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Original Article

A simple and sensitive high-performance liquid chromatography method for determination of ciprofloxacin in bioavailability studies of conventional and gastroretentive prolonged-release formulations

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Abstract

Background: A very simple, sensitive, and accurate high-performance liquid chromatography (HPLC) method with ultraviolet detector was developed and applied to determine ciprofloxacin in human plasma following administration of a gastroretentive formulation developed in our laboratory.

Materials and Methods: HPLC analysis was performed on a C_{18} μ-Bondapack column (250 mm × 3.9 mm) using acetonitrile: potassium dihydrogen phosphate solution 0.1 M (20:80, v/v, pH 3) at a flow rate of 1.5 ml/min and eluate was monitored at 276 nm. After addition of phenacetin as internal standard, plasma samples were treated with 0.1 M phosphate buffer (pH: 7) and followed by extraction with dichloromethane. The method was validated for linearity, precision, accuracy, limit of quantitation (LOQ), robustness, stability, and applied in bioavailability studies of our developed gastroretentive formulation in healthy volunteers.

Results: The calibration curves were linear over the concentration range 0.025–4 μg/ml with the detection limit of 15 ng/ml. Accuracy % were within 93–115 and the coefficient of variance % ranged from 0.20 to 12.8. The very low LOQ (25 ng/ml) allowed avoiding fluorometric detection which is more expensive and is not available in all laboratories. Ciprofloxacin was stable in samples with no evidence of degradation during 3 freeze-thaw cycles and 3 months storage at –70°C.

Conclusion: This validated HPLC method was successfully used for the determination of ciprofloxacin in human plasma following oral administration of controlled release formulation, conventional immediate-release tablets and when administered concomitantly with divalent and trivalent cations such as aluminum-, magnesium-, or calcium-containing products under which the bioavailability of ciprofloxacin is significantly reduced.

Key Words: Bioavailability, ciprofloxacin, high-performance liquid chromatography, plasma, prolonged release

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INTRODUCTION

Ciprofloxacin, structurally (1-cyclopropyl-6-fluoro-1,4-dihydro 4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid) [Figure 1a], is a potent fluoroquinolone chemotherapeutic of the second-generation group of nalidixic acid derivatives. It is faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8 g/mol. Due to the broad spectrum effect, it is widely used both in human and veterinary medicine to treat infectious diseases, caused particularly by Gram-negative and some Gram-positive bacteria. The target of highly selective action of ciprofloxacin is bacterial DNA gyrase, a type of topoisomerase II.[1,2] After peroral administration in human, ciprofloxacin is rapidly absorbed from the gastrointestinal (GI) tract into the systemic circulation and reaches the maximal concentration in 1–2 h. The bioavailability is 56–79%, about 65% of unchanged ciprofloxacin and 10–15% of metabolites is excreted in the urine and about 15% in feces.[3,4] Ciprofloxacin has been available in conventional tablets that require twice-daily administration.[4] Inconvenient regimens, longer duration of therapy, and possible side effects could result in poor patient adherence to the treatment which often leads to sub-therapeutic antibiotic concentrations at sites of infection. This would result in longer persistence of the pathogens, therapeutic failure, and emerging of resistant microorganisms. Therefore, there has been a significant interest in the development of a convenient once-daily formulation of ciprofloxacin. We developed a controlled release floating gastroretentive formulation containing 500 mg ciprofloxacin that could be administered once-daily along with a 500 mg conventional immediate-release tablet to achieve desirable clinical effects.[5] Since the drug is released in a sustained manner from developed floating formulation, lower drug concentrations in the range of 0.025–1 μg/ml in plasma samples are achieved.[5] Thus, the current study details a simple, sensitive, and rapid high-performance liquid chromatography (HPLC) method that we have developed in our laboratory to quantify ciprofloxacin concentrations in human plasma to address pharmacokinetics of ciprofloxacin after ingestion of this novel gastro retentive floating formulation.

