ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 48 (1999) 253–258

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Pharmacokinetics, bioavailability and absorption of flumequine in the rat ☆

Ana Ruiz-García[a], Marival Bermejo[a], Virginia Merino[a], Gloria Sánchez-Castaño[a], Joan Freixas[b], Teresa M[a] Garrigues[a,*]

[a]*Department of Pharmacy and Pharmaceutics, Faculty of Pharmacy, University of Valencia, Valencia, Spain*
[b]*I + D Department, CENAVISA, Reus, Spain*

Received 16 December 1998; accepted in revised form 17 August 1999

Abstract

The study demonstrates that the oral extent of bioavailability of flumequine in the rat, relative to the intravenous injection, is complete (0.94 ± 0.04) and not significantly different from that found by the intraduodenal route (0.95 ± 0.04). The rate of oral bioavailability, however, is slow ($k_a = 1.20 \pm 0.07\ h^{-1}$; $T_{max} = 2.0$ h), but enough to maintain plasma levels above the minimal inhibitory concentration of the most common pathogens for an extended period of time (about 10 h). The reason for the oral absorption slowness could be a slow gastric emptying, an adsorption to the gastric mucosae, a precipitation in the gastric medium or any other feature concerning the stomach as the intraduodenal administration is very quick ($k_{id} = 38.1 \pm 4.7\ h^{-1}$; $T_{max} = 0.05$ h). A possible precipitation of flumequine cannot be discarded as the solubility of flumequine is very low in the pH range of 3 to 6 (mean pH values for rat stomach and rat intestine, respectively; T.T. Kararli, Biopharm. Drug Dispos. 16 (1995) 351–380). Flumequine was shown to be not substantially excreted in bile (2–3% of the dose). Surprisingly, plasma levels and AUC values found for animals with interrupted bile flow always surpass those found for animals with enterohepatic circulation. This could be due to experimental model features, which might bias plasmatic flumequine concentrations if the homeostatic equilibrium of the animal is not completely restored due to the volume reduction induced by biliary extraction.

Keywords: Flumequine; Pharmacokinetics; Bioavailability; Absorption; Biliary excretion

1. Introduction

Flumequine is an antibacterial agent structurally related to other quinolones such as nalidixic acid or ofloxacin. This synthetic antimicrobial was first prepared by Rinker Laboratory in 1976 [2]. It is highly effective in treating uncomplicated and complicated urinary tract infections. It is occasionally used in Europe in both human and veterinary medicine and is usually administered by the oral route. Poor oral bioavailability has been reported in animals [3–5] in contrast to man [6]. The common dose is about 12–30 mg/kg a day, in man and in cattle, depending on the considered species, twice a day. In man, it is recommended to divide the dose three times while in cattle only twice a day seems adequate. Such dosage regimes lead to plasma levels above 6 μg/ml, which are clinically effective for most of the common urinary pathogens [6,7].

The aim of this study was to characterize the pharmacokinetic profile of flumequine in rats in order to evaluate if the reason for its slow oral bioavailability is related to absorption-limiting features taking place in the stomach or in the duodenum. Therefore, an intraduodenal administration and an oral administration were carried out in order to identify the kinetic limiting process. Since it has been reported that flumequine is excreted into faeces (60% of dose in calves [8] and 10% of dose in rats [9]) and in the initial experiences plasma concentrations occasionally raised, we considered worth of investigation the possible influence of enterohepatic circulation.

2. Materials and methods

2.1. Biological technique

Male Wistar rats weighing 280–300 g were anesthetized using a mixture of diazepam (Valium; Roche, Barcelona, Spain) (1.67 mg/kg), ketamine (Ketolar; Parke-Davis, El

☆ The study was approved by the Research Commission of the Department Council.

* Corresponding author. Department of Pharmacy and Pharmaceutics, Faculty of Pharmacy, University of Valencia, Avd. Vte. Andrés Estellés, s/n 46100, Burjassot, Valencia, Spain. Tel.: +34-6-386-4916; fax: +34-6-386-4911.

