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Simultaneous determination of enrofloxacin and ciprofloxacin in animal biological fluids by high-performance liquid chromatography Application in pharmacokinetic studies in pig and rabbit

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Abstract

A simple and rapid high-performance liquid chromatographic method for the simultaneous determination of enrofloxacin and ciprofloxacin has been developed in pig and rabbit samples. Solid-phase extraction was applied from samples on a C_{18} cartridge using a mixture of methanol–hydrochloric acid (98:2, v/v). Analytical separation was performed on a C_{18} column with UV detection at 277 nm under gradient conditions. The mobile phase was a mixture of orthophosphoric acid–triethylamine–acetonitrile. The method has been validated for both molecules in pig and rabbit plasma and adapted for rabbit tissue-cage fluid (TCF). The assay is specific and reproducible within the both drugs and mean recoveries for ciprofloxacin and enrofloxacin, respectively, were 92±6% and 90±5% for pig plasma over the range used. Mean recoveries for enrofloxacin were 108±9% and 102±7% for rabbit plasma and TCF, respectively, over the range used. The suitability of the assay for pharmacokinetic studies was determined by measuring enrofloxacin and ciprofloxacin concentrations either in pig plasma after administration of a single intravenous 5 mg/kg dose of enrofloxacin or in rabbit plasma and TCF during a 24 h infusion of enrofloxacin at a rate of 1.25 mg/kg per hour.

Keywords: Enrofloxacin; Ciprofloxacin

1. Introduction

Enrofloxacin is a fluoroquinolone with broad antibacterial spectrum and high bactericidal activity against major pathogenic bacteria found in diseased animals [1,2]. Enrofloxacin is used in veterinary medicine in cattle, pigs, poultry, dogs and cats. Efficacy and tolerance in rabbits and fish are currently being evaluated [3]. In pigs, enrofloxacin can be used to treat bronchopneumoniae. To study the pharmacokinetic of enrofloxacin in pigs, the first objective was to develop a sensitive, specific and rapid assay to quantify enrofloxacin in plasma of treated pigs. Because ciprofloxacin is the active metabolite of enrofloxacin in several species [4,5], the assay must also be capable of distinguishing between these drugs. To study the penetration abilities of antimicrobial drugs, several investigators were used a tissue cage model, in which a pool of tissue fluid is created by implanting a cage subcutaneously in an experimental animal [6–9]. Our second objective was to use this model in rabbit to study the

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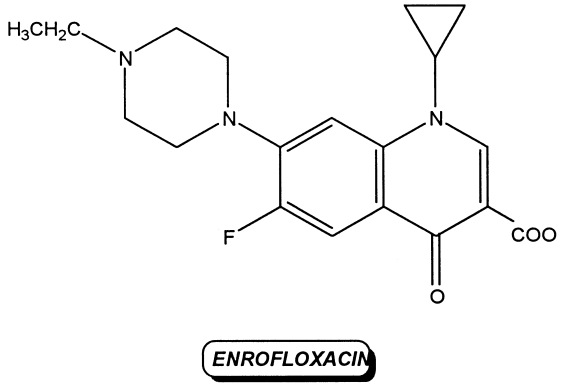
distribution of enrofloxacin in the extravascular liquid. Several methods for detection and quantification of enrofloxacin or ciprofloxacin in biological fluids and tissues have been developed. These methods included microbiological assay [1], reversed-phase high-performance liquid chromatography (HPLC) with UV or fluorescence detection. Some papers compared microbiological assay and HPLC [10–12]. Kung and Riond [10] found no correlation between HPLC and bioassay methods at high enrofloxacin concentrations because ciprofloxacin, the main metabolite of enrofloxacin in dog plasma, influenced bioassay results. Some HPLC methods have been reported for the analysis of either enrofloxacin [13–15] or ciprofloxacin [16,17] in biological tissues or fluids. Fewer methods were developed for used in determining both enrofloxacin and ciprofloxacin in biological tissues or fluids such as bovine and porcine muscle [18], fish muscle, liver and skin [19], fish, chicken, veal and pork tissues [20], canine serum and prostatic tissue [4] or bovine milk and plasma [21,22]. A simultaneous determination of eight quinolones in meat and fish was recently described by Horie et al. [23], a procedure used earlier for enrofloxacin. However, none of these methods appear satisfactory for the rapid and simultaneous determination of enrofloxacin and ciprofloxacin in small samples of pig plasma and rabbit plasma and tissue-cage fluid (TCF) from pharmacokinetic studies.

