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High-performance liquid chromatographic determination of ciprofloxacin in plasma samples

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Abstract

A new bioanalytical high-performance liquid chromatographic (HPLC) method for the determination of ciprofloxacin with norfloxacin as an internal standard was developed and validated for plasma samples. Norfloxacin is structural homologue of ciprofloxacin and exhibits similar retention properties. The quality of respective peak separation is strongly influenced by amphoteric character of ciprofloxacin and norfloxacin as well. In previously published HPLC methods on conventional C18 reversed-phase [F. Belal, A.A. Al-Majed, A.M. Al-Obaid, Talanta 50 (1999) 765–786; G. Carlucci, J. Chromatogr. A 812 (1998) 343–367], ion pair reagents were added into the mobile phase to suppress peak tailing. In comparison with endcapped and high purity silica reversed-phase sorbent (Purospher RP-18e, Merck), which yielded symmetrical peaks, separation efficiency was further enhanced in our method. Gradient elution mode using acetonitril and phosphate buffer pH 3 on the pentafluorophenylpropyl stationary phase (250–4.6 mm Discovery® HS F5, 5 μm, Supelco) was carried out. The resolution of 4.1 for ciprofloxacin–norfloxacin peaks was achieved. Sample preparation by SPE C18 (Supelclean) with recovery 72% was performed. Fluorescence detection with λ_{excit} = 280 nm, λ_{emis} = 446 nm was used. After the validation, the bioanalytical HPLC method was applied to pharmacokinetic studies.

Keywords: Ciprofloxacin; Norfloxacin; Antibacterial agents; HPLC with fluorescence detection

1. Introduction

Ciprofloxacin, structurally (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid), was as a potent fluoroquinolone chemotherapeutic of the second-generation group of nalidixic acid derivatives first commercially introduced in the 1980s. Due to the broad-spectrum effect and systemic matter of action, 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.

After peroral administration in human ciprofloxacin is rapidly absorbed from GIT 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 [1].

Numerous methods for ciprofloxacin determination in biological samples have been referred and reviews [2,3] have been published. High-performance reversed-phase chromatography with fluorescence [4–15], UV [4,8,10,16,18,20,21,23–26], PDA [17], or more recently tandem mass spectrometric [22] detection is preferably used in biological matrix samples as the most specific and sensitive method.

Octadecylsilyl (C18) [4,6,8,9–16,18,22–26] or octylsilyl (C8) [5,7,21] stationary phase are used for separation, exceptionally other one phases [7,19,20]. Composition of mobile

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phases differs more in high-performance liquid chromatographic (HPLC) methods, various type of ionic pair reagens (tetrabutylammonium cations [8,9,13,16,19,23,24], triethylamine [4,8,17,26], dodecanesulfonic [8,17], heptanesulfonic [5] and laurylsulphate anions [14], the mixture of both anions and cations [4,8,17]) were added, or acid mobile phase without ion-pairs were used [6,7,10,12,15,18,20–22,25].

Evaluation with internal standard (pipemidic acid [14,25], clenbuterol and penbutolol [22], difloxacin [13,15], eprofloxacin [24], lomefloxacin [21]), or without [4,5,7–12,16–20,23,26] was performed.

At sample preparation, three ways are principal:

a) Direct injection of deproteinized samples onto chromatographic column. For plasma precipitation, trichloroacetic acid [9], acetonitrile [6,10,12,17], or perchloric acid [21] were used.
b) Liquid–liquid extraction from neutralized aqueous into organic layer, mainly dichloromethane [13,15,16,23–25], followed by organic phase evaporation and residue reconstitution.
c) Solid phase extraction on C18 cartridges [7,26] or others [5,22]. Influence of different types of cartridges on recovery were described [5].

Ciprofloxacin and many others fluoroquinolones, due to the amphoteric character, are in aqueous media present in the ionic form at the whole pH range, which is the reason that the separation on reversed-phase columns is difficult. The main problems are as follow:

- Binding of the analyte to the silanol moiety, broad tailing peaks are produced or eluted too early with low efficiency of separation.
- Extraction into organic media has low recovery dependent on pH.
- Solubility of the zwitterionic form of ciprofloxacin in the water is very low.