E-mail address: teresa.garrigues@uv.es (T.M. Garrigues)

0939-6411/99/$ - see front matter
PII: S0939-6411(99)00057-0

Prat de Llobregat, Spain) (50 mg/kg) and atropine (Atropina sulfato; Braun, Rubí, Spain) (1 mg/kg) to allow cannulae implantation, 24 h before the experiment. All the animals were cannulated in the jugular vein using a previously described technique [10]. The implanted cannula allows blood sampling as well as intravenous administration (i.v.) when needed.

The bile duct and duodenum were also cannulated [11] 24 h before the experiment and both cannulae were connected by a bridge to restore enterohepatic circulation until the compound was administered. At the moment of the administration the cannulae were disconnected to interrupt enterohepatic circulation in animals belonging to groups 2 and 4. If the animal was included in the groups in which enterohepatic circulation was allowed (groups 1, 3 and 5), both cannulae were kept connected.

2.2. Design of the study

The animals were randomly assigned ($n = 6$) to the following groups:

- Group 1. A volume of 2.5 ml was intravenously infused during 10 min; the enterohepatic circulation was allowed
- Group 2. Administration conditions identical to those of group 1; interrupted bile circulation
- Group 3. Animals received 1 ml intraduodenal bolus; the enterohepatic circulation was allowed
- Group 4. Flumequine was intraduodenally administered, as in group 3, with the biliary circulation interrupted
- Group 5. A volume of 2.5 ml was dosed by intragastric sounding allowing enterohepatic circulation

The administered solution was prepared using a mixture of saline and propylene glycol (Sigma, Barcelona, Spain) (50:50) as vehicle. The pH was adjusted to pH 7 to guarantee the solubility. All the animals received a dose of 2 mg of flumequine, donated by Cenavisa, Reus (Spain).

After administration, blood samples were drawn by the jugular cannula at fixed times for a period of 7 h after i.v. administration, 6 h after intraduodenal administration and 34 h after oral administration, in order to characterize the elimination phase in all the groups. Blood samples were collected into heparinized eppendorf tubes and the volume was replaced with heparinized (Heparina Leo 5%, BYK Elmu, Madrid, Spain) (10 IU/ml) saline. Plasma was immediately separated by centrifugation (10 min, 3000 rev./min) and maintained at −20°C until analysis.

In the groups of animals in which the biliary circulation was interrupted, the bile was collected hourly in eppendorf tubes and stored at −20°C until analysis.

2.3. Analysis of the samples

An HPLC procedure developed in our laboratory was used to quantify the flumequine concentration in the samples. The method was carried out on a Hewlett Packard 1045 system (Hewlett Packard, Barcelona, Spain) equipped with a Novapak C18 column (Waters, Barcelona, Spain) (3.9 × 150 mm), using, as mobile phase, a mixture of methanol (Scharlau, Barcelona, Spain) and an aqueous solution consisting of 15 mM phosphate buffer (Scharlau), adjusted to pH 4.0 with orthophosphoric acid (Scharlau) (65:35 v/v). Analysis was done at room temperature, with a flow rate of 1 ml/min. The retention time of flumequine was 3.25 min.

Quantitation was done by fluorimetry, with an excitation wavelength of 233 nm and an emission wavelength of 338 nm. This technique provides a high degree of selectivity and specificity, as it allows separation of the major metabolites of the antibiotic (7-hydroxy-flumequine, flumequine acyl glucuronide).

Before the analysis, plasma samples were deproteinized with methanol (1:3 v/v), and biliary samples were precipitated with an alcoholic solution of 2 M trichloroacetic acid (Merck, Barcelona, Spain) (4:1 v/v) centrifuged and the supernatant diluted (1:2 v/v) with saline. The latter procedure was checked by the plasma method of analysis to assess the lack of influence of the pH on the stability of the metabolites.

The procedure was validated for inter- and intra-day runs before use. Accuracy was calculated by means of the percentage of error associated with measuring 5–8 standards, analysed at least three times. It was demonstrated to be less than 15% regardless of the concentration of analyte. Precision was calculated as the coefficient of variation of five determinations over the same standards. It was shown to be less than 5%. Linearity was established over the range of concentrations present in the samples ($r^2 > 0.999$), using y^{-2} as weighing factor. The low limit of quantitation was shown to be 0.38 μg/ml.

