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*Research Article*

# Electromembrane Extraction of Ofloxacin from Human Plasma and Its Quantification by Capillary Electrophoresis

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**Abstract**

***Background:*** Application of electromembrane extraction coupled with capillary electrophoresis was studied for ofloxacin extraction from plasma samples.

***Methods:*** Ofloxacin migrated from acidic plasma samples through a thin layer of 1-octanol immobilized in the pores of a porous hollow fiber wall into a 10 µL acidic aqueous acceptor solution located inside the lumen of the fiber.

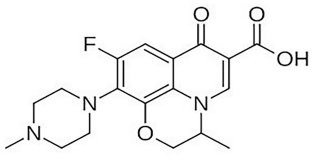
***Results:*** Under optimum conditions influencing electromigration (*i.e.,* 20 min of the operation time, stirring speed of 750 rpm, donor phase pH at 4.0, acceptor pH at 3.0, and applied voltage of 30 V across the supported liquid membrane), ofloxacin was extracted from plasma samples with an enrichment factor of 100-fold corresponding to extraction percent of 24%. The calibration curve showed acceptable linearity in the range of 0.2-7.0 µg.mL$^{-1}$ (R=0.9993). The limits of detection and quantification of 0.05 and 0.2 µg.mL$^{-1}$ were obtained, respectively. The inter- and intra-day precision and accuracy were obtained below 8.65%.

***Conclusion:*** The validated method was successfully processed for the ofloxacin determination in the plasma samples of patients under ofloxacin therapy. There were no interfering peaks which indicates the great selectivity of the developed method.

## Introduction

The development of fluoroquinolone antibiotics has revolutionized the treatment of bacterial infections. Ofloxacin is derivated from nalidixic acid with the addition of an N-methyl piperazine, fluorine, and oxazine ring which results in a tricyclic quinolone.[1,2] It is one of the most frequently used second-generation fluoroquinolones which has a broad spectrum of antibacterial activity, including urinary tract, respiratory tract, and also tissue-based infections.[3] The mechanism of ofloxacin's bactericidal action is through the inhibition of enzymes: DNA topoisomerase IV and DNA gyrase; subunit A of DNA gyrase which catalyzes catenation and decatenation that is necessary for DNA replication, leads to breakage of double-strand DNA in susceptible organisms.[4-7] As shown in Figure 1 the piperazinyl group at position 7 is methylated which could be one of the factors of its great bioavailability of 95% in comparison with other fluoroquinolones.[1,4]

Ofloxacin is widely distributed into body tissues and the peak plasma concentration is achieved within 1-2 hours following oral administration which is observed as excellent dose proportionality. The current reference range of ofloxacin in serum is 2.5-5.5 µg.mL$^{-1}$ with a half-life of 6 hours.[1,8-10] Its metabolism is in the liver and mainly (90%) of ofloxacin following a single oral dose excretes from urine unchanged within 48 h.[4] The amphoteric nature of ofloxacin allows it to be in cationic, anionic, neutral, and zwitterionic states. This molecule is a weak heterocyclic amino acid with two reactive sites: a carboxylic group which can lose a proton and an amino group that can be protonated.[11]




Log P: -0.39, $pK_{a1}$: 5.97, $pK_{a2}$: 9.28

**Figure 1.** Chemical structure of ofloxacin.

It is important to maintain the concentration of an antibiotic in bronchial secretions at an equal level or higher in blood circulation to treat chronic infections since the bacteria are usually found in the airways and around them.

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Because ofloxacin is mainly eliminated through kidneys and excretes unchanged in the urine, in patients with impaired renal function, the dosage should be carefully regulated during chronic administration.[12]

Analytical procedures including spectrofluorometry and microbiological assay,[13-15] high performance liquid chromatography (HPLC)-UV/fluorescence,[11,16-20] micellar liquid chromatography,[21] high-throughput flow system,[12] chemiluminescence,[22-25] thin-layer chromatography,[26] liquid chromatography (LC) with fluorescence detector,[27,28] capillary electrophoresis (CE),[29] LC-mass spectrometry (MS)/MS,[27,30] differential pulse polarography,[31] ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS),[32] anion exchange assay[33] have been used to determine ofloxacin alone and/or in combination with other antibiotics.