The purpose of the present study was to develop a simple, rapid and reliable analytical procedure for detection and quantification of enrofloxacin and ciprofloxacin in plasma samples using a high-performance liquid chromatographic assay with a reversed-phase C_{18} column and specific UV detection. One-step sample preparation procedure involved the use of solid-phase extraction (SPE) with an acidified organic solvent. This method was validated as indicated in the recommendations of analytical methods validation for bioavailability, bioequivalence and pharmacokinetic studies in man and animals [24]. The parameters essential to ensure the acceptability of the performance of an analytical method are stability of the drug in the matrix under study storage conditions, accuracy, precision, sensitivity, specificity, response function and reproducibility [24]. Therefore, this analytical procedure allows the routine analysis of samples and was used to determine the pharmacokinetic profiles of enrofloxacin and ciprofloxacin in pig plasma and in rabbit plasma and TCF.

2. Experimental

2.1. Chemicals and reagents

Enrofloxacin and ciprofloxacin were kindly supplied by Bayer (Leverkusen, Germany). The chemical structure of these compounds is illustrated in Fig. 1. The following quinolones were used for specificity study: flumequine (Sanofi, France), oxolinic acid



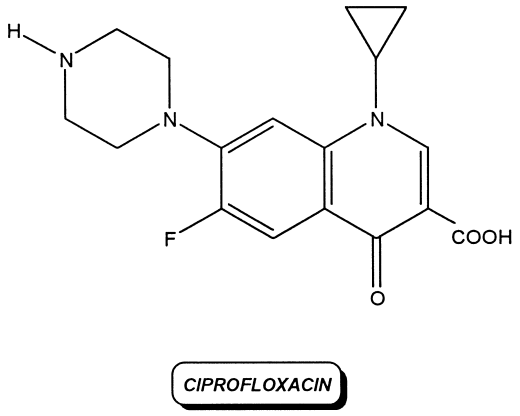


Fig. 1. Chemical structures of enrofloxacin and ciprofloxacin.

(Sigma, USA), nalidixic acid (Sigma), marbofloxacin (Vetoquinol, France), norfloxacin (Sigma), danofloxacin mesylate (Pfizer, France), sarafloxacin (Solvay Duphar, The Netherlands), difloxacin chlorhydrate (Solvay Duphar). Acetonitrile was HPLC-grade (Merck, Nogent-sur-Marne, France). Methanol, triethylamine, orthophosphoric acid and chlorhydric acid were all analytical-reagent grade (Merck). The water was purified by Milli-Q reverse osmosis (Millipore, Milford, MA, USA).

2.2. Chromatography

The HPLC system consisted of a Varian Model 9010 solvent delivery system, a Varian Model 9050 UV detector operating at 277 nm, a Varian Star computing program (Varian, Les Ulis, France) and a Merck Model AS2000 autosampler provided with a Rheodyne vanne and a 200-μl sample loop. Analytical separation of drugs was achieved on a 125×4 mm I.D., 5 μm, prepacked Lichrospher RP18 end-capped column (Merck), protected by a 4×4 mm guard column (Merck). The mobile phase was a mixture of 0.02 *M* orthophosphoric acid–0.008 *M* triethylamine (1:1, v/v) (eluent A) and acetonitrile (eluent B) [18]. At $t=0$, the mixture consisted of A–B (90:10), which changed linearly in 9 min to A–B (69:31). The system remained stable during 2 min and returned to its initial state in 0.5 min, and finally remained stable during 3.5 min. Total run time was 15 min. The flow-rate was 1 ml/min, and drugs were quantified by measuring the peak area. Sample preparation and analysis were performed in a air-conditioned laboratory (22°C).

2.3. Drug standards

Stock standard solution of enrofloxacin was prepared by dissolving 25 mg in 25 ml of methanol in an ultrasonic bath and adjusting to a final volume of 50 ml with water. Stock standard solution of ciprofloxacin was obtained by dissolving 25 mg in 2.5 ml of orthophosphoric acid in an ultrasonic bath and then adjusting to a final volume of 50 ml with water. These solutions were stored at +4°C in brown glass vessels. Working solutions (0.05, 0.1, 0.5, 1, 2.5, 5, 10 μg/ml) were daily prepared by mixing and diluting the stock solutions in water containing 6.7% of methanol–hydrochloric acid (98:2, v/v). Solutions for sample spiking (0.5, 1, 5, 10, 25, 50, 100 μg/ml) were prepared by mixing and diluting the stock solutions in water.