2.4. Solubility determination

Three dark stoppered flasks were shaken overnight, in a water bath at 37°C, with an excess of substance and a volume of a mixture of saline and propylene glycol (50/50) as vehicle, adjusted to pH 1.0 ($n = 3$), pH 3.0 ($n = 3$) and pH 6.2 ($n = 3$) with phosphoric acid. The following day, the suspension was filtered, pH checked and the solution diluted and interpolated on a calibration curve including the concentrations of interest.

2.5. Pharmacokinetic analysis

Individual and mean plasma concentrations versus time were used to estimate the pharmacokinetic parameters of flumequine. As similar conclusions arise from individual fittings and the mean profile fitting in the different groups, only the latter has been reported in order to simplify and evaluate the precision of the parameters. Usual compartmental analysis was carried out by means of PCNONLIN 4.2.

Mean plasma concentrations were calculated for groups 1 and 2 (mean i.v.) and groups 3 and 4 (mean i.d.) to use them as representative of the plasma concentration profiles after

Table 1
Pharmacokinetic parameters of flumequine obtained after fitting of plasma concentration versus time data to two-compartment model, corresponding to intravenous (i.v.) and intraduodenal (i.d.) administration, i.e. groups 1 and 2 and groups 3 and 4, respectively; group 5 column refers to oral administration

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
V_c (ml)	57 ± 4	54 ± 1	57 ± 2	59 ± 3	62.2 ± 1.7
k_{12} (h^{-1})	0.84 ± 0.3	0.84 ± 0.19	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
k_{21} (h^{-1})	1.36 ± 0.5	1.54 ± 0.3	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7
k_{10} (h^{-1})	0.19 ± 0.03	0.185 ± 0.009	0.18 ± 0.03	0.12 ± 0.04	0.155 ± 0.009
k_{01} (h^{-1})	–	–	34.8 ± 5.7	36 ± 8	1.20 ± 0.07
C_{max} (μg ml^{-1})	–	–	31.93	31.95	17
T_{max} (h)	–	–	0.05	0.05	2
$t_{1/2\ \beta}$ (h)	6.07 ± 0.02	5.95 ± 0.007	6.28 ± 0.02	9.27 ± 0.02	7.4 ± 0.4
SS	18.94	5.67	48.63	53.85	42.51
r	0.994	0.999	0.972	0.945	0.995

intravenous and intraduodenal administrations respectively. A simultaneous fit of mean i.v., mean i.d. and group 5 plasma concentration versus time was also carried out. It was performed considering the first order incorporation rate constant (k_{oral}) composed of two constants: k_{id} as the duodenal absorption constant and k_{ed} as an apparent constant including the processes taking place before the absorption (i.e. gastric emptying, desorption from the mucosae, dissolution). The mathematical modelling of the incorporation parameter was achieved considering that all these resistances to absorption are located in series (i.e. $1/k_{oral} = 1/k_{id} + 1/k_{ed}$).

The biliary clearance of flumequine was calculated as the slope of the rate of flumequine bile excretion (computed as the ratio of amount of drug in the biliary fluid to collection time interval) to flumequine plasma concentration in the middle of the considered time interval.

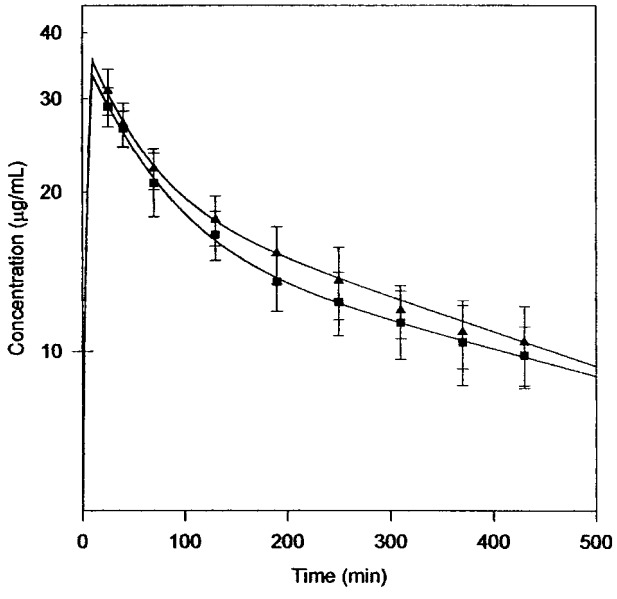

Fig. 1. Mean plasma concentration of flumequine after intravenous (i.v.) administration (■) when the enterohepatic circulation was allowed (group 1) and (▲) when the biliary circulation was interrupted (group 2). The solid lines represent the predicted plasma concentrations according to the two-compartment open model.