Generally, the determination of ofloxacin at its therapeutic concentration using HPLC includes a combination of various preliminary procedures such as concentration in organic solvents pursued by evaporation of the organic phase and dissolution of the residue in a solvent. Thin-layer chromatography methods provide imprecise results due to their diligence. Microbiological assays only show a poor limit of detection and an inability to distinguish between ofloxacin and other antibiotics which results in low sensitivity.[14]

In chemical analysis, sample preparation is required to eliminate interfering matrix components as well as improve the sensitivity of the method.[25] Solid phase extraction,[25,28] solid phase spectrofluorimetry,[15] rotating-disk sorptive extraction,[25] and matrix isopotential synchronous fluorimetry[14] are the sample preparation methods used for the pre-concentration of ofloxacin in real samples.

Electromembrane extraction (EME), is a liquid-phase micro-extraction technique.[34,35] The basis of EME relies on the electrokinetic movement of the charged analytes under the influence of the electric field, from an aqueous solution across a supported liquid membrane (SLM) into the acceptor phase. SLM consists of a small volume of hydrophobic water-immiscible solvent that is immobilized in the pores of the hollow fiber which is a polymeric membrane and acts as a barrier between the donor and acceptor solutions.[36] Utilizing electric potential provides an effective operation of EME results in a quick extraction procedure. Disposable extraction SLMs, high extraction recovery rate, low cost, negligible usage of organic solvents, and small volume of samples are some of the advantages that make EME an appropriate method for analysis of biological samples.[35,37]

CE as a powerful technique is an appropriate method to analysis the pharmaceutical compounds. Its separation mechanism depends on the difference in the migration of analytes under an electric field and electrophoresis operation is performed in a narrow-bore capillary filled with the background electrolyte (BGE). Typically, pharmaceutical compounds have been separated using LC, while CE has proven to be beneficial for many pharmaceutical appliances due to its unique separation mechanism, efficacy, speed, versatility, simple instrumentation in comparison with other techniques, and consisting of sample introduction systems which makes it a powerful analytical instrument for bioanalytical applications.[34,36]

The pharmacokinetics and/or bioequivalence of ofloxacin should be investigated for registering a new formulation. Among biological specimens, plasma is more common and reliable sample to reflect systemic blood circulation. Thus, development of a simple analytical method is in demand for quantifying the levels of ofloxacin in plasma to enable routine antibiotic monitoring.[11,38] So, in this work, the EME was employed for extraction and pre-concentration of ofloxacin in plasma samples and its concentration is quantified by CE analysis.

## Methods

### *Materials*

Ofloxacin was purchased from Temad pharmaceutical company (Tehran, Iran). Sodium hydroxide, sodium dihydrogen phosphate, orthophosphoric acid, hydrochloric acid, 2-nitrophenyl octyl ether (NPOE) and 1-octanol were purchased from Merck (Darmstadt, Germany). De-ionized water was purchased from Shahid Ghazi pharmaceutical company (Tabriz, Iran) and was used to prepare all solutions.

### *Standard solution and biological samples*

The stock solution of ofloxacin was prepared at 1000 $\mu g.mL^{-1}$ in water, sonicated until complete dissolution and stored at 4 °C in a dark place. The working solutions were prepared daily by diluting the appropriate amount of the stock solution with plasma samples. Drug-free plasma samples were provided from healthy volunteers who had not taken any drug and were obtained from the blood transfusion organization of East Azerbaijan province (Tabriz, Iran). For real sample analysis, five plasma samples were collected from the patients who received ofloxacin. To participate in this study, the patients have been informed on details of the project and signed a consent form which has been approved by the ethics committee at Tabriz University of Medical Sciences with the approval code of IR.TBZMED.REC.1402.257. In order to separate plasma from the blood samples; the 4 mL of blood was transferred into an EDTA (as an anticoagulant in the form of di-potassium salt) spiked tubes, containing 7.2 mg of $K_2$EDTA with a final concentration of 1.8 $mg.mL^{-1}$, centrifuged at 600 rpm for 15 min, afterwards plasma was transferred into a polypropylene microtube, and stored at -18 °C until analysis. Frozen plasma samples were thawed at room temperature and shaken before use.

