.0)	−10.4–2.0	68.4	[10]
Human plasma, Human urine	LLE	ACN/ aqueous formic acid 0.1%	HPLC-MS/MS	–	0.005–5.0 0.008–5.3	0.005 0.008	˂ 15.0 Mean 7.6- 15.7		−15.0–15.0 Mean 6.2–11.8	–	–	[12]
Human plasma, Human urine	LLE	–	HPLC-MS/MS	–	0.005– 5 0.05– 10	0.005 0.05	˂ 15.0		−15.0–15.0		–	[13]
Human plasma, Human urine	SPE	ACN/ aqueous formic acid 0.1%	HPLC-MS/MS	–	0.005–5	0.005	≤ 15.0	≤ 15.0	± 15.0	± 15.0	–	[14,15]
Human plasma Rat plasma	PP	ACN (0.1% formic acid)/ aqueous formic acid 0.1% Gradient conditions	UHPLC-/MS/MS	3	0.0029–6.6	0.0029	5.3–10.9	1.2–11.2	−5.6–6.1	−7.5 –9.0	˂72.0	[16]

Abbreviation: LLE, liquid-liquid extraction.

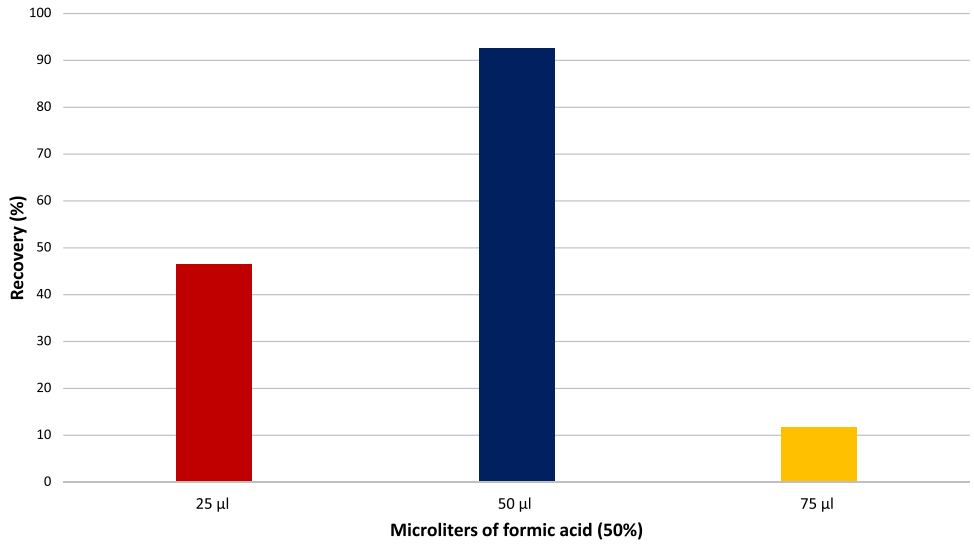

FIGURE 3 Recovery of delafloxacin (500 µg/mL of human plasma) after adding different microliters of formic acid (50%)