2.6. Statistical analysis

Statistical figures (squared residual sum, correlation coefficients between experimental and model-predicted values) and standard error of parameters were calculated as indicative of the goodness of fits, and used to identify the best compartmental model.

The plasma concentrations obtained when biliary circulation was allowed (groups 1 and 3) were compared with those quantified when the bile cycling was interrupted (groups 2 and 4) by means of the multivariant analysis test MANOVA, with the aid of SSPS 6.1.3. This test allows simultaneously the comparison of the plasma concentrations at every sampling time between the groups and the tendency in the change of the concentrations between the groups.

3. Results and discussion

Classic one-compartment and two-compartment open models with elimination from the central compartment were fitted to the plasma concentration versus time curves obtained in the different conditions. The two-compartment open model provided the best fit as demonstrated by lower values of Akaike information criterion [12] and a better correlation between the predicted and experimental plasma levels (data not shown) in both individual and mean data, as expected [3,8,13]. The parameters obtained after two-compartment fit to the mean profiles are shown in Table 1. Figs. 1 and 2 show the plasma concentrations of flumequine obtained as a function of time, after intravenous and intraduodenal administration in both conditions, respectively. The two-compartment predictions (solid lines) have also been plotted. The MANOVA test did not show statistical differences between the plasma levels obtained when the enterohepatic circulation was present and when it was interrupted regardless of the route of administration (groups 1 vs. 2 comparison, significance of $F = 0.281$; and groups 3 vs. 4 comparison, significance of $F = 0.505$, following the

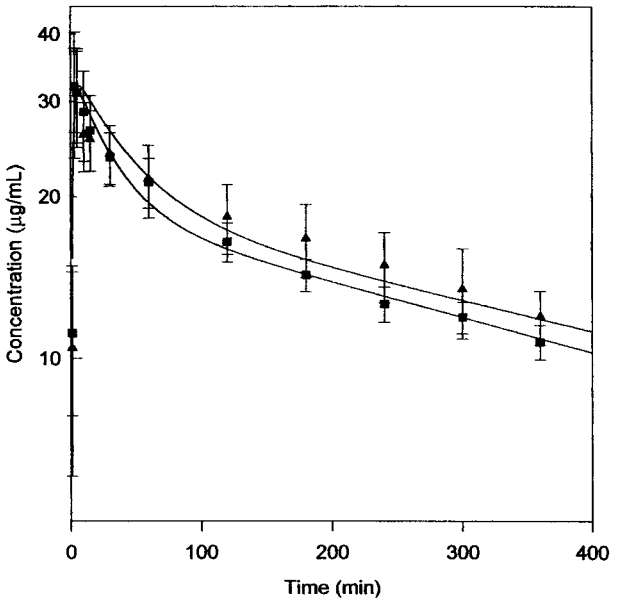

Fig. 2. Mean plasma concentration of flumequine after intraduodenal (i.d.) administration (■) when the enterohepatic circulation was allowed (group 3) and (▲) when the biliary circulation was interrupted (group 4). The solid lines represent the predicted plasma concentrations according to the two-compartment open model.