### *Capillary electrophoresis conditions*

The analysis of the extracted ofloxacin from plasma samples was implemented on an Agilent CE setup 7100 (Waldbronn, Germany), with a diode array detector and the control and data acquisition system of ChemStation

(Waldbronn, Germany). The extraction investigations were accomplished in a 48.5 cm bare fused silica capillary (with effective length of 40 cm) and an internal diameter of 50 µm (Agilent Technologies). Working BGE was 25 mM phosphate buffer adjusted to pH=3.0 using 1 M orthophosphoric acid. The new capillary was rinsed with 1 M NaOH for 45 min, water for 45 min, and BGE solution for 1 hour. At the beginning of the analysis, the capillary was rinsed with 0.1 M NaOH for 10 min, water for 10 min, and BGE for 15 min. Between consecutive runs, the capillary was flushed with 0.1 M NaOH for 2 min, followed by water for 2 min and then BGE for 4 min to eliminate the interferences from the previous analysis. The capillary was flushed for 10 min with 0.1 M NaOH followed by water at the end of the working day and capillary tips were kept in water vials overnight. All working solutions were filtered through a syringe filter (0.22 µm). During the analysis, the capillary temperature was maintained at 25 °C. The samples and standard solutions were hydrodynamically injected at 100 mbar for 10 s. The separation voltage was fixed at +20 kV. Detection of ofloxacin was accomplished at 260 nm as the highest sensitive wavelength.

### *EME setup*

A 9-mL home-made glass, screw-capped vial with an internal diameter of 10 mm and height of 9 cm was employed as the sample compartment. Porous hollow fibers (HFs) are used to house the acceptor solutions, which are polypropylene HFs of PP Q3/2 (Membrana, Wuppertal, Germany), with an internal diameter of 0.6 mm, a wall thickness of 0.2 mm, and 0.2 µm pores size. 0.2 mm diameter platinum wires with a 5 mm inter-electrode distance were used as electrodes in the donor and acceptor solutions. A DC power supply PS858 model with a programmable voltage of 0–300 V providing current output in the range of 0–1000 mA was connected to these electrodes (S.KAR, Tabriz, Iran). A M890C$^+$ multimeter was used for current monitoring during the extraction procedure (Zhangzhou Weihua Electronic Co., China). The EME setup was stirred using a magnetic bar by a heater-magnetic stirrer model RCT basic from IKA company (Germany).

### *EME procedure*

Five cm pieces of HF washed with acetone in an ultrasonic bath, and dried. Following that, the HF was completely immersed in 1-octanol (or NPOE) for 10 s to make 1-octanol impregnate the pores of the HF. The excess amount of 1-octanol in the HF was gently removed with a medical wipe and blown with a medical syringe. The lumen of the HF was filled with 10 µL of the acceptor phase (pH 3.0 aqueous solution, adjusted by adding 1 M hydrochloric acid) by a micro-syringe, and afterward, the lower end of HF was closed with a small segment of nonconductive plastic. The donor phase including 1 mL of plasma samples diluted by 3 mL water with pH of 4.00 (adjusted using 1 M hydrochloric acid) introduced to the sample compartment. For the entire HF to be placed inside the sample, 4 mL of sample is needed, but due to the limitation of the sample volume received from the patients (1 mL), we used water to reach the desired volume. The positive electrode and HF were placed in the donor solution through the cap of the sample compartment. The negative electrode was inserted into the acceptor solution located in HF lumen through a guiding tube. The EME setup was put on the stirrer, and the electrodes subsequently were connected to the DC power supply. Next, optimal extraction conditions were applied. Then, the acceptor solution was collected by a micro-syringe and transferred directly to a microinsert vial for CE analysis.

### *Calculation of extraction percentage, enrichment factor, and relative error*

The extraction recovery percentage (ER%) of ofloxacin from plasma samples was calculated using Eq. 1:

$$ER(\%) = \frac{n_{a.\ \ final}}{n_{s.\ \ initial}} \times 100 = \left(\frac{V_a}{V_s}\right)\left(\frac{C_{a.\ \ final}}{C_{s.\ \ initial}}\right) \times 100$$

Eq. 1

where $n_{a.\ final}$ and $n_{s.\ initial}$ are the mole number of ofloxacin presented in the acceptor solution after the extraction procedure and the mole number of ofloxacin presented in the sample solution (donor phase), respectively. $C_{a.\ final}$ and $C_{s.\ final}$ indicate the final and initial concentrations of ofloxacin in the acceptor and donor phases, respectively, whereas, $V_a$ and $V_s$ denote the acceptor and donor volumes, respectively. Enrichment factor (EF) was calculated using Eq. 2:

$$EF = \frac{C_{a.\ \ final}}{C_{s.\ \ initial}}$$

Eq. 2

In which $C_{a.\ final}$ was computed from the external calibration curve of the direct injection of standard solutions of ofloxacin. Relative error (%RE) was used to obtain accuracy of the developed method by Eq. 3:

$$\%RE = \frac{C_{found} - C_{nominal}}{C_{nominal}} \times 100$$

Eq. 3

Where $C_{nominal}$ and $C_{found}$ were the concentration of the known standard solution and the concentration of the same standard solution measured by the developed method, respectively.