Under the scope of this view, the aim of our work was to develop the more precise, accurate, rugged and reliable method of ciprofloxacin determination in plasma samples for application in bioequivalence study with regards to the great range of concentration (0.05–15 nmol/ml) in plasma samples and great number of samples needed to be analysed in the relatively short time. Structurally close homologue of ciprofloxacin, which exhibits the same spectrum and similar chromatographic separation features, norfloxacin was used as internal standard. Chemical structure of both compounds is given in [29].

Mobile phase without using ion pair reagents was utilized.

Pentafluorophenylpropyl (Discovery HS F5) stationary phase (Fig. 1), which was not previously used for fluoroquinolone analysis was compared with octadecylsilyl (Purospher C18 e) stationary phase and the quality of separation was evaluated. Better resolution on the Discovery HS F5 phase with simple mobile phase acetonitrile–phosphate buffer was achieved. Higher recovery using the solid phase extraction on C18 Supelclean columns in comparison with the liquid–liquid extraction was gained. Method was validated and used in comparative bioequivalence study in 24 human volunteers.

2. Experimental

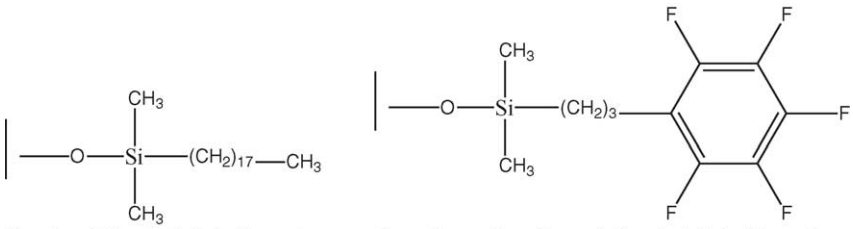
2.1. Reagents and materials

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid, hydrochloride, monohydrate, $C_{17}H_{18}FN_3O_3{\cdot}HCl{\cdot}H_2O$, FW = 385.8 g/mol), Lot No. CIM2001/3, PRO.MED.CS Praha a.s., Czech Republic; norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid, $C_{16}H_{18}FN_3O_3$, FW = 319.3 g/mol), Lot No. 083H0921, Sigma, USA, used as an internal standard for HPLC determination.

Acetonitrile, methanol (both HPLC grade, Merck, Darmstadt, Germany), potassium dihydrogenphosphate (analytical grade, Lachema Brno, Czech Republic), phosphoric acid (85%, analytical grade, Lachema Brno, Czech Republic), ultra-high quality (UHQ) water (prepared using Elgastat UHQ PS apparatus, Elga Ltd., Bucks, UK).

2.1.1. Solutions

Potassium dihydrogenphosphate (0.05 M; pH 3) was prepared mixing 6.8045 g of KH_2PO_4 in 950 ml of water and adjusting pH with H_3PO_4 (2 M) and making up to 1000 ml.



Octadecyldimethylsilyl silica gel
Purospher

Pentafluorophenylpropyldimethylsilyl silica gel
Discovery HS F5

Fig. 1. Structure of the used stationary phases.

Elution reagent was prepared by adding acetonitrile in potassium dihydrogenphosphate (0.05 M; pH 3) 1:1 (v/v). Mobile phase (10% of acetonitrile) was prepared mixing acetonitrile and potassium dihydrogenphosphate (0.05 M; pH 3) 1:9 (v/v). Stock standard solution of ciprofloxacin and norfloxacin was prepared at the concentration level 10^{-3} M, and stored under light-protecting condition at 5 °C. The stability of stock solutions was minimally 1 month. UHQ water was used in preparation of all solutions.

Calibration standard solutions were prepared spiking blank human plasma samples by appropriate amount of diluted stock standard solutions (10^{-5} M) and treated as plasma samples.