Pillais' test, Hotellings' test and Wilks' test). It can therefore be stated that the enterohepatic circulation does not play an important role on the kinetics of flumequine. In fact, the biliary clearance is about 4.5×10^{-4} ml/h in both cases, as can be seen in Fig. 3A,B. Moreover, the mean amount excreted in bile after 7 h was 0.054 (±0.015) mg for group 2, which represents approximately 2.7% of the administered dose. For group 4, the cumulative amount in bile, after 6 h of the administration was 0.058 (±0.027) mg, which is 2.9% of the dose. Nevertheless, as can be observed in Figs. 1 and 2, the plasma concentrations are always higher when the bile is collected than in the normal state. The experimental rat model might be responsible for this slight concentration of the plasma samples, as there is no replacement of the collected bile volume. At the end of the experiment the volume was approximately 7 ml, which is about 17% of the water of highly perfused organs in rats (blood,

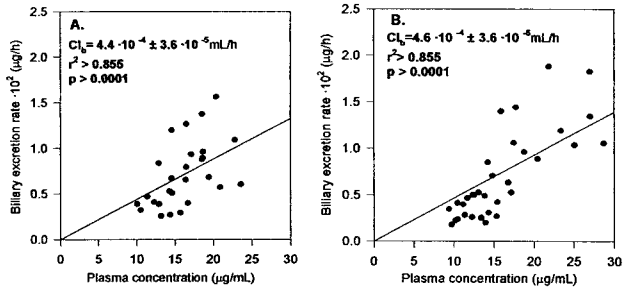

Fig. 3. Biliary excretion rate of flumequine versus plasma concentrations (A) when the substance was i.d. administered and (B) when was administered by i.v. infusion. The parameters of the fit have also been indicated.

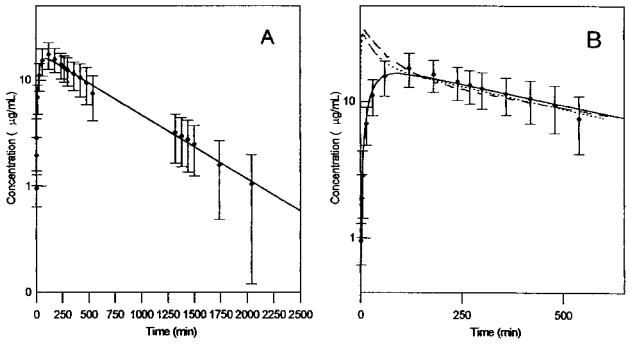

Fig. 4. Observed (◆) and predicted (—) plasma concentration of flumequine as a function of time after oral administration. Predicted plasma concentration after i.v. (- - -) and i.d. (· · ·) administrations are also shown (part B) for better comparison.

gastrointestinal tract, heart, kidney and liver aqueous volumes [14]). This reduction could bias the results when the biliary circulation is blocked, and probably even more so in studies in which the surgical procedures are not identical for all the animals. According to our results, this factor should be taken into account when developing experiments studying the influence of the enterohepatic circulation on drug bioavailability.

As stated, flumequine behaves as a two-compartment drug, that is, its distribution can be explained as a two-rate phenomena due to the access from a central compartment to a peripheral one (with k_{12} first-order rate transfer constant) and the reverse process (k_{21} constant). The k_{12}/k_{21} ratio has a low value (0.58), which means that the drug is not retained in peripheral tissues. Nevertheless, the volume of the peripheral compartment (V_p) is significant (36% of the total volume of distribution, V_d), probably because of the slow elimination rate from plasma ($k_{10} = 0.15\ h^{-1}$). The V_d obtained for flumequine is close to 50% of the total aqueous volume of the rat [14]. This represents a good access of the drug to a large part of the body, and could justify its use in many infections caused by microorganisms sensitive to the antibiotic, as previously suggested by other authors [6].

In Fig. 4A the values of experimental and predicted plasma concentrations of flumequine after oral administration are shown; Fig. 4B superimpose mean i.v. and mean i.d. values for better comparison. Pharmacokinetic parameters of the simultaneous fit of the two compartmental model to mean i.v., mean i.d. and oral plasma concentrations versus time are shown in Table 2.