## Results and Discussion

### *Optimization of CE separation condition*

To reach the optimum separation conditions, four factors including type of buffer, separation voltage, injection time, and injection pressure were investigated. Accordingly, between two acidic buffers, at the same concentration and pH, the phosphate buffer showed good separation and sensitivity in comparison to the acetate buffer. Then, separation voltages of 15, 20, and 25 kV, injection times of 5, 10, 15, and 20 s, and injection pressures of 50, 70, and

100 mbar were tested, respectively. Ultimately, a separation voltage of 20 kV, injection time of 10 s, and injection pressure of 100 mbar were selected as the optimum values which electropherograms demonstrated a proper separation time, and the highest peak area preserving peak symmetry and leading to good peak resolution.

### *Optimization of EME method*

To achieve the highest extraction efficiency of ofloxacin from plasma samples, several parameters influencing EME were optimized and the results were shown in Figure 2.

### *Effect of organic solvent for SLM*

Choosing the proper solvent based on its chemical character is one of the most crucial factors in electrokinetic cross-membrane extraction since it plays a very important role in method's selectivity and efficacy.[39] It must have low vapor pressure, also to prevent solvent loss during the process it should be immiscible with water and have an appropriate viscosity.[40] For this purpose, NPOE and 1-octanol were used as SLM and the results showed that between the examined solvents, the extraction recovery was much higher for 1-octanol as shown in Figure 2A.

### *Effect of pH of the donor and acceptor phases*

In EME, only charged molecules could be extracted efficiently under an electric field. Hence, the target molecule should be in ionized form to extract. As mentioned in the "Introduction" section, ofloxacin is an amphoteric drug (Figure 1). In the donor phase, the potent ionization of the ofloxacin is necessary to supply a reliable electrokinetic migration through the SLM.

Therefore, different pH values (less than $pK_{a1}$=5.97) of the donor phase were investigated to achieve optimum conditions. As ofloxacin contains tertiary amine as a basic functional group, the N atom is given a positive charge when the pH is below $pK_{a1}$. The results depicted in Figure 2B indicate that the extraction efficacy (the highest peak area)

