Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 567–574
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Simultaneous determination of Ciprofloxacin and the active metabolite of Prulifloxacin in aqueous human humor by high-performance liquid chromatography

Roberto Maria Pellegrino [a,*], Fabio Segoloni [b], Carlo Cagini [b]

[a] *Dipartimento di Chimica, Sezione di Chimica Organica, Università di Perugia, Via Elce di Sotto 8, 06100 Perugia, Italy*
[b] *Dipartimento di Specialità Medico-Chirurgiche e Sanità Pubblica, Sezione di Oculistica, Università di Perugia, Via Brunamonti 51, 06100 Perugia, Italy*

Received 30 August 2007; received in revised form 23 January 2008; accepted 29 January 2008
Available online 7 February 2008

Abstract

A rapid and simple method for determining two fluoroquinolones (FQNs), namely Ciprofloxacin and Ulifloxacin, this being the last active metabolite of Prulifloxacin, in aqueous human humor (AHH) has been developed and validated. The calibration data resulted linearly correlated in the 4–500 ng/mL concentration range with 8 ng/mL lower limit of quantification (LLOQ) for Ciprofloxacin, and 5–600 ng/mL concentration range with 6 ng/mL LOD for Ulifloxacin. The proposed analytic procedure has been validated by testing quality control sample (QCS) of AHH probed with the two FQNs at 10, 50, 500, and 1000 ng/mL concentration values. Validation of the method has been checked by accuracy and precision data of intra-day and long-term experiments. The two FQN concentrations have been measured by HPLC technique with UV detection at 278-nm wavelength for the AHH of patients to whom were supplied oral doses of FQNs (500 mg) twice in a day, within 1–24 h before the surgery intervention of cataract. The average concentration of Ciprofloxacin resulted 186 ng/mL and that of Ulifloxacin 78 ng/mL. The nice quality of the proposed analytic procedure means that it may be suitable for *in vivo* studies of pharmacokinetics regarding these substances in the AHH medium.

Keywords: Aqueous human humor; Prulifloxacin; Ciprofloxacin; Ulifloxacin; Reversed-phase chromatography

1. Introduction

1.1. The fluoroquinolones

Fluoroquinolones (FQNs) are synthetic antibiotics largely employed in clinical treatments thanks to their large activity spectrum against gram-positive and gram-negative bacteria. Like quinolones, from which they are derived, the structural molecular unity in these compounds is the nalidixic acid with a fluoro atom attached to the central ring, typically at 6-position (see Fig. 1). Based on their chronological development, FQNs have been classified as first, second, third and fourth generation groups. Ciprofloxacin (CPUFX) is a second-generation fluoroquinolone, largely known and used in clinical practice for its excellent pharmaceutical properties. Very recently, other FQN derivatives with antibiotic activity have been synthesized and introduced into the market such as the Prulifloxacin (PUFX), a pro-drug of Ulifloxacin (UFX) [1]. Nowadays, UFX is subjected to intensive and deep pharmacokinetic studies since it has been shown that such a drug possesses both *in vivo* that *in vitro*, antibiotic activities like those of well-known CPUFX [1,2]. For these reasons, the interest of the scientific community is grown more and more in the past period, and the extension of the knowledge on UFX permeability through various biological tissues currently represents an intriguing task.

1.2. Objective and target analyte

This study is focused on two FQN antibiotics: CPUFX and PUFX. The first objective of this work was to validate a simple and rapid quantification method of CPUFX and UFX in the AHH after oral administration. Secondary, we want to evaluate the permeability of PFUX with respect to CPUFX in AHH as a preliminary clinical study.

* Corresponding author. Tel.: +39 75 585 5557; fax: +39 75 585 5560.
E-mail address: pellerob@unipg.it (R.M. Pellegrino).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.01.043

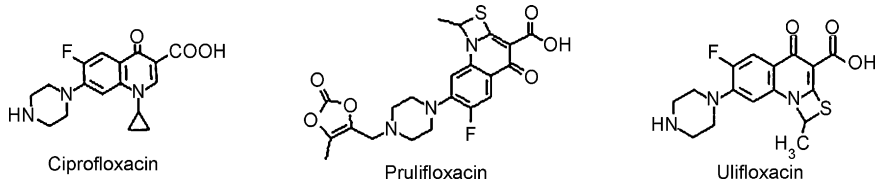

Fig. 1. Structure of Ciprofloxacin, Prulifloxacin and Ulifloxacin.