2.4. Spiked biological fluids

Drug-free pooled plasma or TCF were prepared from a live pig or rabbit. Spiked standards were prepared from 0.05 μg/ml to 10 μg/ml by adding appropriate amounts of enrofloxacin and ciprofloxacin to drug-free pooled plasma or TCF. After 15 min of gentle mixing, spiked standards were shared out in fractions and stored at −20°C.

2.5. Sample preparation

The sample preparation procedure consisted of a SPE. Sep-Pak C_{18} (100 mg) cartridges (Waters, Millipore) were previously activated with 2 ml of methanol and then with 2 ml of purified water before use. Pig plasma samples were thawed out at room temperature and centrifuged at 2000 *g* for 5 min. Supernatant (0.5 ml) was applied to a C_{18} cartridge followed by 0.25 ml of water. After washing with 0.5 ml of water and then 0.5 ml of acetonitrile, the cartridge was properly dried and eluted with 0.1 ml methanol containing 2% of hydrochloric acid. The elution was completed by 1.4 ml of water. The mixture was transferred to autosampler vials.

For rabbit plasma and TCF, the same method was used with, respectively, 0.1 ml of rabbit plasma or 0.05 ml of TCF. Elution was performed with 0.15 ml methanol containing 2% of hydrochloric acid and completed by 1.35 ml of water.

2.6. Calibration and calculation

Analyses of drug-free plasma or TCF spiked with known amounts of enrofloxacin and ciprofloxacin were carried out applying the procedure described above. The sample concentration was calculated by comparison of peak area with the peak area of a nominal concentration of an external standard and corrected by the concentration factor and the recovery rate.

Linear regression of the peak-area ratios as a function of the theoretical concentrations was applied

to each standard curve and spiked curve over the range used (0.05–10 μg/ml for pig plasma and 0.05–5 μg/ml for rabbit plasma). Intra-assay reproducibility was determined for calibration curves prepared in duplicate on the same day using the same stock solutions. The linearity of the method was confirmed using the classical tests by analysis of variance.

Stability of stock standard solutions at +4°C has been verified during two weeks. Stability of 1 μg/ml spiked plasma at −20°C has been verified for ciprofloxacin and enrofloxacin during the samples storage period (two weeks). The influence of three freeze–thaw cycles has been tested in triplicate on 1 μg/ml spiked plasma.

Selectivity of the method against enrofloxacin and ciprofloxacin was verified by comparison with others quinolones (flumequine, oxolinic acid, nalidixic acid, marbofloxacin, norfloxacin, danofloxacin, sarafloxacin, difloxacin).

2.7. Precision and accuracy

Inter- and intra-day repeatabilities in pig plasma were assessed by performing triplicate analysis of spiked plasma with enrofloxacin and ciprofloxacin (0.05, 1, 10 μg/ml) against standard solution, on different days ($n=4$). Inter- and intra-day repeatabilities in rabbit plasma were assessed by performing duplicate analysis of spiked plasma with enrofloxacin and ciprofloxacin (0.05, 1, 5 μg/ml) against standard solution, on different days ($n=3$). The accuracy was expressed as percent deviation of observed concentration from theoretical concentration.

2.8. Recovery

The extraction efficiency (recovery) was determined for the two compounds by comparing peak areas from drug-free samples spiked with known amounts of drugs (in the range of concentrations of the calibration curves) and standard solutions in the mobile phase, injected directly into the analytical column. Each sample was determined in duplicate.

2.9. Limit of quantification and limit of detection

The limit of detection (LOD) is the smallest concentration from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. The LOD is equal to the mean of the measured content of representative blank samples plus three-times the standard deviation of the mean [25].

The limit of quantification (LOQ) in pig and rabbit samples is the lowest concentration from which it is possible to quantify the analyte with reasonable statistical certainty. The LOQ is equal to the mean of the measured content of representative blank samples plus ten-times the standard deviation of the mean. The LOQ can also correspond to the smallest validated concentration within the limits of precision and accuracy set for the method (CV≤15% of precision, ±15% of accuracy).

2.10. Applicability to pharmacokinetic studies

To test the suitability of the assay for pharmacokinetic studies, a single bolus intravenous (i.v.) dose of 5 mg/kg b.w. of enrofloxacin was administered to five crossbreed pigs (two females and three males weighing 30.4±4.0 kg, two months old). Serial blood samples (5 ml) were collected into heparinized tubes at different times after the i.v. administration via a catheter previously implanted in the left jugular vein by surgery. Plasma was immediately separated by centrifugation at 1500 *g* for 15 min, and samples were stored at −20°C until analysis.