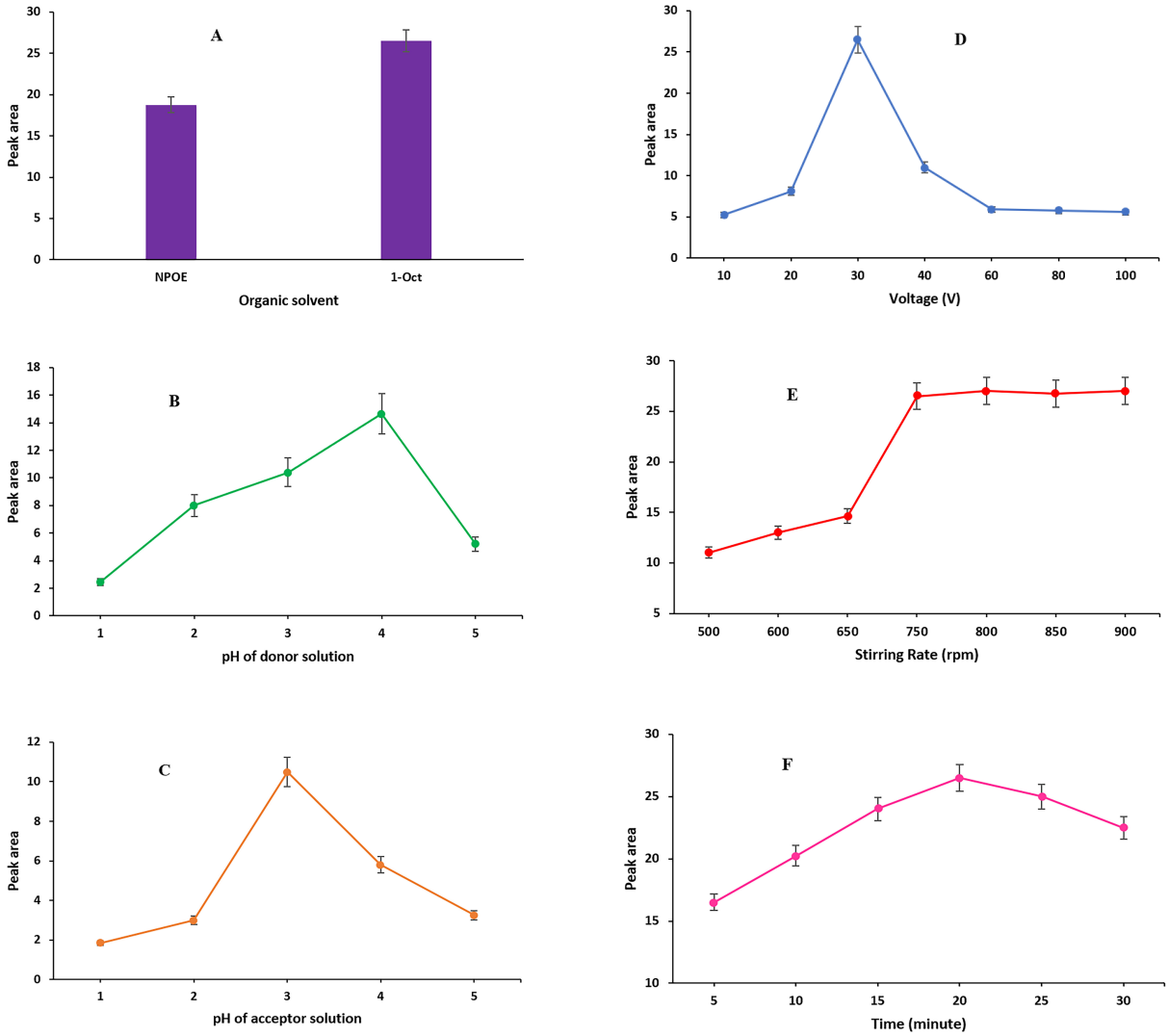


**Figure 2.** Effect of main factors on EME performance. The conditions of the parameters are as follows unless the level of each parameter is changed according to the Figures: 1 μg.mL$^{-1}$ of ofloxacin, 1-octanol as SLM, 750 rpm as stirring rate, voltage of 30 V, acceptor solution pH of 3.0, donor solution pH of 4.0, and 20 min extraction time.

would be more effective at pH=4.0 in the donor phase. At pH 4.0, ofloxacin is completely ionized. On the other hand, to prevent back extraction of the extracted drug, the pH in the acceptor phase must be the same as the donor phase. To investigate the effect of the pH of the acceptor solution, a range of pH values were tested and results indicate a pH value of 3.0 was required for the acceptor phase to obtain the effective extraction (Figure 2C). It should be noted that in the EME technique, in addition to the effect of donor and acceptor pHs, analyte flux may be controlled by the ratio of the total ionic concentration in the donor and acceptor phases, which is referred to as ion balance. There is no doubt that the maximum peak area is achieved for the highest ion concentration in the acceptor phase in comparison to the donor phase.[41] Therefore, a concentrated acceptor solution is favored (pH 3.0) compared to a less concentrated one (pH 4.0).

### *Effect of the voltage*

For the EME method, the voltage is the most important parameter for driving the electrokinetic migration of analytes across the SLM.[41] It was discovered that increasing voltages higher than 100 V led to bubble formation around the electrodes due to electrolysis, which reduced the extraction recovery. Thus, to find the optimum voltage, it was varied from 10 to 100 V. As shown in Figure 2D, the applied voltage of 30 V was chosen as the optimum voltage in which the maximum peak area was achieved.

### *Effect of stirring rate*

The stirring rate has a crucial role in having an effective extraction through the reduction of double-layer thickness around the SLM and an increase in mass transfer, stirring rates between 500 and 900 rpm were implemented to reach optimum efficacy. The results revealed that 750 rpm was the optimum value for utilization for the subsequent investigations (Figure 2E).