2.2. Sample preparation and solid-phase extraction of plasma

The frozen plasma samples were thawed at room temperature, mixed, centrifuged (10 min, 1000 × *g*) and submitted to the solid phase extraction on the reversed-phase (C18) cartridges (1 ml, 500 mg) under the following steps:

1) *Conditionation*: One milliliter of water followed with 1 ml of methanol was drown slowly through the column under taking care for not to let the column dry.
2) *Sample application*: Mixture of 100 μl of plasma and aliquot amount of internal standard solution (10^{-5} M) norfloxacin was applied on the column.
3) *Washing*: Gradually, 1 ml of water and 1 ml of methanol solution (10% in water) was drown through the column until dryness.
4) *Elution*: The analytes were eluted by slow application of 1 ml of elution reagent, the eluate was collected into the glass tube and evaporated at 45 °C under the stream of nitrogen. The sample residue was reconstituted by 1 ml of mobile phase and after mixing and centrifuging, injected onto the chromatographic column and analysed.

2.3. Comparative pharmacokinetics application

Twenty-four healthy adult volunteers both males and females, were participated in the bioequivalence study. All subjects were healthy on the basis of their medical history, clinical and laboratory examination (haematology, biochemistry and urine analysis).

The subjects were instructed to abstain from any medication, alcohol, xanthine containing food or beverages and grapefruit juice during the study.

After the overnight fast, one 500 mg ciprofloxacin tablet of the test or reference preparation was administered orally. The blood samples (5 ml) were drawn from cubital vein into heparinized syringe at 0 (baseline), 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24 h. After collecting (up to 30 min), the samples were centrifuged, plasma separated into polypropylene tubes and stored at −70 °C until analysis.

2.4. Apparatus

Routine chromatographic analyses were performed using a Thermo Electron (formerly Thermo Finnigan) chromatograph (San Jose, CA, USA). The chromatographic system was composed of an SCM1000 solvent degasser, P4000 quaternary gradient pump, AS3000 autosampler with a 100 μl sample loop, FL3000 fluorescence detector, SN4000 system controller and a data station (Intel-Pentium 4, CPU 1.6 GHz, RAM 256 MB, HDD 40 GB) with the ChromQuest 4 analytical software (Thermo Electron Inc., San Jose, CA, USA) working under the Windows 2000 operating system (Microsoft Corporation).

For sample preparation, SPE apparatus Visiprep Solid Phase Extraction Vacuum Manifold (12-port, Supelco) and columns Supelclean LC-18, volume 1 ml; 500 mg of filling, Supelco, Bellefonte, USA) were utilized.

2.5. Chromatographic assay of ciprofloxacin and norfloxacin (IS)

Two types of analytical columns were used for the method development:

Purospher® C18e (250–4) mm, 5 μm (Merck, Darmstadt, Germany) with guard column Purospher® C18e (4–4) mm and Discovery HS F5 (250–4.6) mm, 5 μm (Supelco, Swiss) with guard column 4L–3 mm C18 (Phenomenex, USA).

Different option in chromatography assay were investigated to find the best separation efficiency:

I. Mobile phase: Nonylamine (0.01 mol/l, pH 3.5 adjusted with H_3PO_4) and acetonitrile 95:5 (v/v); mode: isocratic, flow rate 1 ml/min; column: Purospher C18e.
II. Mobile phase: Potassium hydrogenphosphate (0.05 M, pH 2.5 adjusted with H_3PO_4) and acetonitrile 85:15 (v/v) mode: isocratic, flow rate 1 ml/min; column: Purospher C18e.
III. Mobile phase: Potassium hydrogenphosphate (0.05 mol/l, pH 3 adjusted with H_3PO_4) and acetonitrile, mode: gradient–10% acetonitrile in phosphate buffer increased to 50% in 20 min then back to 10% in 5 min, equilibration time 5 min, flow rate 1 ml/min; column: Purospher C18e.
IV. Mobile phase composition, gradient mode and flow rate was the same as referred in III; column: Discovery HS F5.