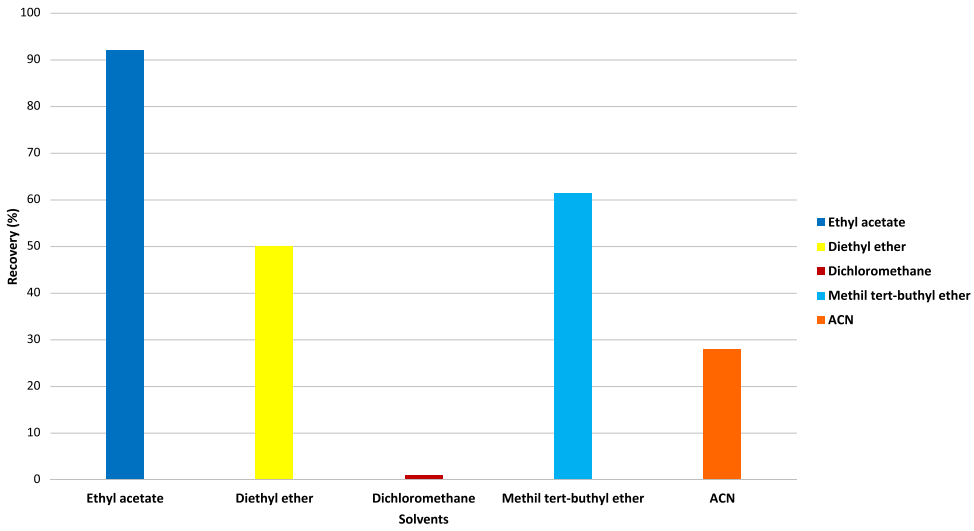

FIGURE 4 Recovery of delafloxacin (500 µg/ml of human plasma) after using 1 mL of different solvents as extract agent

curves were obtained by plotting the area ratio of DF/IS versus nominal concentrations of DF in µg/ml. The concentration range was linear from 0.1 to 2.5 µg/mL and good linearity was observed ($r^2 \geq 0.9994$). The linear regression equation was $y = 0.4037x + 0.007$. The LOD and LOQ values were 0.05 and 0.1 µg/mL, respectively. These values indicated that this proposed method is suitable and has adequate sensitivity for determining DF concentrations in human plasma with HPLC-FL detection. Quantification limits obtained by other authors (Table 1) in different biological matrices with the HPLC-MS/MS were lower than the present method because the sensitivity of HPLC-MS/MS is better than HPLC-FL detection. Nevertheless, it is not a disadvantage since most of the minimum inhibitory concentration values of DF [9] are over the LOQ of the method described here and consequently, quantification of concentrations under these minimum inhibitory concentrations are not useful from a clinical point of view.

4.2.2 | Intra- and inter-day precision and accuracy

Accuracy and precision results are presented in Table 2. Intra- and inter-day precision were evaluated at four QCs plasma concentration levels 0.1, 0.5, 1, and 2 µg/mL, and five replicates measurements were recorded. The CV precision in plasma samples was <10.0% for intra-day and <11.0% for inter-day values. The accuracy of the method was determined by replicate analysis of five sets

TABLE 2 Intra-day and inter-day accuracy (bias) and precision (coefficient of variation [CV]) of delafloxacin in human plasma ($n = 5$)

Concentration (μg/mL)	Intra-day ($n = 5$) Mean ± SD (μg/mL)	CV (%)	Bias (%)	Inter-day ($n = 5$) Mean ± SD (μg/mL)	CV (%)	Bias (%)
0.1	0.11 ± 0.01	5.55	11.53	0.10 ± 0.01	4.53	0.20
0.5	0.49 ± 0.05	9.73	−1.72	0.48 ± 0.05	10.20	−4.02
1	0.94 ± 0.05	4.40	−5.55	1.08 ± 0.08	7.11	8.16
2	2.05 ± 0.08	4.14	2.62	1.99 ± 0.21	10.70	−0.59

Abbreviation: CV, coefficient of variation.

of samples spiked with four different concentrations of DF (QCs, 0.1, 0.5, 1, and 2 μg/mL), and comparing the difference between the spiked value with the nominal value. The intra-day accuracy results were within the range of −5.55 to 11.56%. The inter-day accuracy ranged from −4.02 to 8.16%. On the other hand, intraday and inter-day accuracy and precision results are similar to those reported for DF in human plasma samples analyzed by HPLC-MS [11–15]. These results have not been shown in the literature, but these values are ≤15% of nominal concentration