### *Effect of extraction time*

Another parameter in the EME technique is the extraction time which affects mass transfer. To investigate the mass transfer from the donor solution to the acceptor phase over time, the extraction time was studied from 5 min to 30 min. As indicated in Figure 2F, the peak area was significantly increased from 5 min to 20 min and reduced slightly after 20 min, so 20 min was considered as the best extraction time. It is probably a consequence of the Joule heating phenomenon, which can result in dissolution or evaporation of the organic solvent in the sample solution and lead to back-extraction of ofloxacin to the SLM.[42]

### *Analytical performance*

After optimization of the effecting conditions on EME technique, the optimal conditions were employed to evaluate the performance of this method for the extraction of ofloxacin from plasma samples. Method validation was performed according to the US FDA guidelines.[43] Linearity, limit of detection (LOD), lower limit of quantification (LLOQ), upper limits of quantification (ULOQ), accuracy, precision, selectivity, specificity, recovery, and stability were studied using standard solutions of ofloxacin in diluted plasma samples.

### *Linearity*

The calibration curve was developed to check the relation between analytical responses based on the peak area of extracted ofloxacin from matrix-matched samples. Samples were prepared by spiking known amounts of the drug on 4 mL of sample solution containing 25 (%v/v) plasma in water with a final pH of 4.0 used as a donor solution. As shown in Table 1, linearity was studied over a range of 0.2-7.0 $\mu g.mL^{-1}$ at five calibration points (n=3) with the correlation coefficient (R) of 0.9993. The accuracy and precision of all calibration points, were less than 15% (based on back-calculated values), meeting FDA requirements for bioanalysis. The LOD was calculated with a signal-to-noise (S/N) ratio of 3:1 and was 0.05 $\mu g.mL^{-1}$. Moreover, the EF and ER% of the method were calculated from Eqs. 1 and 2, and a summary of the results is presented in Table 1. It should be noted that the EF for the entire developed EME method is 100, but in the case of samples taken from patients, it is 25 because the received patient sample is diluted with water by a factor of 4.

### *Precision and accuracy*

In order to evaluate the method's repeatability (intraday precision), intermediate precision (inter-day precision), and accuracy, plasma samples at three concentrations levels 0.2, 3, and 5.6 $\mu g.mL^{-1}$ (five replications) were subjected to the entire analytical procedure and measured in one single day and over three consecutive days, respectively. Precision and accuracy are indicated by the RSD of the replicated measurements and relative error (RE) which are calculated from Eq. 3, respectively. Table 2 summarizes the results, which indicate acceptable accuracy and precision for the reported method based on the guideline.[43]

**Table 1.** Validation data for EME-CE method for determination of ofloxacin from plasma samples.

| Linear equation | R | LOD[a] | LOQ[b] | LR[a, b] | ER%[c] | EF[d] |
|---|---|---|---|---|---|---|
| y = 27.239x + 1.7128 | 0.9993 | 0.05 | 0.2 | 0.2-7.0 | 24 | 100 |

[a] Limit of detection ($\mu g.mL^{-1}$)
[b] Limit of quantification ($\mu g.mL^{-1}$)
[c] Extraction Recovery for 1 $\mu g.mL^{-1}$ of ofloxacin
[d] Enrichment factor

**Table 2.** Precision and accuracy of proposed method (n = 5).

| Concentration (μg.mL$^{-1}$) | Precision (RSD%[a]) | | Accuracy (RE%[b]) | |
|---|---|---|---|---|
| | Inter-day | Intra-day | Inter-day | Intra-day |
| 0.2 | 3.43 | 5.97 | +8.7 | +3.86 |
| 3 | 1.14 | 4.54 | +5.34 | +4.01 |
| 5.6 | 2.03 | 3.70 | +1.80 | -0.45 |

[a] Relative standard deviation
[b] Relative error

### *Selectivity and specificity*

Detection of a desired analyte in the presence of other potential species in the blank biological matrix is referred to the selectivity of a bioanalytical method, while specificity indicates the capability of the method to distinguish and measure the analyte from other species like related metabolites, medications, and endogenous matrix compounds. To investigate the selectivity, plasma samples were collected from different volunteers (6 individuals) who did not use ofloxacin. To assay the specificity, an analytical procedure was established 1.0 μg.mL$^{-1}$ of each of the different drugs usually taken together with ofloxacin including lisinopril, furosemide, cotrimoxazole, spironolactone, omeprazole, hydrochlorothiazide, metoprolol, cefalexin and captopril. For this purpose, EME method was first performed under optimum conditions, on blank plasma samples. EME was then performed from plasma samples, containing 1 μg.mL$^{-1}$ of each drug. In the blank plasma sample as shown in Figure 3A, no interfering peak was observed for ofloxacin. Except for hydrochlorothiazide, no peak was observed in the recorded electropherograms for the studied drugs. However, as indicated in Figure 3B, in the presence of hydrochlorothiazide, there is no overlap or interfering peak at the migration time of ofloxacin.