The CPUFX, when supplied orally or systemically to a patient, was scarcely metabolized and showed a direct antibiotic activity [3]. PUFX, on other side, when orally administered, was rapidly metabolized into its active metabolite UFX thanks to the intestinal esterase [1,4]. This is the reason for which the analytic detection of the PUFX permeability in fluid biological tissues must be referred to the UFX concentration.

2. Materials and methods

2.1. Reagents

Ciprofloxacin [3-quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-CAS 85721-33-1], *ortho*-phosphoric acid 85%, water and acetonitrile HPLC grade and 1-mL eppendorf vials were purchased from Sigma–Aldrich; Ulifloxacin [1*H*,4*H*-[1,3]-thiazeto-[3,2-*a*]quinoline-3-carboxylic acid, 6-fluoro-1-methyl-4-oxo-7-(1-piperazinyl), CAS 112984-60-8] was gently supplied from Angelini S.p.A.

2.2. Analytical techniques for CPUFX and UFX determination

Two reviews [3,5] deals with the state-of-the-art techniques employed in the analysis of FQN antibiotics. Among FQNs the CPUFX is among the most studied substances. Quantitative determination of CPUFX in complex biologic fluids as plasma, urines, serum, milk, aqueous and vitreous humor is possible with a very nice sensitivity through HPLC, using UV, fluorimetric (FL), and spectrometric detectors. The HPLC separation of CPUFX from other FQNs [6], or from the naturally present in the matrix substances, is conducted with a very good resolution by means of inverted phase C_{18} columns. Generally the limit of detection (LOD) with UV detectors is around 2–40 ng/mL and it depends very much on both the employed volume and sample preparation's steps. Even if bioassay methods also have been used to measure CPUFX in aqueous and vitreous humor, precision of these procedures was much low [7].

UFX was determined after PUFX administration with HPLC and UV detector [8]. Also HPLC–tandem mass spectrometry was used for human plasma samples [4]. The LOD was around 25–40 ng/mL in both cases.

Taking into account the existing literature data, the method was based on the HPLC–UV analytical technique by using a reversed-phase column because its use is a very common, inexpensive and does not require specialized personnel.

2.3. Chromatographic apparatus and conditions

The HPLC instrument was a Hewlett-Packard 1100 model, consisting of a degaser system, a quaternary pump, a Rheodyne injector with 20 μL loop and an UV–vis detector. The detector was operated at a wavelength of 278 nm. Data were collected with a HP 3395 integrator. The mobile phase consisted of an isocratic acetonitrile/85% aqueous phosphoric acid at a mixture ratio of 15:85, respectively and the flow was set at 0.5 mL/min. Chromatographic separation was achieved at room temperature with a Hewlett Packard ZORBAX Eclipse XDB-C_8 column, dimensions: 4.6 mm × 150 mm, 5 μm (PN 5063-6600). Before each injection, rheodyne valve was washed three times with 50 μL mobile phase.

2.4. Concentration range of CPUFX and UFX in various biological tissues after supply

Several investigations have been carried out to study FQNs penetration in tissues and into different biological media, both human and animal, after systemic administration [8–12]. Usually, the detected concentration level of various Floxacines in plasma, milk, and urine of patients treated with single or multiple doses of these antibiotics, ranges from 50 to 1000 ng/mL. Some authors [13–17] reported the HPLC measurements of CPUFX and other FQN concentrations, in AHH and the vitreous humor, but only after a topic application. In AHH, the detected CPUFX concentration was in these circumstances comprised between 150 and 1000 ng/mL. CPUFX and other Floxacines have been measured by bioassay in AHH of patients who received two oral doses of 500 mg before cataract surgery [7]. In their paper, the authors report that average CPUFX concentration was found to be 560 ng/mL. Again, in an *in vivo* pharmacokinetic study of CPUFX penetration in serum has been performed in AHH and vitreous humor onto *New Zealand white rabbit* after systemic supply of a 40 mg/kg dose [18]. Maximum detected CPUFX concentration has been about 250–300 ng/mL.