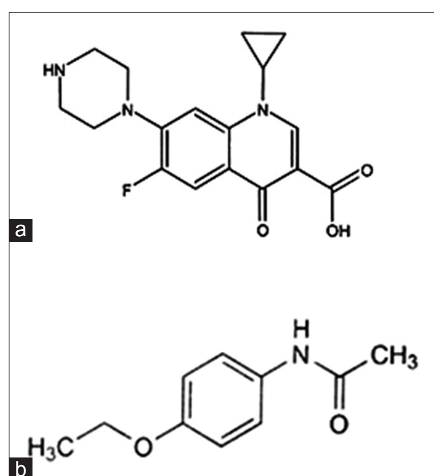

Figure 1: Chemical structure of ciprofloxacin hydrochloride (a) and internal standard, phenacetin (b)

A literature review revealed that the HPLC method has been the technique of choice for the separation and determination of ciprofloxacin in biological fluids. Thus far, several HPLC methods have been described to analyze ciprofloxacin in various body fluids following oral administration of conventional immediate-release dosage forms using different clean-up procedures including protein precipitation (PP),[6-11] filtration,[12-14] solid-phase extraction (SPE),[15,16] liquid-liquid extraction (LLE)[17-19] either with ultraviolet (UV)[7,11,14,16-18] or fluorimetric detection.[6,8-10,12,13,15,20] Although direct injection of samples after precipitation of the plasma proteins is a simple and rapid procedure, this method could deteriorate the chromatographic column due to inadequate PP. Endogenous compounds can also overload the column, which interfere with the peak of interest or appear as late eluting peaks and consequently lead to a long run time. In addition, sample dilution, which occurs after deproteinization, reduces the sensitivity of the assay.[21] To increase the column durability and efficiency and to remove the interferents coming from proteins, the use of SPE has been reported. SPE technique is a fairly expensive procedure and suffers from sorbent drying between washing procedures resulting in cracking of the packing materials. Moreover, due to the strong bonding of ciprofloxacin to the sorbent, the use of acidic aqueous extractant[15,16] seems essential reflecting to a decrease in sensitivity of the assay. In most instances, LLE has been considered as the main procedure for the isolation and determination of drug substances from biological matrices. Although some studies have been performed with regard to HPLC determination of ciprofloxacin in plasma after LLE, none of them are appropriately developed and validated to be entirely applicable and reproducible to the pharmacokinetic studies of this drug. Nessem *et al*.[17] proposed a HPLC method for determination of ciprofloxacin in plasma samples using LLE and UV detection. The calibration curve was linear in the range of 1–10 μg/ml and the method was not validated according to the International Conference on Harmonization (ICH) guidelines. In some other similar studies,[18,19] after LLE of plasma samples, the limit of quantitations (LOQs) of ciprofloxacin were reported

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between 100 and 250 ng/ml. The quantification limits in the aforementioned studies is not sufficiently low to precisely characterize the drug plasma profiles, especially at later time points after oral administration of the drug where the concentration of ciprofloxacin is quite low. To overcome the low sensitivity of the assay procedures, fluorescence detections have been proposed and utilized for analysis of ciprofloxacin. However, fluorescence detectors require extremely pure solvents and are not readily available and affordable at most laboratories.

In the current study, it was of interest to us to develop a reliable HPLC method using UV detection and LLE for determination of ciprofloxacin in human plasma in the range of 0.025–4 μg/ml. The described method does not utilize fluorescent detection and yet more sensitive, making the method rapid, simple, and appropriate for pharmacokinetic and bioequivalence studies of this drug following the oral administration of controlled release and conventional immediate-release tablet formulations. This method can also be utilized in the determination of plasma concentration of this antibiotic when administered concomitantly with divalent and trivalent cations such as aluminum-, magnesium-, or calcium-containing products under which the bioavailability of ciprofloxacin is significantly reduced. This method was developed and validated for its accuracy, precision, limit of detection (LOD), LOQ, robustness, and stability as per ICH guidelines.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride was provided by Pars Daru (Iran, Tehran). Phenacetin (internal standard [IS], Figure 1b), HPLC-grade acetonitrile and methanol, potassium dihydrogen phosphate, orthophosphoric acid 85% were purchased from Merck (Germany) 500 mg prolonged-release gastroretentive tablets of ciprofloxacin were prepared in our laboratory.

Chromatographic conditions

The apparatus used was a Waters HPLC system model 746 (Milford, US), consisting of a model 515 intelligent solvent delivery pump, a 100 μl injection loop, a computerized system controller, and a Waters 2487 UV detector. HPLC assay was carried out on a μ-Bondapack C_{18} column (250 mm × 4.6 mm, 10 μm, Waters, Ireland). The mobile phase consisted of potassium dihydrogen phosphate (0.1 M)/acetonitrile at 80/20 (pH, 3 ± 0.1) eluted at flow rate 1.5 ml/min. Column effluent was detected at 276 nm with a UV detector. Column temperature was set at 40°C, and 50 μl of samples was injected to the HPLC system. Quantitation was achieved by measurement of the peak area ratios of the drug to the IS.