When flumequine is administered orally, the absorption rate constant is slow ($k_{oral} = 1.01 \pm 1 \times 10^{-5}\ h^{-1}$), and the peak in plasma (T_{max}) is delayed about 100 min. The intraduodenal administration produces a quick absorption ($k_{id} = 38.07 \pm 4.70\ h^{-1}$). Despite the fact that this value was not accurately determined because the process is very fast and can not be properly quantified from the plasma curves, the difference between the intraduodenal and oral routes is highly significant. This phenomenon could be explained by different factors such as the influence of the

Table 2
Pharmacokinetic parameters of flumequine obtained after simultaneous fit of plasma concentration versus time data after i.v., i.d. and oral administration of the antibiotic

Parameters of simultaneous fit			Statistical figures
k_{10} (h^{-1}) = 0.15 ± 0.05	V_c (ml) = 57.2 ± 5	k_{id} (h^{-1}) = 38.075 ± 4.70	r = 0.9907
k_{12} (h^{-1}) = 0.49 ± 0.25	Cl (ml h^{-1}) = 8.94 ± 2.16	k_{ed} (h^{-1}) = 1.03 ± 0.11	SS = 55.73
k_{21} (h^{-1}) = 0.71 ± 0.54	$t_{1/2\beta}$ (h) = 7.91 ± 3.6	k_{oral} (h^{-1}) = 1.01 ± 0.00	
		F_{oral} = 94.02 ± 0.04	
		F_{id} = 95.54 ± 0.04	

gastric emptying rate, adsorption to gastric mucosae and/or the possible precipitation of the drug in the gastric medium. The rate constant quantifying those processes (k_{ed}) is about 1 h^{-1}, which is similar to the reported gastric emptying rate constant [1].

The solubility of flumequine was determined in order to ascertain whether precipitation of the compound occurs when administered to the stomach. As can be seen in Table 3, the solubility of flumequine is comparable at pH 6.2 (typical for the duodenal fraction of the intestine, [1]) and at pH 3.0 (typical for the rat stomach, [1]). Nevertheless, the stomach shows a high ability to lower pH in the medium, producing a massive precipitation of the 2 mg dose. Other gastric environment factors (adsorption to mucosae, emptying rate) are also likely to limit the rate of oral absorption of the antibiotic, as they can delay the access of the flumequine to the actual absorbing membrane (i.e. the duodenum).

It should be born in mind that the flumequine solubility is significantly lower at pH 1.00, typical of fasted conditions in the stomach in man, where a massive precipitation of the antibiotic would probably occur, delaying the gastric emptying and slowing even more the absorption process.

Due to the facts that (i) the enterohepatic circulation of flumequine is very low in the rat and (ii) no statistical differences between plasma concentrations were demonstrated when comparing the two experimental conditions, mean values of groups 1 and 2 (mean i.v.) and groups 3 and 4 (mean i.d.) were used for the determination of intraduodenal and oral bioavailability. The results are outlined in Table 2. As can be observed, both intraduodenal and oral administrations provide complete bioavailability. So that, the gastric medium does not produce any degradation or loss in absorbability. The main difference between intraduodenal and oral groups is related to the rate of absorption that generates differences in peak plasma levels. The oral route provides only a slow incorporation rate that produces more sustained plasma concentration profiles. Considering that the flumequine minimal inhibitory concentration for most of the susceptible bacteria is about 6 μg/ml [15], the oral administration produces active plasma levels for about 10 h. It can be concluded that it is undoubtedly a feasible and suitable route of administration for rats.

Table 3
Solubility values of flumequine at 37°C obtained at different pH values; statistical differences are also indicated

pH	Solubility ± standard error (μg/ml)	Statistical differences ($P < 0.05$)		
		pH 6.2	pH 3	pH 1
6.2	217 ± 8	–		
3	194 ± 9	NS	–	
1	162 ± 6	S	S	–

4. Conclusions

The enterohepatic circulation of flumequine is negligible in rat. The antibiotic exhibits a high oral bioavailability if solutions of the antibiotic are administered. Oral administration produces a slow incorporation rate that is responsible of plasma concentrations above 6 μg/ml for an extended period of time, which can be very valuable. The reason for such a phenomenon is located at the stomach (gastric emptying and/or flumequine precipitation), since the intraduodenal administration produces higher and quicker plasma peaks than the oral administration.

Acknowledgements

This work was supported by the CICYT of the Ministry of Education and Science of Spain (project SAF-96-1710). The authors are also indebted to the Ministry of Education and Science of Spain for grants to A.R.-G. and G.S.-C. We would like to express our gratitude to Prof. Plá-Delfina for his constructive criticism and discussions, and Jane Heilker for the review of the manuscript.