The UFX concentration in human plasma, after oral administration of 600 mg PUFX ranges between 110 and 660 ng/mL [4,8]. At our best knowledge, there is no report in the literature concerning the measure of UFX concentration level in AHH except only one example where the UFX concentration was detected in the aqueous humor of *pigmented rabbit* achieving 14 ng/mL as a maximum value, after an oral supply of 20 mg/kg of PUFX [19].

2.5. Calibration range

The forecast concentration range for CPUFX and UFX, either in quality control samples (QCSs) and in the real samples, is comprised between 50 and 1000 ng/mL. However, as shown below, the preparation procedure of AHH requires a 1:6 dilution ratio so that the calibration range of 5–500 ng/mL has been taken as adequate. In this paper the analytes' peak areas have been directly correlated with their concentrations expressed in ng/mL.

2.6. Validation parameters

The validation of the method was performed by establishing the limit of detection (LOD), lower limit of quantification (LLOQ), linearity of calibration curve and the method's reproducibility and repeatability. In order to obtain these validation data two types of samples were prepared: calibration standard solution (CSS) and quality control samples. The LOD was expressed as the lowest dilution value of CS that presents a %RDS that did not exceed 15% and the LLOQ was expressed as the lowest dilution value of QCs that presents a %RDS that did not exceed 20%. Linearity was checked with correlation coefficient r^2 and with homoscedasticity of calibration curve. Repeatability and reproducibility were checked, respectively, by intra- and inter-day determinations of accuracy and precision. Accuracy, expressed as average percent of recovery of five replicates QCs at various concentrations, should be in the ranges of 80–110% for samples concentration between 100 and 1000 ng/mL and 60–115% for samples concentration between 10 and 99 ng/mL [20]. Precision, expressed as percentage of the %RDS of five replicates, should not exceed 15% in all cases [21].

2.7. Preparation of the calibration standard solution (CSS)

0.0130 g of CPUFX and 0.0157 g of UFX were exactly weighted each in a different volumetric flask of 25 mL and the relevant volume was adjusted with an 85% aqueous solution of *ortho*-phosphoric acid. The two standard solutions have been named A-CPUFX and A-UFX, respectively. To facilitate the solid dissolving the two flasks were submerged into an ultrasounds bath (VWR International, USC-300TH model) at room temperature for a time of 5 min. Next, by mixing and diluting opportune volumes of these solutions we achieved six CSS that we named B, C, D, E, F, and G and that contained both substances at conc. 500, 380, 250, 125, 41.6, and 4.16 ng/mL for CPUFX and 600, 450, 300, 150, 50.2, and 5.02 ng/mL for UFX. The volumes of these solutions were adjusted to the mark with mobile phase.

2.8. Preparation of standard-pool of AHH samples (SPAHH)

During some surgery treatments of cataract, aliquots of 20–150 μL of AHH have been withdrawn from 22 randomized conscious patients (healthy and not floxacines treated). Each sample, after transferring into a 1 mL *eppendorf* vial, was frozen at −20 °C just soon after the sampling. The collected AHH samples were restored to room temperature and put all together in order to reach a total volume of ca. 1.2 mL.

2.9. Preparation of quality control samples (QCSs)

0.0501 g of CPUFX and 0.0500 g of UFX have been exactly weighted, each in a 10 mL volumetric flask, and relevant volumes were adjusted to the mark with the mobile phase. 1 mL of each solution has been transferred in a 10 mL volumetric flask and volume was adjusted to the mark with the mobile phase. From this solution, containing both floxacines at an overall concentration of 500 μg/mL, through subsequent dilutions, we prepared one solution having 10 μg/mL (CU-10) and another with 1 μg/mL (CU-1). Afterward, we prepared four glass test tubes labeled QC-1000, QC-500, QC-50 and QC-10. We transferred in the first two 20 and 10 μL of CU-10 solution and in the third and fourth 10 and 5 μL of CU-1 solution. Solvent was evaporated by fluxing gently dry N_2 and soon after we added (200 μL of SPAHH) to each glass test tube. After 5 min of gentle stirring to ensure the total antibiotics solubilization, the four QCSs QC-1000, QC-500, QC-50 and QC-10 were each divided in 10 aliquots of 20 μL with the transferring in *eppendorf* vials of 1 mL each. Finally the remaining SPAHH volume (about 150 μL) was divided in some aliquots of 20 μL named QC-B and used as blank samples. All aliquots were frozen at −20 °C until the analysis time.