Standard solutions of ciprofloxacin and internal standard

A standard stock solution of ciprofloxacin hydrochloride at 100 μg/ml was prepared by dissolving 11.64 mg of the drug (equal to 10 mg ciprofloxacin base) in 100 ml double-distilled water. A series of working solutions at concentrations of 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 20, 30, and 40 μg/ml were prepared by further dilution of the standard stock solution in double-distilled water. Stock solution of phenacetin (IS) at a concentration 100 μg/ml was prepared in methanol.

Calibration procedure

To 0.5 ml of blank plasma, 50 μl of ciprofloxacin standard solutions at concentrations of 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 20, 30, 40 μg/ml and 50 μl of IS at fixed concentration of 100 μg/ml were added to obtain ciprofloxacin standard concentrations ranging from 0.025 to 4 μg/ml. A 0.5 ml of 0.1 M phosphate buffer (pH: 7.4), 5 ml of dichloromethane were added, vortexed, and centrifuged at 3000 rpm for 10 min. The upper aqueous layer was removed by aspiration and discarded. The organic layer was evaporated to dryness under nitrogen gas and the residue reconstituted with 75 μl of mobile phase, and 50 μl aliquot was injected to the HPLC system. Two calibration curves were obtained by linear least-squares regression analysis by plotting peak area ratios (ciprofloxacin/IS) versus two different ranges (0.025–0.5 and 0.5–4 μg/ml) of ciprofloxacin plasma concentrations.

Method validation

Linearity

Calibration plots were constructed by plotting the ratio of ciprofloxacin peak area to that of IS, using five different concentrations of each sample on five separate days. Distribution of the residuals (% difference of the back-calculated concentration from the nominal concentration) was determined to validate the correlation. The calibration model will be accepted if the residuals are within ± 20% for the lower limit of quantification and within 15% for all other calibration levels and at least 2/3 of the standards should meet this criterion. The calibration curves were evaluated by the correlation coefficient, slope, and intercept.

Limit of detection and limit of quantitation

The parameter LOD was determined using the signal-to-noise ratio by comparing the results of test samples with known concentrations of analyte to the blank samples. The analyte concentration that

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produced a signal-to-noise ratio of 3:1 was accepted as the LOD. The LOQ was identified as the lowest plasma concentration of the standard curves that could be quantified with acceptable accuracy, precision, and variability.[22]

Precision, accuracy, and recovery

The intra- and inter-day variation of the assay was determined by replicate analysis (n = 5) of samples at concentrations within the range of calibration curves in a single analytical run on the same day and at five different days, respectively, using the same stock solutions and plasma batches. Percentage coefficient of variance (% CV) was used as the measure of precision, and percentage accuracy (% accuracy = [measured concentration/nominal concentration] ×100) was also determined. The extraction recovery of ciprofloxacin was estimated at 0.025, 1, 4 μg/ml concentrations in plasma. Plasma samples (in six replicates) containing ciprofloxacin were extracted and analyzed. Six samples containing similar concentrations of the compound in mobile phase were directly injected, and peak areas were measured. Absolute recovery was calculated by comparing the peak areas for direct injection of pure ciprofloxacin solution with those obtained by plasma containing the same amount of ciprofloxacin.

Robustness

The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the percentage of mobile phase acetonitrile, in the pH, in the mobile phase flow rate and in the temperature. The effect on retention time and peak parameters were studied.[23]

Stability

Stability of ciprofloxacin was examined by keeping replicates of plasma samples at room temperature for 12 h. Freeze-thaw stability of analyte in human plasma samples were studied over three freeze-thaw cycles, by thawing at room temperature for 2–3 h and refrozen for 12–24 h. Stability of the drug in human plasma was also tested after storage at below – 70°C for 3 months. In all stability studies, plasma samples were spiked with ciprofloxacin at three levels of concentration, low (0.025 μg/ml), medium (1 μg/ml), and high (4 μg/ml). For each concentration and each storage condition, six replicates were analyzed. The concentration of ciprofloxacin after each storage period was related to the initial analyte concentration of freshly prepared samples. Samples were considered stable if the assay values were within the acceptable limits of accuracy and precision.[22]