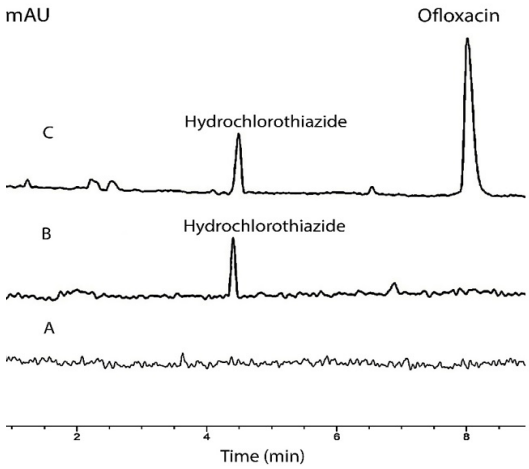


**Figure 3.** Specificity of the developed method. Electropherogram of blank plasma (A), a plasma sample spiked by 1 μg.mL$^{-1}$ of hydrochlorothiazide, lisinopril, furosemide, co-trimoxazole, spironolactone, omeprazole, metoprolol, cefalexin, and captopril (B), a plasma sample spiked by 1 μg.mL$^{-1}$ of ofloxacin, hydrochlorothiazide, lisinopril, furosemide, cotrimoxazole, spironolactone, omeprazole, metoprolol, cefalexin, and captopril (C). EME conditions; pH of donor: 4.0, pH of acceptor: 3.0, applied voltage: 30 kV, stirring speed: 750 rpm, extraction time: 20 min. Separation conditions; uncoated fused-silica capillary, 48.5 cm (effective length: 40 cm) × 50 μm i.d.; BGE, 25 mM phosphate buffer (pH = 3.0); detection, UV at 260 nm; temperature, 25 °C; applied voltage, +20 kV.

### *Stability*

To evaluate the chemical stability of ofloxacin, stability tests were conducted. Three types of stability tests were carried out; stock solution stability, short-term room temperature (bench-top), and freeze-thaw stability. To study stock solution stability, the fresh diluted stock solution and diluted solutions (in the linear range of method) at different time intervals of five days which were kept in the dark under 4 °C, were extracted under optimum conditions of EME method and analyzed afterward. To investigate the temperature stability over a short period, three spiked aliquots of low, medium, and high concentrations were extracted from plasma samples and the donor solution was thawed and left at room temperature for 10 hours before analysis. Three replicates were analyzed for each concentration. To study freeze-thaw stability, extracted ofloxacin in the donor phase underwent five freeze-thaw cycles, each cycle consisted of 1-hour freezing (-18 °C) and 30 minutes of thawing at 25 °C, and then analyzed. Based on FDA guidelines where accuracy is under ± 15 it is considered as a stable analyte. As shown in Table 3, the obtained results met the criteria indicating that extracted drug from plasma samples by EME method is stable under-investigated conditions.

**Table 3.** Stability tests for EME-CE method for determination of ofloxacin from plasma samples.

| Storage condition | Concentration (μg.mL$^{-1}$) | Accuracy (RE%) |
|---|---|---|
| Fresh diluted stock solutions | 2 | +8.83 |
| | 5 | -0.91 |
| | 7 | +0.32 |
| Diluted stock solution (after 1 month) | 2 | +3.93 |
| | 5 | -2.62 |
| | 7 | -6.15 |
| Short term stability (bench-top) at room temperature for 10 h | 2 | -5.25 |
| | 5 | -4.58 |
| | 7 | -1.95 |
| Freeze-thaw stability (after four cycles of freezing and thawing) | 2 | +2.10 |
| | 5 | -2.38 |
| | 7 | -4.57 |