2.10. QC samples processing and analysis

Just before the analyses, samples of QCs were brought and conditioned at room temperature and directly added with 100 μL of mobile phase, thus 1:6 dilution was performed. After the vigorous agitation for 30 s by hand, the sample was centrifuged for 5 min at 1000 × *g* with a microfuge-18 Beckman. 30–35 μL of supernatant liquid was injected three times into the chromatographic apparatus. Thus, the CPUFX and UFX concentrations were deduced by using averaged values of peak areas.

2.11. Patients and samples collection

50 healthy patients (25 males and 25 females) aging from 61 to 87 years and having cataract surgery intervention were divided in two groups of 25 in a random manner. To all these patients were supplied two oral doses of FQNs, the first dose the day before the cataract intervention and the second dose just the day of surgery. The first group received two oral doses of 500 mg CPUFX, the second two oral doses of 500 mg PUFX, both in a time of 1–12 h before the surgery treatment. Just soon before surgery, 0.02–0.150 mL of AHH was aspirated by paracentesis. The samples were immediately transferred into pre-weighted *eppendorf* vials and stored at −20 °C until the analytic action.

2.12. Clinical samples processing and analysis

The AHH collected samples were brought back at room temperature and the coarse weight was measured. The density of aqueous humor practically that of water being 1000 g/mL [22], the weight and the volume of liquid coincide in their numerical values for samples put in the vials after the withdraw. The samples with less than 20 μL were discarded. 20 μL of AHH was put into eppendorf test tube and the samples were treated as described in the previous paragraph.

3. Results and discussion

3.1. Matrix sample volume

Antibiotics are taken from plasma, serum, milk, and urine starting with volumes of sample of order of 0.2–1 mL [8,9,23–25]. These matrices are easily withdrawn and are available in relatively abundant quantities. By contrast, AHH can be obtained only from voluntary and allowing patients, subjected to ophthalmology surgery. This fact limits the sample's availability very much. Moreover we have appreciated that the AHH volume to be withdrawn is highly variable among the patients with an average of 71 ± 40 μL. So, it was decided to fix the sample volumes at 20 μL and to discard samples not reaching this threshold.

3.2. Sample preparation

In comparison with plasma, serum, urine, or milk, the AHH is a matrix relatively poor of salts and proteins, being essentially constituted by water at 99% [22]. On the other hand, such an AHH characteristic allows avoiding complex pre-treatments to extract and deproteinize the sample in the step preceding HPLC analysis of the FQNs. Basci et al. have proposed a completely opposite treatment, which consisted of the simple dilution of AHH [15]. However, about 15–50% of FQNs is linked to proteins [1,3], and only the action of a denaturizing agents assures their complete solubilization.

As a mild way to precipitate proteins, the dilution with mobile phase followed by centrifugation step was proposed. This mixture is able to precipitate proteins, thanks to acetonitrile and the low pH of the aqueous layer, simultaneously ensuring solubilization of the drugs. In fact, as shown by the validation method, the accuracy in analyte recovery is more than satisfactory (Tables 3 and 4). Compared to other methods of preparation it is extremely simple and can significantly reduce the time of sample's elaborations. Moreover, the tube of sample analysis is the same of sample preparation: this eliminates the loss of analyte.

3.3. Selectivity

The selectivity was investigated analyzing the work solution F, the *blank* sample of AHH (QC-B) and the samples with CPUFX and UFX at 10 ng/mL conc. (QC-10). We tested a set of HPLC columns and Zorbax C_8 resulted as the most efficient. With columns C_{18} phase type the group of substances naturally present in the AHH showed a very good resolution but in a range of retention times that override that CUPFX and UFX. A similar result was obtained by Basci et al. who solved the peaks overlay by using a selective detector [15]. Instead, with Zorbax C_8 column, at the chromatographic conditions reported below, the retention time of the two FQNs was relatively shorter (5.7 and 7.8 min) with good peaks shape that did not interfere with those of substances naturally present in the AHH, as shown in Fig. 2.