The fluorescence detector was programmed as follows: excitation/emission wavelengths were adjusted to 280/446 nm for the time interval 0–25 min. The lamp flash rate was adjusted to 100 Hz, PMT voltage 600 V. Optimal excitation and emission wavelengths of ciprofloxacin (norfloxacin has the equal fluorophore and exhibits similar spectra) were find using the scanning spectra mode Fig. 4.

Typical chromatograms of standard solution ciprofloxacin and norfloxacin, recorded under the option described above, are shown in Fig. 2.

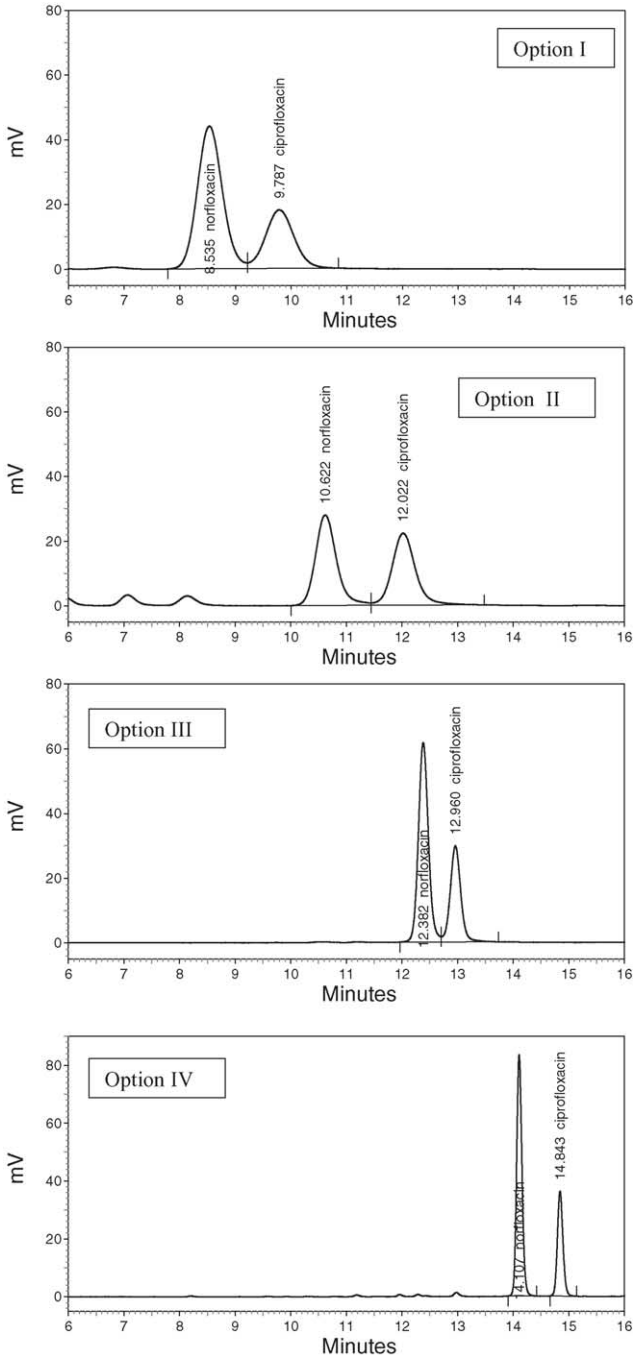


Fig. 2. Chromatograms of ciprofloxacin an IS norfloxacin showing the separation method development, using chromatographic options I–IV.

The best separation of ciprofloxacin and norfloxacin was at condition IV achieved. This chromatographic option was chosen for method validation and application to ciprofloxacin assay in plasma samples of comparative pharmacokinetic studies.

2.5.1. Calibration

A 9-level calibration series with six samples at each concentration level was prepared spiking 100 μl of blank human plasma samples with appropriate amount of ciprofloxacin at the concentration level 0.05; 0.2; 2; 4; 6; 8; 10; 12; 14 nmol/ml of plasma and internal standard norfloxacin at the same concentration level 8 nmol/ml of plasma, which corresponded approximately to the range 0.5–160% of expected maximal concentration of ciprofloxacin in the average human pharmacokinetic (C_{max}). The solid-phase extraction procedures were the same as described in Section 2.2. Calibration series was measured using the fluorescence detector under the condition IV mentioned in Section 2.5. On-line statistical processing of the calibration analyses by the least-squares regression method was performed automatically using the ChromQuest software.