Application of the method

The present method was applied in human pharmacokinetics study of ciprofloxacin after ingestion of 500 mg ciprofloxacin immediate-release tablet as well as the developed gastro retentive controlled release formulation in two separate groups.[5] The study was conducted in accordance with ethical principles and standards described in the Declaration of Helsinki and the ICH/Good Clinical Practice. Guidelines were approved by an independent Medical Bioethics Committee at the Isfahan University of Medical Sciences. Twelve healthy adult volunteers aged between 21 and 26 years and weighted from 56 to 70 kg participated in each group. Clinical examinations and laboratory tests revealed that no subject had an evidence of any acute or chronic disease or drug allergy. The subjects were asked to avoid taking any medication at least 2 weeks prior to and during the study period. No milk or dairy products were served during the study. Each subject was enrolled after signing an informed consent form. After oral administration of dosage forms, in predetermined intervals, the blood samples were collected in heparinized tubes. The blood samples were centrifuged at 3000 rpm for 20 min; plasma was separated and kept frozen at –20°C in glass tubes. For determination of Ciprofloxacin concentration in plasma of volunteers, 50 μl of IS solution, 0.5 ml phosphate buffer (pH, 7.4), and 5 ml of dichloromethane were added to 0.5 ml plasma. All samples were taken through the same extraction procedure described earlier. Final sample concentrations were calculated by determining the peak area ratio of ciprofloxacin related to IS and comparing the ratio with the standard curve, obtained after the analysis of calibration samples.

Pharmacokinetic analysis

The peak plasma concentration (C_{max}) and the corresponding peak time (T_{max}) were obtained directly from individual plasma concentration-time profiles. The AUC_{0-48} was calculated by the trapezoidal rule and the total $AUC_{0-\infty}$ was calculated according to the following equation.

$$AUC_{0-\infty} = AUC_{0-48} + C_{48}/K_E$$

Where, C_{48} is the drug concentration after 48 h and K_E is the elimination rate constant. The K_E value was estimated from the terminal slope of plasma concentration versus time plot through the logarithmic transformation of the concentration values and application of linear regression. We considered four points in the terminal log-linear phase to obtain an accurate estimate of K_E from linear regression. The mean residence time (MRT) was also calculated using following equation.

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$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$$

$AUMC_{0-\infty}$ is the area under the first moment of the concentration-time curves and was calculated according to the following equation.

$$AUMC_{0-\infty} = AUMC_{0-48} + (C_{48} \times t_{48})/K_E + C_{48}/K_E^2$$

The $AUMC_{0\text{-}48}$ was calculated by the trapezoidal rule

$$AUMC_{0-48} = \Sigma(t_i - t_{i-1}) \times \frac{(C_i \times t_i - C_{i-1} \times t_{i-1})}{2}$$

RESULTS

Calibration curve

The calibration curves were constructed by plotting the ratio of ciprofloxacin peak areas to that of IS against standard ciprofloxacin concentrations [Figure 2]. Acceptable linear relationships were found in both low (0.025–0.5 µg/ml) and high ciprofloxacin concentrations (0.5–4 µg/ml). Since the distribution of residuals was within ± 5%, no weight factor was applied. Although blank plasma samples (with zero concentrations) were not selected in constructing the calibration curves, the 95% confidence interval of the intercept encompassed the origin. The linear regression equation for the low concentration range were Y = 0.5573 × (±0.0313) + 0.0028 (±0.0042). The coefficient of the linear regression analysis was 0.996 ± 0.0022. For calibration curves prepared at high ciprofloxacin, concentration range results were as follows: Y =0.6873 × (±0.02025) – 0.0842 (±0.0047) and coefficient of the linear regression analysis of 0.997 ± 0.0013.