3.4. Validation data

According to the criteria earlier mentioned, we have checked validation parameters analyzing appropriate QC and CS samples, as follows.

3.4.1. LOD and LLOQ

The LOD and LLOQ for the two FQNs determined by empirical method measured a series of dilution of the CSS, with mobile phase, and QCS, with SPAHH. LOD of 4 ng/mL for CPUFX and 5 ng/mL for UFX were the lowest concentrations that presented a %RDSs value that did not exceed 20%. The LLOQ of 8 ng/mL for CPUFX and 6 ng/mL for UFX were the lowest concentration that present an RDS that did not exceed 10%. These values were adequate for a correct quantification of two drugs in AHH.

3.4.2. Linearity and calibration data

The six calibration solutions B–G, soon after preparation, were injected in the HPLC instrument. Each concentration level was analyzed in triplicate fashion. Averaged peak-area values for CPUFX and UFX were directly correlated to the corresponding substance concentration. Table 1 collects the results concerning these analyses.

The r^2 correlation coefficient was 0.9999 for CPUFX and 0.9997 for UFX. Values of slope and intercept of the linear regression reported in Table 1 were used to calculate the concentration of the two FQNs in the unknown samples using the two following equations:

$$\text{CPUFX (ng/mL)} = \left(\frac{0.3189 \times \text{area CPUFX}}{1000} + 0.0797\right) \times 6,$$

$$\text{UFX (ng/mL)} = \left(\frac{0.527 \times \text{area UFX}}{1000} + 0.6917\right) \times 6$$

Being "6" the dilution factor in both cases.

Given considerable extent of the calibration range (two orders of magnitude), the homoscedasticity of the analytical method was evaluated with Cochran's test. In order to achieve homoscedasticity, the Cochran C of 4 standards with 3 replicates of each standard should be less than the critical values of 0.768 [26]. Since the largest and smallest values of variance usually appear at the extremities of the calibration curve in the heteroscedastic case, the two lowest concentrations (F and G) and the two highest concentration (B and C) standards were included in the tests. The results are shown in Table 2.

The two calibration curves pass the homoscedasticity test since the $C_{3,4}$ values were less than the critical value.

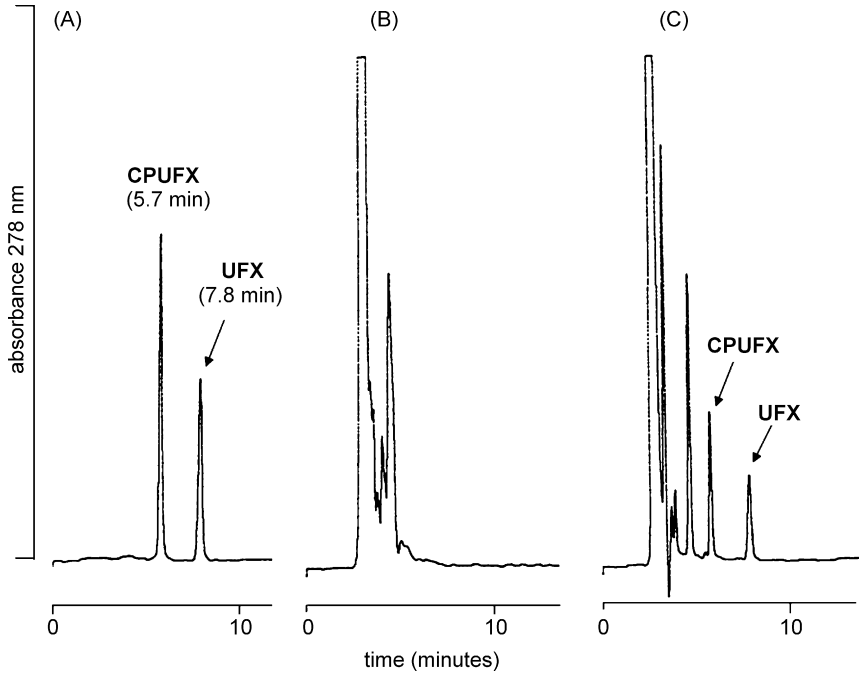

Fig. 2. Chromatograms of calibration solution 20 ng/mL (A); QC-B (B); QC-10 (C).