This HPLC method has been applied to quantify enrofloxacin and ciprofloxacin secondly in plasma and TCF of four rabbits (New Zealand White females weighing 2.25–2.50 kg, two months old). Four sterile silicone (Silastic, Prolabo, France) tubes (40×8 mm I.D.) perforated by regularly spaces 1-mm diameter holes were aseptically implanted subcutaneously into the flanks as previously described [7]. The experiment was started three weeks after surgery when the interstitial fluid that had accumulated in tissue cages was stable. Rabbits received a 24 h infusion of enrofloxacin at a rate of 1.25 mg/kg per hour by a catheter implanted in the central vein

of the ear. A second catheter was inserted in the opposite ear to take blood (0.5 ml) at different times during and after the end of infusion. Plasma was recovered after centrifugation at 1500 *g* for 15 min, and samples were stored at −20°C until analysis. Capsular fluid (200 μl) was taken for enrofloxacin assay at different times during and after the end of infusion, and samples were stored at −20°C until analysis.

3. Results and discussion

Results of validation for pig and rabbit plasma were presented. This method was applied for rabbit tissue-cage fluid after having verified the linearity, recovery, accuracy and precision (through lack of samples). Results of recovery and limits of detection and quantification were only presented for rabbit TCF.

3.1. Sample preparation and HPLC separation

Initially the procedure of Charriere et al. [20] was attempted, utilizing a gradient method with orthophosphoric acid and acetonitrile as phase mobile using a conventional C_{18} column allowed the separation of enrofloxacin and ciprofloxacin. Moreover, both molecules appeared as separated peaks without tailing with triethylamine. It is generally known that quinolone derivatives give severely tailing peaks in reversed-phase chromatography, but this can be reduced by using mobile phases which have a high ionic strength or high acidity [11]. As a result, a prepacked Lichrospher RP18 end-capped column protected by a guard column was chosen with a mixture of 0.02 *M* orthophosphoric acid–0.008 *M* triethylamine (1:1, v/v) and acetonitrile as the mobile phase under gradient conditions. This separation was simple (UV detection), rapid (observed retention times about 8.9 and 9.7 min for ciprofloxacin and enrofloxacin, respectively), and efficient (resolution between the two compounds of 4.6). In addition, this method was selective between the following quinolones: flumequine, oxolinic acid, nalidixic acid, marbofloxacin, norfloxacin, danofloxacin, sarafloxacin and difloxacin; and was not expansive using a conventional C_{18} column and polar solvent as phase mobile.

Due to the simple nature of the plasma matrix, one-step sample preparation was used contrary to tissues or milk matrix in the literature [18–22]. The sample preparation procedure consisted of a solid-phase extraction easier to use for plasma matrix and little volumes of samples were applied on cartridge. On the contrary, liquid–liquid extraction was time-consuming sample preparation, more expansive because of great solvent volume and such multiple extraction steps cause loss for molecules with low recoveries for ciprofloxacin [18,20].

For the SPE step, the C_{18} bonded phase, a non-polar sorbent widely used, was chosen because of an high retention capacity for both enrofloxacin and ciprofloxacin. Others sorbents like phenyl and more polar phases were not tested because of the good results of the Sep-Pak C_{18} cartridge, as Brinkmann et al. [19] noticed. This solid-phase sorbent resulted in improvement recoveries and elimination of interfering substances.

For the choice of the extractant, several solvents or mixtures of organic solvents in acidic medium were tested. Extractions with phosphoric acid alone or a mixture of orthophosphoric acid–triethylamine–acetonitrile, as in the mobile phase, did not lead to elution of the two compounds. Extraction with acetonitrile–phosphoric acid (1:9, v/v) was not effective enough with elution of around 40% of both molecules, while elution with acetonitrile alone led to retention of both molecules. So acetonitrile at 100% was used like an washing solvent and led to improved drying of the cartridge before the elution. The clean-up procedure consisted of a sequence of washing in water and acetonitrile, very effective and original prior to elution, and led to reduced amounts of contaminants, because lots of molecules are soluble either in water or in acetonitrile alone, but not enro- and ciprofloxacin.