References

[1] T.T. Kararli, Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals, Biopharm. Drug Dispos. 16 (1995) 351–380.
[2] F.P. Schena, L. Gesualdo, G. Caracciolo, A multicentre study of flumequine in the treatment of urinary tract infections, J. Antimicrob. Chemother. 21 (1988) 101–106.
[3] G.M. Dorrestein, H. van Gogh, M.N. Buitelaar, J.F.M. Nouws, Clinical pharmacology and pharmacokinetics of flumequine after intravenous, intramuscular and oral administration in pigeons (Columba livia), J. Vet. Pharmacol. Ther. 6 (1983) 281–292.
[4] R. Mountafchieva, J.F.M. Nouws, H.J. Keukens, C.A. Kan, W.M.J.

Beek, E. Streutjens, L.A.P. Hoogenboom, The effects of co-medication with sodium salicylate on plasma disposition, metabolism and tissue distribution of flumequine in broilers, Proc. 6th EAVPT Congress (Edinburgh) O15 (1994) 78.

[5] G. Ziv, S. Soback, A. Bor, B. Kurtz, Clinical pharmacokinetics of flumequine in calves, J. Vet. Pharmacol. Ther. 9 (1986) 171–182.

[6] M. De Bernardi, F. Chioleno, A. Goi, R. Canclini, Farmacocinetica e biodisponibilità della flumechina nell'uomo, Riv. It. Biol. Med. 4 (1984) 159–162.

[7] D. Schuppan, L.I. Harrison, S.R. Rohlfing, H.L. Miller, M.L. Funk, C.S. Hansen, R.E. Ober, Plasma and urine levels of flumequine and 7-hydroxiflumequine following single and multiple oral dosing, J. Antimicrob. Chemother. 15 (1985) 337–343.

[8] D.J. Mevius, H.J. Breukink, P.J.M. Guelen, T. Jansen, B. De Grève, Pharmacokinetics, metabolism and renal clearance of flumequine in veal calves, J. Vet. Pharmacol. Ther. 13 (1990) 159–169.

[9] L.L. Harrison, D. Schuppan, S.R. Rohfling, A.R. Hansen, C.S. Hansen, M.L. Funk, S.H. Collins, R.E. Ober, Disposition of radiolabeled flumequine in rat and dog, Drug Metab. Dispos. 14 (1986) 555–558.

[10] F. Torres-Molina, J.C. Aristorena, M.C. García-Carbonell, L. Granero, J. Chesa-Jiménez, J.M. Plá-Delfina, J.E. Peris-Ribera, Influence of permanent cannulation of the jugular vein on pharmacokinetics of amoxicilin and antipyrine in the rat, Pharm. Res. 9 (1992) 1587–1591.

[11] J.E. Peris-Ribera, F. Torres-Molina, M.C. García-Carbonell, J.C. Aristorena, J.M. Plá-Delfina, Pharmacokinetics and bioavailability of diclofenac in the rat, J. Pharmacokinet. Biopharm. 19 (1991) 647–665.

[12] J.G. Wagner, The measures of fit (Chapter 17), Pharmacokinetics for the Pharmaceutical Scientist, Technomic, Lancaster, 1993.

[13] T.B. Vree, E.W.J. van Ewijk-Beneken Kolmer, J.F.M. Nouws, Direct-gradient high-performance liquid chromatographic analysis and preliminary pharmacokinetics of flumequine and flumequine acyl glucuronide in humans: effect of probenecid, J. Chromatogr. 579 (1992) 131–141.

[14] L. Granero, J. Chesa-Jiménez, V. Monserrat, M. Almela, M.J. Gimeno, F. Torres-Molina, J.E. Peris-Ribera, Physiological pharmacokinetic model for ceftazidime disposition in the rat and its application to prediction of plasma concentrations in humans, Eur. J. Pharm. Sci. 1 (1993) 3–11.

[15] S.R. Rohlfing, J.F. Gerster, D.C. Kvam, Bioevaluation of the antibacterial flumequine for urinary tract use, Antimicrob. Agents Chemother. 10 (1976) 20–24.