2.6. Validation of the method

Validation parameters are according to the recommendation of the guidance CDER and CVM established [27].

2.6.1. Accuracy

The accuracy was determined as a relative error bias (%) calculated from the following equation: accuracy (%) = $100(C_{real} - C_{determined})/C_{real}$ was calculated from the calibration curve equation.

The range of the applicability of HPLC method was enclosed within the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ). The lower limit of quantification was determined as the lowest concentration on the standard calibration curve which was measured with a precision of 20% and accuracy of ±20%. Upper limit of quantification was equal to the highest concentration in 9-level human plasma calibration.

2.6.2. Precision

The precision, as the measure of intra-day repeatability, was expressed as the coefficient of variance CV_{intra} (%) of six identically prepared and measured calibration samples during 1 day measurement series.

Inter-day precision (reproducibility) was performed as a coefficient of variance CV_{inter} (%) of 24 between-days measurements of plasma samples. The samples were prepared spiking blank human plasma at three concentration levels and performed as the quality control points incorporated into the series after every three to four pharmacokinetics samples during the routine everyday measurements.

2.6.3. Recovery

To determine the recovery of the extraction procedure, water calibration series at the same concentration levels as in Section 2.5.1 mentioned plasma calibration series was prepared and directly injected into the chromatograph. The recovery of ciprofloxacin was calculated as the percent ratio of the external standard calibration (ESTD) slopes of plasma and water samples.

2.6.4. Linearity

The linearity of the calibration curve from the extracts of a drug-free human plasma spiked with the above-mentioned analytes was tested and evaluated using both linear and quadratic regression model of internal standard calibration (ISTD) curve. Coefficients of calibration equation and the correlation coefficient were expressed.

2.6.5. Stability tests

To ensure the reliability of the results in relation to the handling and storing of plasma samples and stock standard solutions, following stability tests were carried out:

1) Long-term stability of ciprofloxacin in plasma samples stored at −70 °C minimally for 4.5-month period.
2) Freeze and thaw stability for three cycles.
3) Twenty-four hours autosampler stability of plasma samples extracts at room temperature.
4) Standard stock solution stability stored at 5 °C for minimally 1-month period.
5) Diluted standard solution stability exposed to the daylight at room temperature for 6-h period.

3. Results and discussion

3.1. Chromatography

3.1.1. Method development: optimization of chromatographic separation

Ciprofloxacin and norfloxacin as well exhibits amphoteric properties. In chromatographic reversed-phase separation mixed effects, both hydrophobic and ionic interactions are taken part. This is in compliance with observations [20], that the functional dependence of capacity factor on pH of mobile phase differs from the ideal sigmoidal shape of predicted clearly hydrophobic interactions, which indicates additional donor–acceptor interactions. These interactions of protonated amino group of piperazine moiety with residual silanol groups of stationary phase in acidic media often cause the problems in chromatographic separation performance, manifesting mainly with bad peak shape (broadening and tailing), low efficiency of separation and insufficient resolution. In developing our method, choosing suitable reversed-phase column and suitable chromatographic mobile phase option showed to be essential in adequate separation for given purpose. Various types of "special base" stationary phase columns, which considerably differ in their chromatographic separation properties [28], are commercially available. Comparison of the separation characteristics of ciprofloxacin (and internal standard norfloxacin) on endcapped octadecylsilyl stationary phase with monomeric bonding and high purity synthetic silica support (Purospher, Merck) and pentafluorophenyldimethylsilyl endcapped stationary phase (Discovery HS F5, Supelco) is given in Table 1. Chromatograms of the respective peaks are shown in Fig. 2.