Table 1
Calibration data of CPUFX and UFX

Analyte	Work solution	Concentration (ng/mL)	Area (average)[a]	RDS%	Type	Slope	Intercept	r^2
CPUFX	B	500	1567	0.29	Linear	0.3189	0.0797	0.9999
	C	380	1191	0.00				
	D	250	783	0.20				
	E	125	400	1.26				
	F	41.6	123	1.65				
	G	4.16	14	8.69				
UFX	B	600	1136	0.24	Linear	0.527	0.6917	0.9997
	C	450	852	0.00				
	D	300	578	1.20				
	E	150	297	0.73				
	F	50.2	84	7.41				
	G	5.02	12	3.09				

[a] $n = 3$, the area value was divided by 1000.

Table 2
Cochran's test results

Concentration (ng/mL)	S.D.	Square of S.D.	Sum of square S.D.[a]	C[b]
Calibration data of CPUFX				
500	5.590	31.243	42.203	0.740
380	1.617	2.615		
41.6	2.489	6.194		
4.16	1.466	2.151		
Calibration data of UFX				
600	3.396	11.530	41.376	0.682
450	1.196	1.430		
50.2	5.312	28.222		
5.02	0.440	0.194		

[a] Standard deviation of the three replicates.
[b] C: Cochran's C.

Table 3
Repeatability by intra-day accuracy and precision observed with QC samples

Analyte	Concentration added (ng/mL)	Concentration found (ng/mL) ± S.D.	Accuracy[a] (%)	Precision[a] (%RDS)
CPUFX	1000	997 ± 16	99.7	1.6
	500	493 ± 6	98.6	1.2
	50	49 ± 4	98.4	8.2
	10	9.2 ± 0.6	91.6	6.2
UFX	1000	994 ± 19	99.4	1.9
	500	487 ± 10	97.4	2.1
	50	48 ± 2	95.6	5.0
	10	9.3 ± 0.5	92.5	5.7

[a] $n = 5$.

Table 4
Reproducibility by inter-day accuracy and precision observed with QC samples

Analyte	Concentration added (ng/mL)	Concentration found (ng/mL) ± S.D.	Accuracy[a] (%)	Precision[a] (%RDS)
CPUFX	1000	978 ± 22	97.8	2.2
	500	472 ± 11	94.4	2.3
	50	48 ± 5	96.4	9.8
	10	8.6 ± 0.7	86.2	8.0
UFX	1000	981 ± 10	98.1	1.0
	500	464 ± 6	92.9	1.2
	50	47 ± 3	94.8	6.9
	10	8.3 ± 1.3	84.0	13.1

[a] $n = 5$.

3.4.3. Determination of repeatability and reproducibility

Repeatability and reproducibility of the method was tested with intra-day and inter-day (until 25–45 days) experiments, determining accuracy and precision in the analyses of five QCSs as described below: five samples of QC-1000, QC-500, QC-50 and QC-10 were subjected to intra-day analysis. The remaining samples were analyzed within 25–45 days to test long-term parameter control. The first set of experiments allowed determining the method's repeatability and the second the method's reproducibility through the measure of accuracy and precision. Table 3 collects data for first set of analyses whilst Table 4 reports data of the second set.

The tables show that in both cases the method of analysis reached the required levels of accuracy and precision.

The goodness of the obtained results on validation procedure enabled us to apply the proposed method to the clinical study of comparison of CPUFX and UFX FQNs permeability in the AHH.

3.5. Clinical applications

As a preliminary application, we have used this analytic method to measure the concentration of CPUFX and PUFX in AHH of 45 patients treated with these two antibiotics. This study is described below. Our results are given in Tables 5 and 6 which refer to data of analyses performed on samples collected in the Oculist Clinical of the Perugia University, following the procedures described in detail through the above paragraphs. Samples having volume lower than 20 μL were not included among those subjected to the analysis. Only sample corresponding to patient labeled with number 9 resulted lower than 20 μL and was then discarded.