Severals extractants also studied by Brinkmann et al. [19]: dichlormethane, methanol and ethanol, were suitable for enrofloxacin (recovery approximately 70%) but not for ciprofloxacin (recovery maximum 30%). Ethanol acetic acid (1%) as extractant resulted also in good recoveries for ciprofloxacin [19]. So, elution of the cartridge with an acidified organic

solvent was rapidly imposed on us because enrofloxacin was soluble in organic solvent and ciprofloxacin was soluble in acid solution. Experimentally, we have determined the mixture of organic solvent in acidic medium: 0.1 ml methanol containing 2% of hydrochloric acid, which resulted in the best recoveries (above 90%) for each drug.

Direct injection (without evaporation) of the eluate allowed us to complete the elution using a greater volume of water. Moreover, evaporation of the eluate was time-consuming and not necessary with high antibiotic concentrations in pharmacokinetic studies, contrary to screening methods for the determination of antibiotics in animal tissues [18–20]. The extraction and clean-up procedure, without evaporation, was rapid and more samples can be analysed simultaneously. The extraction procedure was also applied to small volumes of TCF.

In other developments, the extraction procedure of enrofloxacin and ciprofloxacin were improved for complex matrix such broth medium, for example, used to study the in vitro action of antibiotics against bacteria. We successfully tested the capacity of other sorbents such as Sep-Pak tC2 and Diol cartridges, with recoveries around 100% and little interfering substances.

Chromatograms obtained after extraction of pig plasma and rabbit plasma and TCF under the established conditions are shown in Figs. 2 and 3. As shown in this chromatograms, the chromatographic method is suitable for the complete separation and quantification of both molecules. The drug-free sample did not contain substances that would interfere at retention times of the respective analytes.

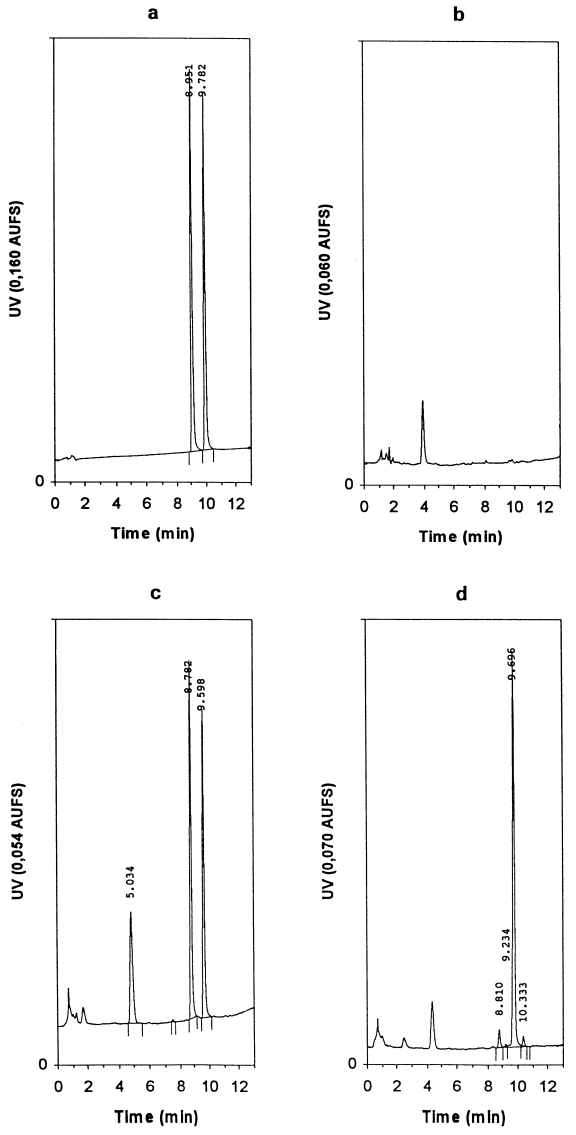


Fig. 2. Chromatograms of standard solution at 1 μg/ml of ciprofloxacin and enrofloxacin (a), drug-free pig plasma (b), pig plasma spiked with 1 μg/ml of ciprofloxacin and enrofloxacin (c) and pig plasma from one treated animal after the i.v. administration of 5 mg/kg of enrofloxacin (d).

3.2. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD estimated by measuring the response of 12 drug-free pig plasma samples was determined to be 0.019 and 0.021 μg/ml for ciprofloxacin and enrofloxacin, respectively. The LOD for drug-free rabbit samples was only determined for enrofloxacin and was determined to be 0.010 and 0.050 μg/ml for plasma ($n=8$) and TCF ($n=57$), respectively.