**Table 4.** Determination of ofloxacin in plasma samples.

| No. | Gender | Age | Interval between sampling and drug administration | Daily dose (g) | Found concentration ($\mu g.mL^{-1}$) | Co-prescribed drugs |
|---|---|---|---|---|---|---|
| 1 | Male | 20 | 3 h | 0.2 (1 per day) | 3.06 | None |
| 2 | Male | 29 | 45 min | 0.2 (1 per day) | 1.41 | Indomethacin, omeprazole |
| 3 | Male | 27 | 10 h | 0.2 (1 per day) | 0.67 | Rifampin |
| 4 | Male | 51 | 4 h | 0.2 (1 per day) | 2.84 | Doxycycline, warfarin, aspirin |
| 5 | Male | 27 | 2 h | 0.2 (2 per day) | 3.24 | Fluoxetine |

### *Real sample analysis*

In order to examine the applicability of the developed method for quantification of ofloxacin in plasma, five real samples were obtained from the patients, and the details of the patient, dose of administration, and the concentration of ofloxacin in plasma are listed in Table 4. The explained method was utilized to extract ofloxacin from plasma samples successfully. 1 mL of plasma samples of the patients was diluted with 3 mL water with pH of 4.0 and used as a donor phase. The pH of the plasma sample was adjusted at 4.0 by the addition of HCl solution. After implementing the EME procedure in the optimal extraction conditions, the acceptor solution was introduced to CE and its results can be seen in Figure 4.

## Conclusion

In the present study, we have demonstrated the use of hollow fiber-based EME coupled with a CE analysis providing a simple, rapid, low-cost, accurate, selective, as well as being a greener approach by consuming small amounts of organic solvents and high-sensitivity method for the quantification of ofloxacin from plasma samples. Results include a low LOD of 0.05 $\mu g.mL^{-1}$, acceptable reproducibility demonstrated by intra-day accuracy and precision which obtained lower than 8.65%, and high enrichment factor, show that this selective method has made it possible to isolate and analyze ofloxacin from real-patient plasma samples. The detection and quantitation of ofloxacin is important for drug monitoring in ofloxacin therapy. Poor sensitivity of electromigration methods is a limiting factor in applying the proposed method for analysis of lower concentrations of the analyte in biomedical samples.

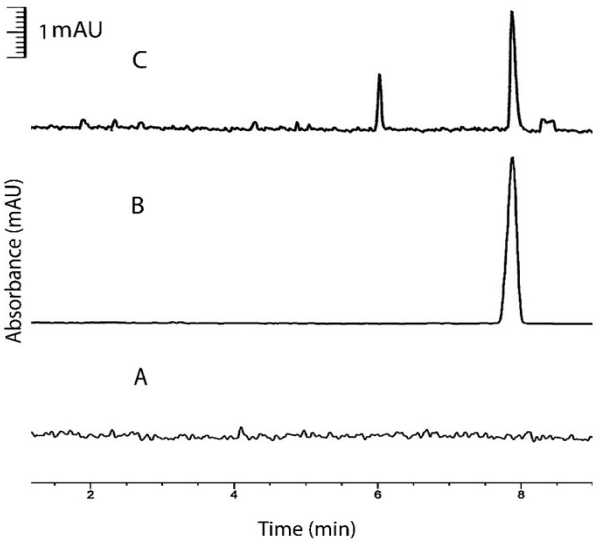


**Figure 4.** Electropherograms of a blank plasma sample (A), a plasma sample spiked by 1 $\mu g.mL^{-1}$ ofloxacin (B), and plasma sample of the patient (C). EME condition; pH of donor: 4.0, pH of acceptor: 3.0, applied voltage: 30 kV, stirring speed: 750 rpm, extraction time: 20 min. Separation condition; uncoated fused-silica capillary, 48.5 cm (effective length: 40 cm) × 50 µm i.d.; BGE, 25 mM phosphate buffer (pH = 3.0); detection, UV at 260 nm; temperature, 25 °C; applied voltage, +20 kV.

## Ethical Issues

The sample donors have been informed on details of the project and signed a consent form approved by the ethics committee at Tabriz University of Medical Sciences (IR.TBZMED.REC.1402.257).

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## Author Contributions

Mahsa Javan: Investigation, Data curation, Writing – Original Draft. Behrouz Seyfinejad: Investigation, Data Curation, Writing – Review & Editing. Elaheh Rahimpour: Data Curation. Mojtaba Varshochi: Resources. Abolghasem Jouyban: Conceptualization, Supervision, Funding Acquisition, Writing – Review & Editing.

## Conflict of Interest

The authors declare that they have no conflict of interests.

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