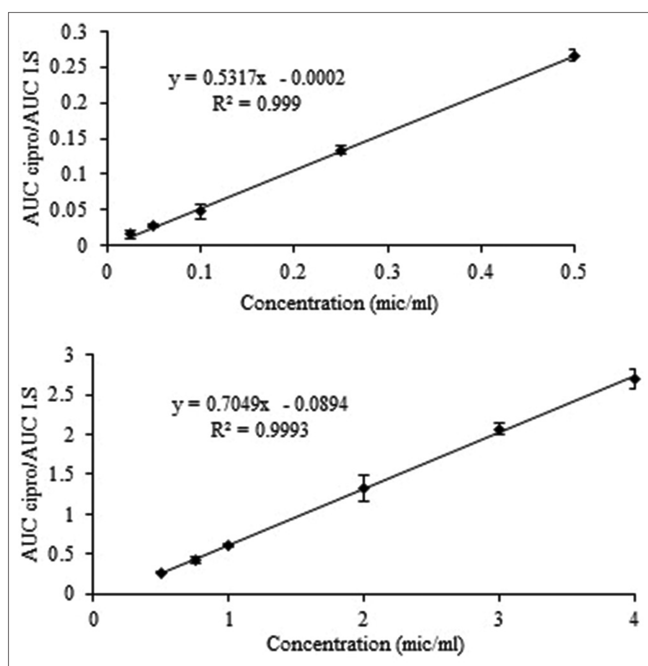

Figure 2: Peak area ratios (ciprofloxacin/internal standard) versus two different ranges (0.025–0.5 and 0.5–4 µg/ml) of ciprofloxacin plasma concentrations

Limit of detection and limit of quantitation

The LOD was 15 ng/ml at a signal-to-noise ratio of 3:1, using 0.5 ml of the plasma sample. The LOQ was 25 ng/ml in plasma with precision, expressed as a CV%, of 7.09% and accuracy of 115%. Representative chromatograms of blank human plasma, (a) blank plasma spiked with IS, phenacetin, (b) the lowest standard concentration (0.025 µg/ml), (c) the highest standard concentration (4 µg/ml), (d) and human plasma 6 h after oral ingestion of a 500 mg gastro retentive prolonged-release formulation are shown in Figure 3. All samples were spiked with 50 µl of IS at a concentration of 100 µg/ml. No interfering substances were observed at the retention time of ciprofloxacin and IS, and both compounds were eluted completely and appeared as two separate resolved peaks (resolution factor: 3.2) without peak tailing (tailing factor: 1.05, 1.2, respectively). An optimum flow rate of 1.5 ml/min for the mobile phase resulted in the retention times of 5.15 min for ciprofloxacin and 8.21 min for phenacetin.

Precision, accuracy, and recovery

The precision and accuracy of calibration standard concentrations were within acceptable limits as defined in the ICH guidelines. The inter- and intra-day precision and accuracy values of the assay method are presented in Table 1. Accuracy % was within 93–115% and the CV% ranged from 0.20 to 12.8, which indicate the method is reproducible within a day and between days. The mean recoveries of ciprofloxacin after plasma extraction were 97.4% ±3.6%.

Robustness

The results of the robustness of the assay method are listed in Table 2. Method robustness that was checked after deliberate alterations of the mobile phase composition, flow, pH, and temperature showed that the changes of the operational parameters did not lead to any essential changes in the performance of the chromatographic system. The tailing factor for ciprofloxacin and phenacetin always ranged from 1 to 1.3, and the eluents were well-separated under all the changes carried out (resolution factor: 3.12–3.26). The percent recoveries of ciprofloxacin were good under most conditions and did not show a significant change when the critical parameters were modified. Considering the result of modifications in the system suitability parameters and the specificity of the method, it would be concluded that the method conditions are robust.