Table 5
CPUFX concentration in AHH

Sample	Body weight of patient (kg)[a]	CPUFX (ng/mL)	±S.D.[b]
1	66	156	2
2	54	233	17
5	–	189	7
6	60	148	6
8	80	96	1
11	–	105	9
12	60	18	7
15	84	152	3
17	62	32	5
18	68	505	9
22	84	252	6
23	65	259	3
24	52	125	4
25	80	173	2
28	76	38	8
29	–	258	4
30	76	118	7
31	72	62	5
32	58	99	4
36	62	109	2
37	55	498	7
38	–	58	6
42	80	291	4
45	–	153	6
46	–	514	2
Average		186	5.4

Average sample volume: 80 μL.
[a] Data not collected for all patients.
[b] $n = 3$.

Table 6
UFX concentration in AHH

Sample	Body weight of patient (kg)[a]	UFX (ng mL^{-1})	±S.D.[b]
3	64	20	3
4	80	43	3
7	70	17	5
9	90	Not determined[c]	–
10	–	14	4
13	67	5	3
14	70	104	4
16	65	152	4
19	62	42	3
20	80	148	4
21	50	59	2
26	57	124	4
27	58	78	3
33	68	31	5
34	65	57	7
35	75	22	9
39	80	33	5
41	61	24	5
43	95	13	8
44	115	40	4
89	–	133	8
90	–	194	5
91	–	169	8
31	72	294	9
Average		78	5.2

Average sample volume: 75 μL.
[a] Data not collected for all patients.
[b] $n = 3$.
[c] The sample volume was less than 20 μL.

The average concentration of CPUFX in AHH of patients supplied with two oral doses of 500 mg is equal to 186 ng/mL (number of cases = 25). The average concentration of UFX in AHH of patients treated with two 500 mg oral doses of PUFX is equal to 78 ng/mL (number of cases = 24). No remarkable correlation was observed between body weight of patients and FQNs concentration in AHH.

Tables 5 and 6 show that the concentration of CPUFX and UFX in AHH varies also 10-fold between patients. This data suggest that, in accordance with pharmacokinetic studies in human plasma of two fluoroquinolones [1,23], the concentration in AHH subsequent oral administration, after a maximum, decrease rapidly. In this work the data time between oral administration and subsequent sampling was not collected but it was within 1–24 h. This time is much higher than the long half-life of these fluoroquinolones in human plasma, which is about 4–10 h, whatever the dose. Future pharmacokinetics study of this fluoroquinolones in AHH will be necessary for clarifying these aspects.

Moreover, Tables 5 and 6 show that CPUFX presents an average concentration of about 2.4 times higher than UFX. This was not expected because it is inconsistent with the data available in the literature showing a concentration of plasma UFX similar to that of CPUFX after oral administration. This can be explained or with a less penetration velocity of UFX into AHH or in a more rapid excretion of the drug respects the CPUFX. Further inquiries on the concentration of UFX in AHH must take into account these results.

4. Conclusions

4.1. Analytical methods

The procedure described in the present article represents a validated HPLC-based method to detect Ciprofloxacin and Ulifloxacin, the latter being the active metabolite of Prulifloxacin, in the aqueous human humor of patients in surgery cataract treatment.

The sample preparation is simple, inexpensive and rapid. It permits the elaboration of a large number of samples in a relatively shorter time compared to another method. In fact the typical laborious deproteinization procedure has been replaced by a simple dilution of the sample with mobile phase and subsequent centrifugation in the same analysis tube. This allows minimizing the loss of analyte. The small and very variable volume of AHH withdrawn from each patient (20–150 μL) does not constitute a limit at the whole sensitivity.

The use of non-selective detector UV does not affect the result thanks to chromatografic conditions that allow a very good separation of the two antibiotics from the other substances naturally dispersed in the aqueous human humor.

It is worthy to notice that HPLC separation procedure is very simple and can be easily reproduced because it has been performed under isocratic conditions by using an extremely simple eluant (acetonitrile/phosphoric acid). Due to its features the present method is suitable for the pharmacokinetic studies of CPUFX and UFX in human healthy volunteers.