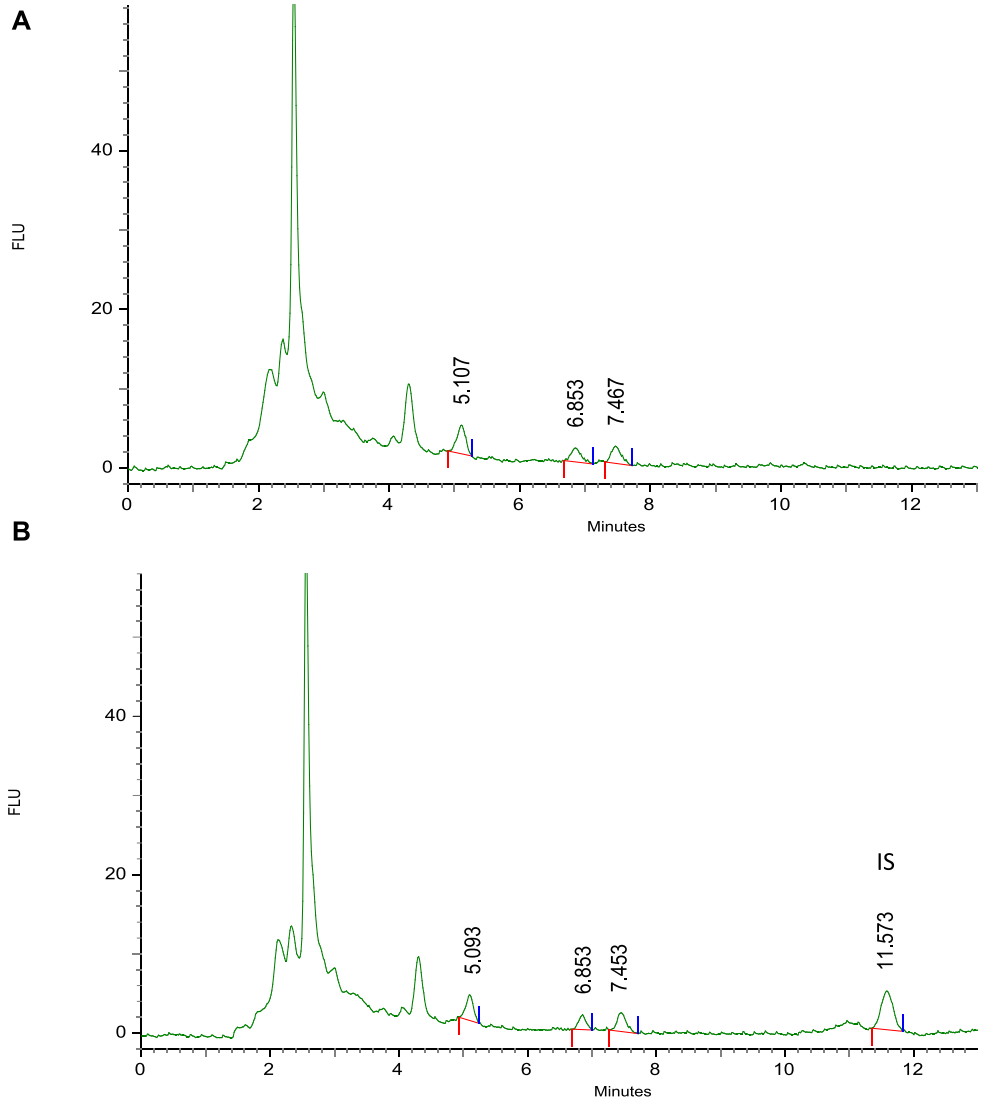

FIGURE 5 Chromatograms of delafloxacin and IS in human plasma by HPLC with fluorescence detection (HPLC-FL). (A) Blank plasma. (B) Blank plasma spiked with 20 μL of internal standard (10 μg/mL). (C) Blank plasma spiked with delafloxacin at LOQ (0.1 μg/mL) and 20 μL of internal standard (10 μg/mL)