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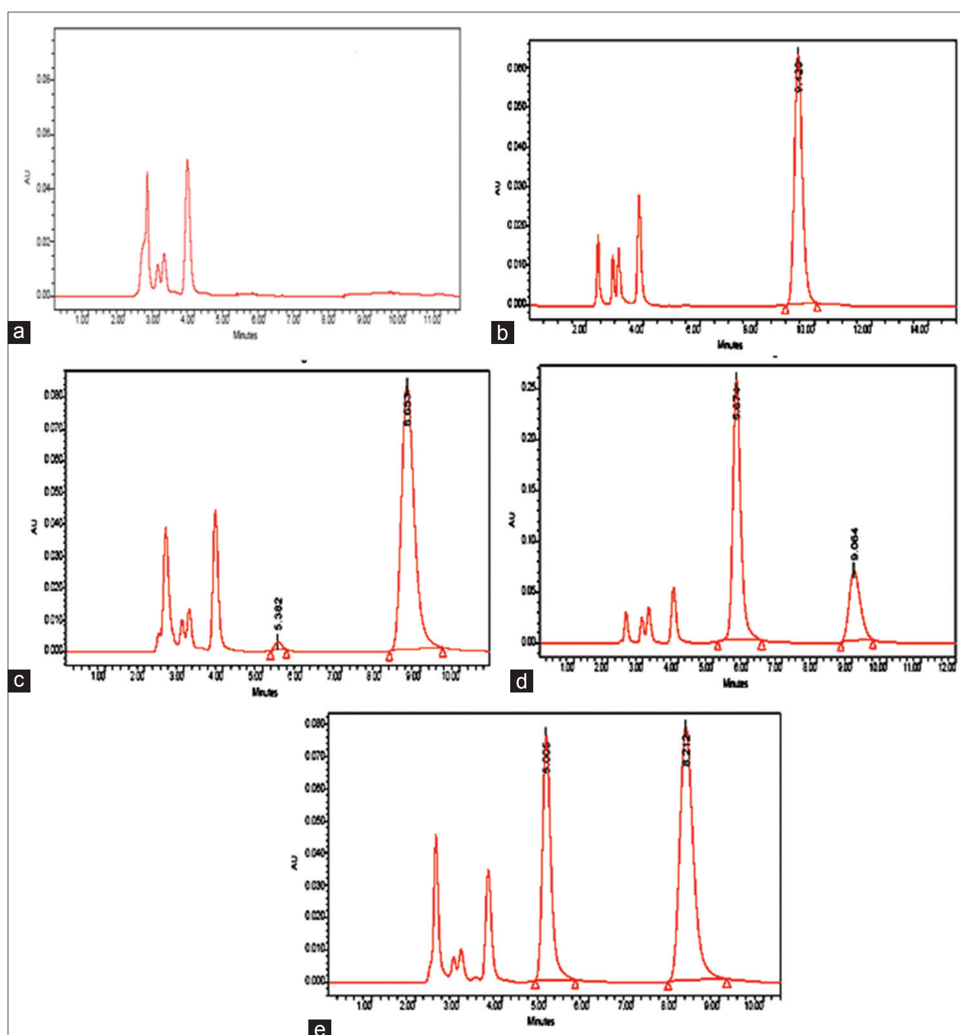

Figure 3: Chromatograms of blank human plasma, (a) blank plasma spiked with internal standard, phenacetin, (b) the lowest standard concentration (0.025 μg/ml), (c) the highest standard concentration (4 μg/ml) (d), and human plasma 6 h after oral ingestion of a 500 mg gastroretentive prolonged-release formulation

Table 1: Intra- and inter-day precision and accuracy of the high-performance liquid chromatography assay developed for determination of ciprofloxacin concentrations in plasma

Analyte (μg/ml)	Intra-day (n=5)			Inter-day (n=5)		
	Mean±SD[a]	CV %[b]	Accuracy %	Mean±SD	CV %	Accuracy %
0.025	0.028±0.001	4.069	112	0.029±0.002	7.094	115
0.05	0.048±0.005	11.04	96	0.050±0.006	12.87	100
0.1	0.092±0.006	7.330	92	0.088±0.005	6.073	88
0.25	0.271±0.003	1.072	108	0.265±0.013	3.950	106
0.5	0.493±0.007	1.492	98	0.496±0.005	1.031	99
0.75	0.695±0.001	0.203	93	0.709±0.023	3.253	94
1	0.984±0.062	6.374	98	0.985±0.044	4.505	98
2	2.051±0.008	0.397	102	2.035±0.025	1.266	101
3	3.112±0.167	5.605	103	3.117±0.125	4.163	103
4	3.912±0.114	2.870	97	4.115±0.085	2.157	103

[a]SD: Standard deviation, [b]CV: Coefficient of variation

Stability

The results of the stability study determined at various storage conditions are summarized in Table 3. No tendency of degradation of ciprofloxacin after storage at room temperature was observed. In three freeze-thaw cycles, the drug was stable in plasma, indicating no significant substance loss during repeated thawing and freezing. Acceptable analyte

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Table 2: Influence of changes in experimental parameters on the performance of chromatographic system