Table 1
Chromatographic separation characteristics of ciprofloxacin analyzed under different conditions I–IV mentioned above in Section 2.5

Option	I	II	III	IV
Theoretical plates (USP)	1670	4132	22742	103034
Capacity factor	2.91	3.81	4.18	4.94
Resolution of ciprofloxacin and norfloxacin (USP)	1.39	1.95	1.71	4.06
Width at 50% height (min)	0.55	0.43	0.20	0.11
Asymmetry (10%)	0.99	1.09	1.03	1.08

Isocratic mode and base-competing reagent, nonylamine in mobile phase (I) yielded symmetric but the less resolved peaks with low separation efficiency. Changing base-competing reagent for potassium phosphate buffer (II) improved the resolution and gradient mode (III) gave better efficiency of separation and still resolved peaks ($Rs > 1.5$). The same conditions, but different stationary phase (HS F5), yielded entirely the "baseline to baseline" (IV) and most efficient separation in 25 min.

Pentafluorophenylpropyl stationary phase is the type of phase, which provides separation similar to reversed-phase, but π–π interactions are involved in separation mechanism particularly of aromatic compounds. It is probable, that this type of interactions are more dependent on the shape and geometry of the molecule, and thus the structural homologues, which differ more in the shape of side chain parts, could be better resolved than on common C18 phases.

3.2. Sample preparation procedure development

Three ways of plasma sample preparation (Table 2), liquid–liquid extraction in dichlormethane, solid phase extraction (C18 cartridges) and deproteination with acetonitrile, were tested and compared. As some small interference peaks occurred in the retention time of the analytes in plasma deproteinated samples, SPE extraction with lower recovery, but better removing balast peaks, was used for the assay. The dependence of the recovery of SPE (C18) extraction on the amount of acetonitrile in elution reagent is given in Fig. 3. Due to the strong bonding of ciprofloxacin/norfloxacin to the

Table 2
Comparison of sample preparation methods by recovery % of ciprofloxacin from plasma samples: liquid–liquid extraction into dichlormethane at pH 7, deproteination with acetonitrile precipitation, solid phase extraction on C18 cartridges

	Recovery %		
	Dichlormethan extraction	Acetonitrile deproteination	SPE-C18
Norfloxacin	7.5	94.3	72.0
Ciprofloxacin	8.8	94.7	72.3

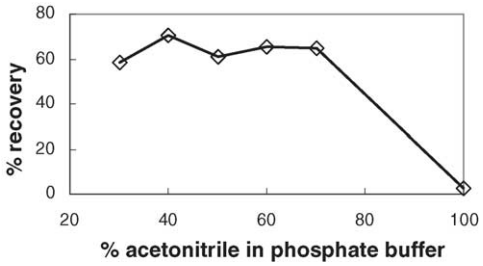


Fig. 3. Recovery % of ciprofloxacin from plasma samples using SPE with various content of acetonitrile in elution reagent.

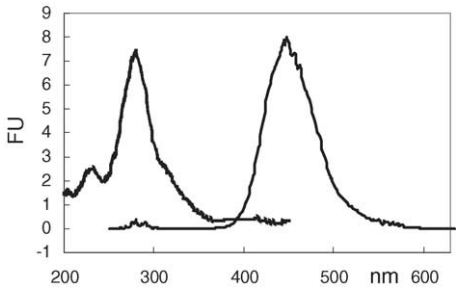


Fig. 4. Excitation and emission spectra of ciprofloxacin (the spectra of norfloxacin are similar).

sorbent, pure nonpolar solvent was not efficient enough to elute the respective analytes.

3.3. Validation parameters

3.3.1. Accuracy and precision

Accuracy determined during 1 day measurement of nine concentration levels for six spiked plasma samples was expressed (Table 3) as relative error difference between measured and actual concentration. Precision was as relative standard deviation for each level for six measurements evaluated. Lower limit of quantification for ciprofloxacin of 50 pmol/ml plasma was determined as the last measured concentration level for which the accuracy or precision is less or equal than 20%. The range of applicability was from 50 to 14,000 pmol/ml estimated.

The inter-day accuracy and precision was at three concentration levels during 24 day consequent measurements evaluated (Table 4).