4.2. Clinical results

The developed method allows the comparative assessment of the two drugs in AHH by *in vivo* experiments: the comparative assessment of penetration of CPUFX and UFX in the AHH allowed establishing that, as a consequence of an equal oral administration, CPUFX presents an average concentration about 2.4 times higher than UFX. These results, although preliminary, together with the pharmacologic properties of Prulifloxacin should be taken in serious consideration in the clinical applications of this drug.

Acknowledgments

We thank Angelini S.p.A. for gently supplying of UFX sample and Dr. L. Vaccaro of University of Perugia for his support and encouragement during the development of our analytical procedure.

References

[1] M.G. Matera, Pulm. Pharmacol. Ther. 19 (2006) 20–29.
[2] M. Cazzola, E. Salvatori, P. Dionisio, L. Allegra, Pulm. Pharmacol. Ther. 19 (2006) 30–37.
[3] F. Belal, A.A. Majed, A.M. Al-Obaid, Talanta 50 (1999) 765–786.

[4] L. Guo, M. Qi, X. Jin, P. Wang, H. Zhao, J. Chromatogr. B 832 (2006) 280–285.
[5] G. Carlucci, J. Chromatogr. A 812 (1998) 343–367.
[6] E. Turiel, G. Bordin, A.R. Rodríguez, J. Chromatogr. A 1008 (2003) 145–155.
[7] M.C. García-Sáenz, A. Arias-Puente, M.J. Fresnadillo-Martinez, C. Carrasco-Font, J. Cataract Refract. Surg. 27 (2001) 1969–1974.
[8] O.R. Idowu, J.O. Peggins, J. Pharm. Biomed. Anal. 35 (2004) 143–153.
[9] H.J. Mascher, C. Kikuta, J. Chromatogr. A 812 (1998) 381–385.
[10] G.S. Rao, S. Ramesh, A.H. Tripathi, L.D. Sharma, J.K. Malik, Vet. Res. Commun. 25 (2001) 197–204.
[11] K.M. Sowinski, M.B. Kays, J. Clin. Pharm. Ther. 29 (2004) 381–387.
[12] N.E. Basci, S. Hanioglu-Kargi, H. Soysal, A. Bozkurt, S.O. Kayaalp, J. Pharm. Biomed. Anal. 15 (1996) 663–666.
[13] R. Solomon, E.D. Donnenfeld, H.D. Perry, R.W. Snyder, C. Nedrud, J. Stein, A. Bloom, Ophthalmology 112 (2005) 466–469.
[14] J.P. McCulley, D. Caudle, J.D. Aronwicz, W.E. Shine, Ophthalmology 113 (2006) 955–959.
[15] N.E. Basci, A. Bozkurt, D. Kalayci, S.O. Kayaalp, J. Pharm. Biomed. Anal. 14 (1996) 353–356.
[16] I.S. Yalvac, N.E. Basci, A. Bozkurt, S. Duman, J. Cataract Refract. Surg. 29 (2003) 487–491.
[17] F.A. Bucci Jr., Am. J. Ophthalmol. 137 (2004) 308–312.
[18] A.A. Madu, M. Mayers, R. Perkins, W. Liu, G.L. Drusano, R. Aswani, C.N. Madu, M.H. Miller, Exp. Eye Res. 63 (1996) 129–136.
[19] O. Masao, M. Masuya, A. Tatsuya, O. Yoshio, Atarashii Ganka 14 (1997) 1055–1058.
[20] G.C. Hokanson, Pharm. Technol. 18 (1994) 118–130.
[21] V.P. Shaw, et al., Eur. J. Drug Metab. Pharmacokinet. 16 (1991) 249–255.
[22] A. Eriksson, B. Svedbergh, Ger. J. Ophthalmol. 212 (1980) 187–197.
[23] M. Kamberi, K. Tsutsmi, T. Kotegawa, K. Nakamura, S. Nakano, Clin. Chem. 44 (1998) 1251–1255.
[24] S. Imre, M.T. Dogaru, C.E. Vari, T. Muntean, L. Kelemen, J. Pharm. Biomed. Anal. 33 (2003) 125–130.
[25] G.J. Krol, G.W. Beck, T. Benham, J. Pharm. Biomed. Anal. 14 (1995) 181–190.
[26] D.L. Massart, B.G.M. Vandenginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997.