Parameter	Modification	Recovery*	Tailing factor	Resolution factor
Mobile phase ratio (v/v)	85:15	85.6	1.08	3.14
Buffer: acetonitrile	82.5:17.5	99.1	1.05	3.18
	80:20	97.4	1.08	3.21
	77.5:22.5	89.5	1.12	3.28
	75:25	89.1	1.29	3.26
pH	3.5	89.8	1.06	3.24
	3	97.4	1.08	3.21
	2.5	85.5	1.12	3.21
Flow rate (ml/min)	1.9	88.9	1.05	3.13
	1.5	97.4	1.08	3.21
	1.2	82.2	1.23	3.22
Temperature (°C)	45	88.8	1.05	3.28
	40	97.4	1.08	3.21
	35	91.7	1.25	3.23

*Recovery=(measured concentration/nominal concentration) × 100

Table 3: Stability of ciprofloxacin in human samples at different storage conditions (*n*=6)

Stability	Concentrations (µg/ml)	Ciprofloxacin		
		Concentration (mean±SD)	Coefficient of variation %	Accuracy %
Short term stability for 12 h in plasma	0.025	0.026±0.002	1.08	104
	1	1.16±0.061	5.35	116
	4	4.59±0.12	2.31	114
Three freeze-thaw stability	0.025	0.021±0.001	3.91	84
	1	1.24±0.13	8.41	124
	4	4.23±0.31	6.32	105
3 months stability at <−70°C	0.025	0.024±0.001	3.09	96
	1	1.03±0.05	3.81	103
	4	4.36±0.19	4.06	109

SD: Standard deviation

stability was demonstrated for all phases of storage and processing.

Pharmacokinetics and tissue distribution in human

The method described here was successfully employed to quantify ciprofloxacin in plasma following administration of a conventional tablet formulation and a sustained release GI floating tablet containing 500 mg ciprofloxacin to healthy human volunteers. The concentration-time profile following oral administration of floating gastroretentive formulation was presented in Figure 4. Peak plasma concentrations (C_{max}), time taken to reach the maximum concentration (t_{max}), area under the plasma concentration-time curve (AUC_{0-48}), and MRT of ciprofloxacin following ingestion of floating gastroretentive formulation were 2.1 ± 0.49 µg/ml, 1.4 ± 0.59 h, 8.56 ± 1.87 µg h/ml, and 3.64 ± 0.39 h, respectively.

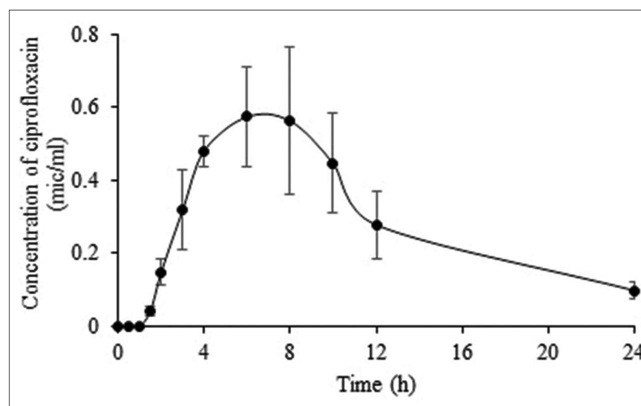

Figure 4: Mean plasma concentration-time profile of ciprofloxacin after oral administration of 500 mg prolonged-release gastroretentive tablet formulations in healthy volunteer

DISCUSSION

The present study describes a very sensitive, accurate, and reproducible HPLC method for the determination of ciprofloxacin in human plasma. Several HPLC methods have so far been reported for the analysis of ciprofloxacin in biological fluids using various extraction procedures including PP,[6-11] filtration,[12-14] SPE,[15,16] LLE.[17,18] Some of these methods use UV detection,[7,11,14,16-18] whereas others use expensive fluorometric detection.[6,8-10,12,13,15,19] A literature review indicated that PP has been the main clean up producer for determination of ciprofloxacin from plasma samples. However, PP will result in low recoveries due to drug precipitation, low sensitivity because of unavoidable dilution, column deterioration, back pressure increment, and late eluting peaks due to endogenous compounds. In different studies conducted by Kamberi *et al*. and Krol *et al*., total chromatography run time was prolonged to 20 min to allow for late eluting peaks.[24,25] Moreover, the interfering peak from plasma in the chromatograms demonstrated inadequate PP, which could diminish peak resolutions and sensitivity of the assays after a long run time. In other similar studies, the plasma sample containing ciprofloxacin was deproteinized with perchloric acid and the supernatant was directly injected to the HPLC column.[26,27] Perchloric or trichloroacetic acid causes PP without significant dilution of the sample, but acidic condition can decrease column durability. To overcome the low sensitivity associated with PP fluorescence detectors have been utilized which are not readily available in most laboratories due to their financial limitations and requires extremely pure HPLC grade solvents. In the study conducted by Sowinski and Kays, the quantification limit of 100 ng/ml was reported by HPLC with UV detection and PP followed by ultrafiltration; however, using