Table 4
Inter-day precision of ciprofloxacin determination in plasma samples at three concentration levels

Inter-day ($n = 24$)		
Concentration (nmol/ml plasma)	Accuracy (%)	Precision CV_{inter} (%)
0.2	−3.40	5.40
4	−0.65	3.57
8	−0.34	1.53

3.3.2. Linearity

Though the correlation coefficient using linear regression model of calibration curve is acceptable $r^2 = 0.99944$, there was a bias error at estimating the value of lowest two levels from calibration curve. Quadratic regression with correlation coefficient $r^2 = 0.99999$ gives closer approximation of the calibration curve. Results are given at the Tables 5 and 6.

3.3.3. Recovery

Recovery was as the ratio of the slopes of linear plasma and water ESTD calibration plots expressed. Nine levels and six analysis at each level for both plasma and water calibration was performed (Table 7).

3.3.4. Selectivity

Blank plasma samples from six volunteers were analysed, no interference peaks in the retention time of ciprofloxacin and internal standard norfloxacin were observed (Fig. 5).

3.3.5. Stability tests

Stability test of ciprofloxacin in plasma were carried out at three concentration levels (low, medium, high) with six equally prepared samples at each level.

Ciprofloxacin in plasma samples proved to be stable stored at −70 °C for minimally 4.5 month period as well as three freeze and thaw cycles did not change the concentration levels of respective analyte significantly (e.g. more then of 15%).

Stock standard solutions were stable stored under light-protecting condition at 5 °C for 1 month period. Stability of diluted standard water solutions minimally for 6 h at room temperature and daylight access was equally proved.

Table 3
Intra-day accuracy and precision of ciprofloxacin determination in plasma samples at nine concentration levels

Actual (nmol/ml plasma)[a]	Calculated ($n = 6$) ± S.D. (nmol/ml plasma)[a]	Precision (R.S.D., %)[a]	Accuracy (%)[a]
0.05	0.0465 ± 0.0039	7.74	−7.07
0.2	0.223 ± 0.0023	1.16	11.60
2	1.98 ± 0.014	0.70	−1.03
4	3.98 ± 0.029	0.74	−0.46
6	6.01 ± 0.064	1.06	0.09
8	8.02 ± 0.034	0.42	0.27
10	10.00 ± 0.092	0.92	0.01
12	11.99 ± 0.027	0.23	−0.05
14	14.00 ± 0.12	0.85	−0.02

[a] Concentration.

Table 5
Expression of the bias of actual (spiked) concentration and concentration calculated from ISTD calibration curve

Concentration level (nmol/ml plasma)	0.05	0.2	2	4	6	8	10	12	14
$(C_{actual}-C_{calculated})/C_{actual}$ (%)									
Linear regression model	75.0	27.4	0.5	−1.9	−2.4	−1.9	−0.7	0.3	1.3
Quadratic regression model	7.1	−11.6	1.0	0.5	−0.1	−0.3	0.0	0.1	0.0

Table 6
ISTD calibration regression equation coefficients, correlation coefficient and range of applicability

	Calibration equation	Correlation coefficient	Range of applicability (pmol/ml plasma)
Linear model	$y = 0.84358x + 0.01249$	0.99944	693æ14000
Quadratic model	$y = -0.04110x^2 + 0.91189x - 0.00092$	0.99999	50æ14000

Table 7
ESTD calibration curve coefficients for ciprofloxacin in spiked plasma and water samples

	ESTD calibration		
	Slope	Intercept	Correlation coefficient
Plasma matrix	49222	4229.7	0.99965
Water matrix	68436	9246.5	0.99936
Recovery (%)		72	

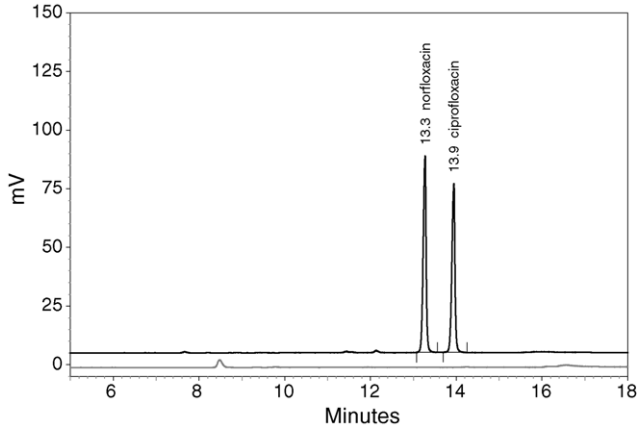


Fig. 5. Typical chromatograms ciprofloxacin and norfloxacin in plasma samples spiked at the level 8 nmol/ml plasma (upper curve), blank plasma sample (lower curve).