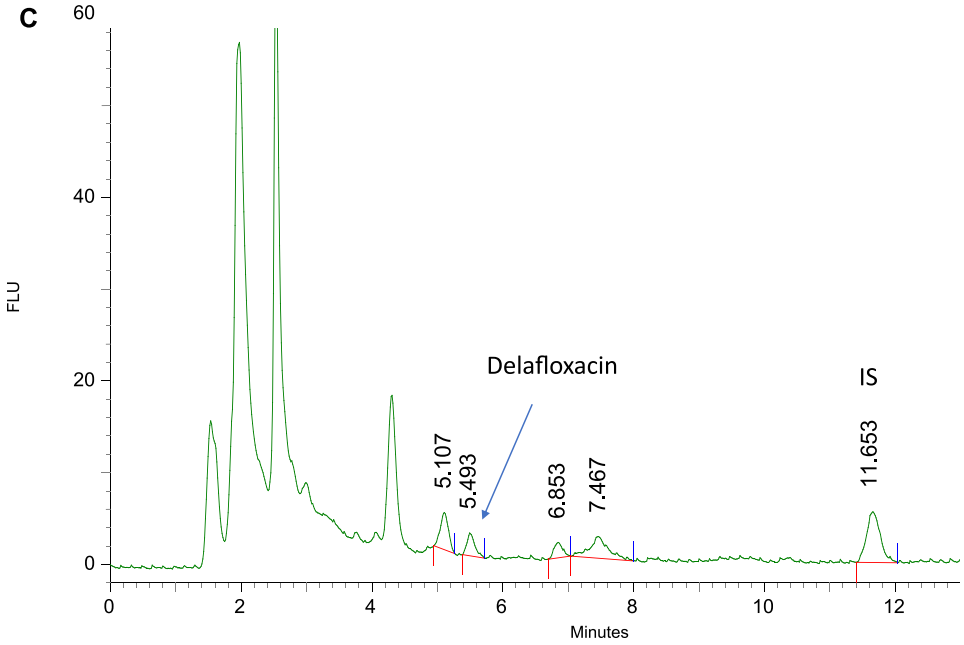

FIGURE 5 Continued

TABLE 3 Recovery of delafloxacin in human plasma

Concentration (μg/ml)	*n*	Recovery (%)	
		Mean ± SD	CV (%)
0.5	5	102.97 ± 1.72	1.68
1	5	93.99 ± 6.95	7.40
2	5	97.92 ± 3.28	3.35

Abbreviation: CV, coefficient of variation.

for both precision and accuracy. Recent studies (Table 1) for DF determination in human plasma by HPLC-MS/MS [16], and in rat plasma and rabbit aqueous humor by UHPLC-MS/MS [10] showed values of precision and accuracy ≤ 15% according to FDA guidance [23]. Therefore, the precision and accuracy results of this proposed method have demonstrated reliable values for the quantitative analysis of DF in human plasma in accordance with the established standards.

4.2.3 | Recovery

Recoveries of DF were measured at low, mid, and high QC levels 0.5, 1, and 2 μg/mL, respectively, by comparing extracted samples ($n = 5$) with blanks, spiked with the analyte post-extraction. The values were close to 100% with a CV < 8.0%. The results are shown in Table 3. In the case of recovery test, our results of mean recovery q(98.3%) were higher than those obtained in rat plasma (82.3%) [10], rabbit aqueous humor (68.4%) [10], and human plasma (<72.0%) [16]. Recovery test results in human plasma and urine were not reported by other authors [11–15]. In summary, excellent recoveries were obtained, indicating that this method was efficient and reproducible and confirming the adequacy of the analytical methodology.

4.2.4 | Selectivity, specificity, and carry-over

Six blank plasma samples were analyzed and there were no endogenous interferences with the same retention times as DF and IS (Figure 5A). Furthermore, well-resolved peaks for DF and IS were observed. The specificity of the method was evaluated through the analysis of some fluorescence antibiotics and no interferences were found. Adequate results were obtained, indicating a high selectivity and specificity of the method for human plasma. Finally, no significant interferences were observed at the same retention time of DF, after running a set of high concentration samples (5 μg/mL, $n = 6$). Similar results for selectivity, specificity, and carry-over have been reported previously in different biological matrices [10,16].

4.2.5 | Stability

Stability results of QCs and stock solutions are summarized in Tables 4 and 5, respectively. DF in QCs was stable at 4°C and room temperature for 24 h. Surprisingly, when QCs of DF were stored at −40°C for 15 and 30 days, showed instability. The accuracy of the low QC (0.5 μg/mL) at 15 and 30 days were −21.30% (CV 7.06%) and −31.33%

TABLE 4 Short term stability, long term stability, and freeze/thaw stability of quality controls (QCs) of delafloxacin