The LOQ for pig plasma estimated by measuring the response of 12 drug-free pig plasma samples was determined to be 0.040 and 0.044 μg/ml for ciprofloxacin and enrofloxacin, respectively. The LOQ estimated by the smallest validated concentration within the limits of precision and accuracy set for the method was determined to be 0.050 μg/ml for the

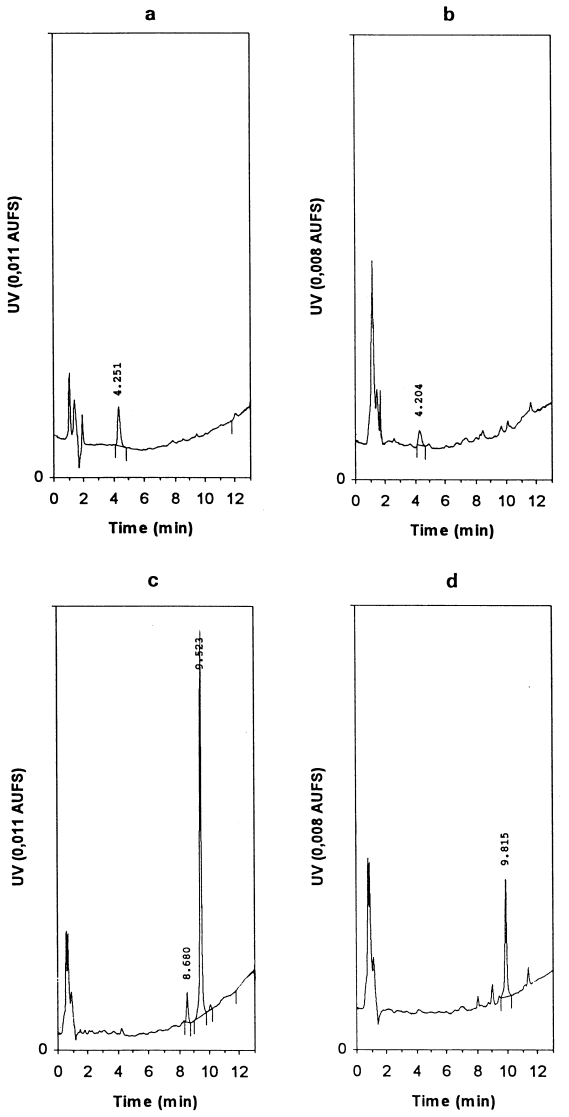


Fig. 3. Chromatograms of drug-free rabbit plasma (a) and TCF (b). Chromatograms of rabbit plasma (c) and TCF (d) from one treated animal during the i.v. infusion of enrofloxacin.

both molecules. For the purpose of enrofloxacin depletion in the pig plasma during a pharmacokinetic study, a quantitative limit of 0.050 μg/ml is considered satisfactory. The LOQ for rabbit samples was determined for enrofloxacin to be 0.020 and 0.120 μg/ml for plasma (n=8) and TCF (n=57), respectively.

3.3. Assay validation

The calibration standards (0.050 to 10 μg/ml) for pig plasma gave correlation coefficients of 0.9999 for both drugs and, for spiked plasma over the same range correlation coefficient were 0.9986 and 0.9979 for enrofloxacin and ciprofloxacin, respectively. In plasma samples, the peak area ratios of enrofloxacin and ciprofloxacin varied linearly with concentrations over the range used. Relationships between observed concentrations and theoretical concentrations for pig plasma are shown in Table 1 for enrofloxacin and ciprofloxacin. The intra-day average slope of the fitted straight lines, the correlation coefficient and the mean intercept are presented in these tables. The results in this Table show that enrofloxacin and ciprofloxacin are determined with good precision for both pig and rabbit plasma.

The accuracy, repeatability and reproducibility of enrofloxacin and ciprofloxacin in pig and rabbit plasma are shown in Table 2 for the three tested concentrations representing the entire range of the calibration curve (low, medium and high concentrations). For pig plasma, repeatability of the method did not exceed 6.4 and 5.0% for enrofloxacin and ciprofloxacin, respectively; and the reproducibility of the method did not exceed 8.1 and 10.2% for enrofloxacin and ciprofloxacin, respectively. For rabbit plasma, the repeatability of the method did not exceed 2.6 and 2.0% for enrofloxacin and ciprofloxacin, respectively; and the reproducibility of the method did not exceed 3.5 and 3.3% for enrofloxacin and ciprofloxacin, respectively.