3.3.6. Comparative pharmacokinetic studies

The method was applied to comparative pharmacokinetic studies of oral 500 mg ciprofloxacin preparation in 24 healthy volunteers. The pharmacokinetic parameters for test and reference preparation were evaluated (Table 8). The maximum ciprofloxacin concentration (C_{max}) and the corresponding time of peak plasma concentration (T_{max}) were taken directly from the individual plasma data. The elimination rate constant (k_e) was estimated as the slope of the semilogarithmic plot of the 3–4 last points of the plasma concentration versus time curve calculated by linear regression. The area under the plasma concentration versus time curve AUC_{0-24} was calculated by linear trapezoidal method and the elimination constant k_e was used to extrapolate area from the last measurable concentration to infinity. Elimination half-life ($t_{1/2}$) was calculated as $\ln 2/k_e$. Mean plasma concentrations of ciprofloxacin after oral administration of the test and reference preparations versus time curves are shown in Fig. 6.

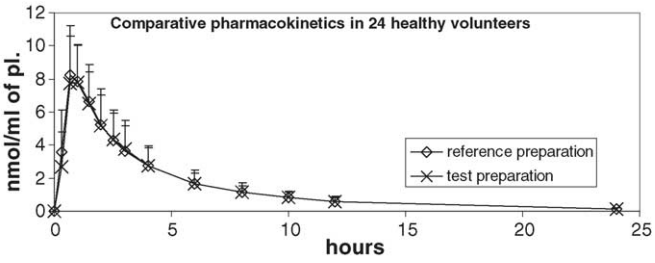


Fig. 6. Comparison of the arithmetic means of ciprofloxacin plasma concentration vs. time curves after p.o. administration test and reference ciprofloxacin preparation (500 mg).

Table 8
Pharmacokinetic parameters for the test and reference preparations (500 mg of ciprofloxacin) after oral administration to 24 healthy volunteers

	Test preparation					Reference preparation				
	C_{max} (μmol h/l)	T_{max} (h)	AUC_{0-24} (μmol h/l)	$AUC_{0-\infty}$ (μmol h/l)	$t_{1/2}$ (h)	C_{max} (μmol h/l)	T_{max} (h)	AUC_{0-24} (μmol h/l)	$AUC_{0-\infty}$ (μmol h/l)	$t_{1/2}$ (h)
Mean	8.92	1.00	32.89	34.24	5.58	9.11	0.86	33.32	34.58	5.42
S.D.	2.11	0.54	10.08	10.34	1.00	2.43	0.30	12.59	12.94	1.13
CV (%)	23.7	54.3	30.6	30.2	17.9	26.7	35.4	37.8	37.4	20.9
Minimum	2.87	0.67	7.64	8.16	3.81	4.93	0.33	14.82	15.28	2.95
Median	8.86	0.84	31.54	33.27	5.69	8.94	0.67	29.16	30.61	5.53
Maximum	15.07	3.00	53.00	54.78	7.66	13.83	1.50	70.93	73.80	7.67

4. Conclusions

Analytical method for ciprofloxacin (and norfloxacin) in plasma samples determination using high-performance liquid chromatography with fluorescence detection was developed and validated. Due to the excellent separation efficiency, the method would be possibly suitable for more complex mixture of fluoroquinolones (or their impurities) determination, which has not been tested yet. Accuracy and precision of the method has been proved, the method is reliable and rugged and suitable for routine analysis. The assay has been applied to the comparative pharmacokinetic study in 24 volunteers.

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