Analyte	Validation	*n*	Conditions	QC low 0.5 (μg/ml) Mean ± SD (μg/mL)	CV (%)	Bias (%)	QC high 2.0 (μg/ml) Mean ± SD (μg/mL)	CV (%)	Bias (%)
Delafloxacin	Short-term stability	5	0 h	0.44 ± 0.02	4.03	−11.50	2.17 ± 0,13	6.16	8.25
		5	24 h, (RT)	0.43 ± 0.04	9.70	−14.30	1.82 ± 0.26	14.5	−9.22
		5	24 h, (4°C)	0.47 ± 0.07	14.75	−7.00	2.09 ± 0,22	10.60	4.48
	Long-term stability (−40°C)	5	15 days, (−40°C)	0.39 ± 0.03	7.06	−21.30	2.13 ± 0.19	9.00	6.08
		5	30 days, (−40°C)	0.34 ± 0.03	8.40	−31.33	1.38 ± 0.08	5.94	−31.02
	5 cycles Freeze and Thaw (−40°C)	5	5 days freeze and thaw, (−40°C)	0.35 ± 0.03	8.05	−31.50	2.20 ± 0.09	4.46	10.10

Abbreviation: RT, retention time.

TABLE 5 Short and long term stability of stock solutions of delafloxacin

Analyte	Validation	*n*	Condition	Stock solution low 2.5 (μg/mL) Mean ± SD (μg/mL)	CV (%)	Bias (%)	Stock solution high 25 (μg/mL) Mean ± SD (μg/mL)	CV (%)	Bias (%)
Delafloxacin	Short-term stability	5	0 h	2.24 ± 0.07	3.30	−10.50	24.70 ± 0.78	3.17	−1.17
		5	7 days, (−40°C)	2.06 ± 0.08	4.04	−17.50	24.00 ± 0.28	1.18	−4.01
	Long-term stability (−40°C)	5	15 days, (−40°C)	1.99 ± 0.28	13.90	−20.37	22.60 ± 0.50	2.20	−9.57
		5	30 days, (−40°C)	0.95 ± 0.02	2.60	−62.00	16.95 ± 0.62	3.70	−32.17

Abbreviation: CV, coefficient of variation.

(CV 8.40%), respectively. Equally, the values obtained for the high QC (2.0 μg/mL) showed degradation of 6.08% for 15 days (CV 9.00%) and 31.02% (CV 5.94%) after 30 days storage. Besides, freeze-thaw stability (five cycles at −40°C) was analyzed and a nominal concentration of DF decreased to 31.50% (CV 8.05%) for the QC of 0.5 μg/mL and 10.10% (CV 4.46%) for the QC of 2.0 μg/mL. Similarly, stock solutions at 2.5 and 25 μg/mL were stored at −40°C for 7, 15, and 30 days and stability results were reported in Table 4. Stock solutions (2.5 μg/mL) of DF at 7 days were unstable with a loss of nominal concentration of 17.50%. Similarly, the stability of DF in low stock solution decreased from nominal concentration in 20.37 and 62.00% following storage of 15 and 30 days, respectively. Contrary to what was expected, only a high concentration of the stock solution of DF was degraded at 30 days, and it may be due to the high amount of DF in the solution. Stability results of QCs of DF have shown stability for 24 h according to European Medicine Agency and FDA [1–31] guidelines, which only recommends storing DF for 24 h without freezing. Freeze-thaw stability (three cycles) in human plasma [16] has shown stability at −80°C. Long-term stability studies previously reported in rat plasma, aqueous humor, and human plasma [10,16] showed good results at −80°C for 2 months. Moreover, other authors ensured the stability of DF in human plasma and urine up to 35 and 384 days at −80°C, although the stability results were not shown [13,14]. Our data clearly suggest the opposite at −40°C. Due to this, future studies should be encouraged to evaluate the stability of DF in different situations to find the best storage conditions for future analysis.

5 | CONCLUDING REMARKS

A novel, simple, rapid, sensitive, and reproducible method for DF determination in human plasma was developed by HPLC-FL using the native fluorescence of the compound and according to FDA guidelines. Furthermore, this validated method could be applied as a verified and reliable alternative to HPLC-MS in clinical studies, routine analyses, therapeutic drug monitoring and, pharmacokinetic studies using a short running time.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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