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fluorescence detection resulted in a sensitivity limit of 20 ng/ml.[7] Similar quantification limits (>20 ng/ml) have been reported using PP procedure and fluorescence detection.[8-10] These reported quantification limits even by fluorescence detection is very close to 25 ng/ml which was observed in our study.

To overcome the mentioned difficulties associated with PP, LLE may be a potential alternative. Some studies have been published with regard to HPLC determination of ciprofloxacin in plasma samples following LLE. In all of these studies, the quantification limits obtained higher than 100 ng/ml, which could not precisely characterize the drug plasma profiles, especially at later time points after oral administration of the drug where the concentration of ciprofloxacin is quite low or following ingestion of sustained release forms. Therefore, in the current study, we aimed to develop a very sensitive HPLC method using UV detection and LLE for the determination of ciprofloxacin in human plasma which can be applied in pharmacokinetic studies of this drug when lower ciprofloxacin plasma concentrations are attained. During the development of analytical method, short analysis time, and simple procedures are especially important. The retention times of ciprofloxacin and phenacetin achieved in this work were comparable or even much shorter than those have been yet reported. It is generally known that quinolones give tailing peaks in reversed-phase chromatography, which can be prevented by acidic mobile phase as employed in this study.[16] In some previous studies, the mobile phase has been modified by tetrabutylammonium salts or sodium dodecyl sulfate to reduce retention time and peak tailing.[6,9,10,16,18] However, these compounds can be bound to the analytical HPLC column irreversibly and may cause some problems in column maintenance. Ciprofloxacin is an amphoteric compound which due to the presence of two ionizable groups in its molecule, exhibits ionic properties in all ranges of the pH. Only the zwitter ionic form of ciprofloxacin is soluble in organic solvent. As a result, adjustment of the sample pH to the isoelecteric pH of around seven prior to extraction of the drug can improve the recovery of the eluent. Direct extraction with organic extracting solvents from biological fluids may not result in an efficient extraction of the drug from matrix without pH adjustment. The first reported HPLC method for ciprofloxacin analysis involved the direct methylene chloride extraction of ciprofloxacin from serum without prior adjustment of the pH.[17] The percent recovery of ciprofloxacin following LLE with dichloromethane was reported to be around 8.8%.[14] The low recovery of the analyte could be attributed to the pH of the samples which not adjusted to the isoelectric pH of the analyte ciprofloxacin. In spite the pH adjustment, Idowu and Peggins[8] were unable to recover ciprofloxacin from plasma and milk by LLE using dichloromethane.[8] No explanation was provided for such unrealistic observation. In the current study, we adjusted the pH of the samples to about 7.4, which resulted in an efficient recovery of about 97.4%. The very low quantification limit obtained with UV detection (25 ng/ml) which is comparable[6,7,10,18] or even less[9,12,14-17] than that of most previous reports even when fluorescence detection[6,9,10,12,15] was used allowed us to avoid using fluorometric detection which is more expensive and not available in most laboratories. UV detector produces more reproducible responses in compression with fluorometric detection. Another advantage of our method is the simple composition of mobile phase which reduces the risk of column deterioration associated with the use of ion-pair reagents such as tetrabutylammonium salt and sodium dodecyl sulfate. It is considered that this advantage is favorable for clinical routine applications.

CONCLUSION

In the current study, we developed a very sensitive, precise, accurate, and beneficial HPLC method using LLE and UV detection for determination of ciprofloxacin in human plasma which does not utilize fluorescent detection but still sensitive, making the method rapid, simple and appropriate for pharmacokinetic studies of this drug following oral administration of controlled release formulations for research purposes, conventional immediate-release tablets and when administered concomitantly with divalent and trivalent cations such as aluminum, magnesium, or calcium containing products under which the bioavailability of ciprofloxacin is significantly reduced.

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Conflicts of interest
There are no conflicts of interest.

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