The recoveries obtained for pig plasma were stable over the range used (0.05–10 μg/ml). They varied from 84.1 to 99.5% for ciprofloxacin and from 83.4 to 98.9% for enrofloxacin. The mean recovery was 91.5±6.3% for ciprofloxacin and 89.6±5.5% for enrofloxacin. The mean recovery for rabbit samples, determined for enrofloxacin, was 108.5±8.9% and 102.5±6.9% for plasma and TCF, respectively.

3.4. Pharmacokinetic studies

The plasma concentration–time profile after a bolus i.v. dose of 5 mg/kg enrofloxacin to a pig is shown in Fig. 4. The small amounts of ciprofloxacin below the limit of the quantification were not

Table 1
Calibration curves[a] of enrofloxacin and ciprofloxacin for the range concentrations of 0.05–10 μg/ml in standard, spiked pig plasma and their estimated concentrations corrected by the recovery rate

	Enrofloxacin	Ciprofloxacin
Standard	$y=152\,280.20x+24\,791.02$	$y=168\,796.44x+16\,257.07$
Spiked plasma	$y=138\,937.91x-1366.76$	$y=165\,256.42x-42\,249.62$
Estimated concentration	$y=1.0148x-0.0524$	$y=1.0290x-0.0828$
r[b]	0.9986	0.9979

[a] Each curve was constructed based on seven concentration levels. Peak area (y) was expressed as a linear function of the antibiotic concentration (x, μg/ml): $y=ax+b$ where a corresponded to slope and b, the intercept.
[b] r: Coefficient of the linear regression analysis.

represented. The single one above the LOQ at 0.5 h was corresponded to less than 4% of the total. In pigs, residues in tissues consisted mainly of enrofloxacin. Twelve hours after the intravenous administration, the plasma concentration of enrofloxacin was 94.3±21.7 ng/ml which corresponded to a rapid elimination.

Plasma and TCF concentration–time profiles of enrofloxacin in four rabbits during and after a 24 h infusion of enrofloxacin at 1.25 mg/kg per hour are presented in Fig. 5. In rabbits, like in pigs, enrofloxacin was very slightly metabolised to ciprofloxacin.

4. Conclusions

We have described an HPLC method for analysing both enrofloxacin and ciprofloxacin in little sample volume of pig plasma and rabbit plasma and TCF. This method is in accordance with guidelines for

Table 2
Repeatability (CV_r), reproducibility (CV_R) and accuracy of enrofloxacin and ciprofloxacin from pig and rabbit plasma[a]

	Enrofloxacin		Ciprofloxacin	
	Pig	Rabbit	Pig	Rabbit
Low concentration				
n	10	6	12	6
Measured concentration (mean±SD) (μg/ml)	0.055±0.004	0.055±0.002	0.053±0.005	0.049±0.002
CV_r (%)	4.4	2.6	5.0	2.0
CV_R (%)	7.4	3.5	10.2	3.3
Accuracy (%)	110.3	110.7	107.1	97.9
Medium concentration				
n	11	6	11	6
Measured concentration (mean±SD) (μg/ml)	0.99±0.08	0.93±0.02	0.96±0.02	0.99±0.01
CV_r (%)	6.4	0.8	2.1	0.6
CV_R (%)	8.1	2.1	2.1	1.2
Accuracy (%)	99.2	92.9	96.0	98.6
High concentration				
n	12	6	12	6
Measured concentration (mean±SD) (μg/ml)	9.52±0.17	4.82±0.06	9.42±0.35	5.17±0.06
CV_r (%)	1.8	0.9	3.7	0.3
CV_R (%)	1.8	1.2	3.7	1.1
Accuracy (%)	95.2	96.4	94.2	103.5

[a] n: Number of analysis during the study of the precision; coefficient of variation of repeatability (CV_r) and reproducibility (CV_R).

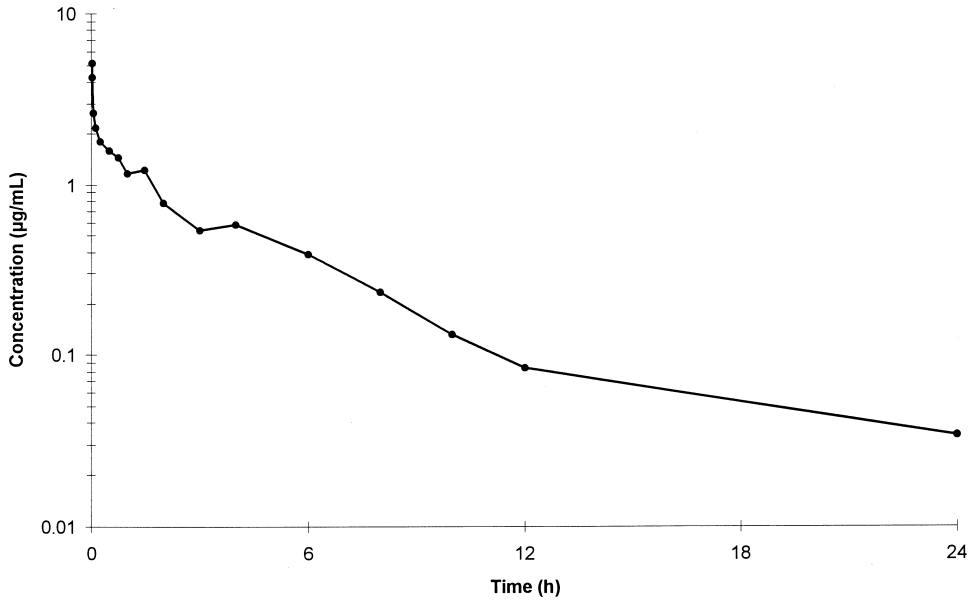


Fig. 4. Plasma concentration–time profile of enrofloxacin in a pig following a single i.v. 5 mg/kg dose of enrofloxacin.

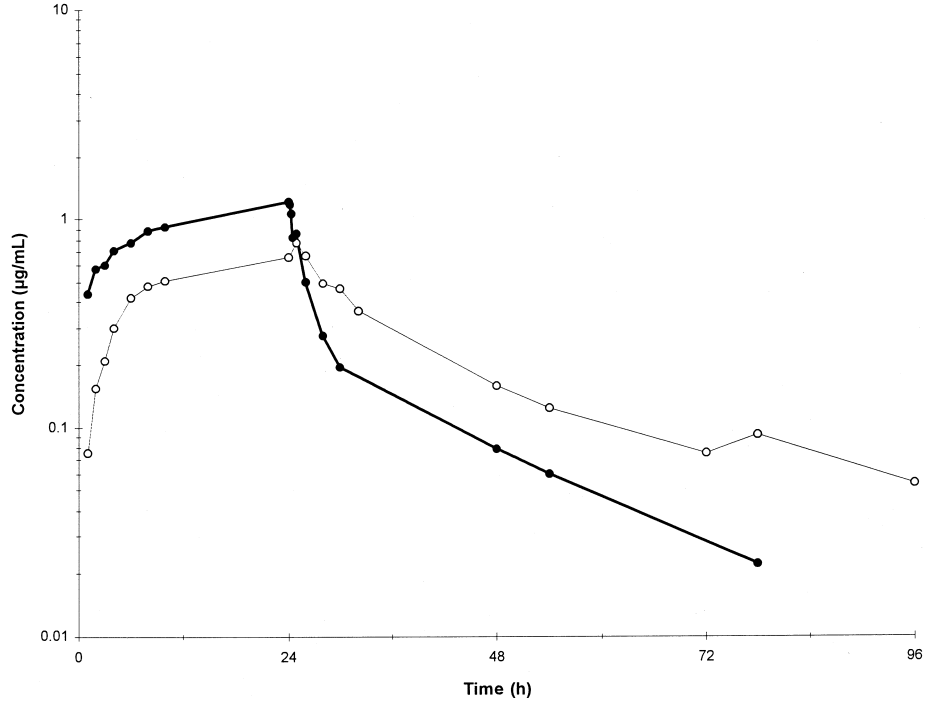


Fig. 5. Semi-logarithmic plot of mean (±SD, $n=4$) plasma and tissue-cage fluid concentrations of enrofloxacin vs. time (h) in rabbits given a perfusion dose of enrofloxacin (1.25 mg/kg per hour during 24 h). ●, Plasma; ○, TCF.

validation of analytical methods employed in bioavailability, bioequivalence and pharmacokinetics studies in man and animals [20]. This method was simple, specific and reproducible, with a good accuracy and precision, and allowed for numerous samples to be processed in a short period of time. Furthermore, the assay is applicable to pharmacokinetic studies of enrofloxacin in both pigs and rabbits.

Testing of the suitability of the method for analysis the same molecules in plasma of other species or in other tissues is in progress in our laboratory. The method could be successfully applied to monitor structurally related novel fluoroquinolone antibiotics in plasma. This method could be also developed with a fluorimetric detector for dosing smaller amounts in bacteria during pharmacodynamic studies in a experimental tissue cage model of infection with rabbits.

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