ELSEVIER

Journal of Chromatography B, 704 (1997) 207–214

JOURNAL OF CHROMATOGRAPHY B

Disposition of human drug preparations in the horse VI. Tiaprofenic acid

F.T. Delbeke*, K. Baert, P. De Backer

Vakgroep Farmacologie, Farmacie en Toxicologie, Faculteit Diergeneeskunde, Universiteit Gent, Salisburylaan 133, B-9830 Merelbeke, Belgium

Received 12 May 1997; received in revised form 12 August 1997; accepted 8 September 1997

Abstract

Urinary and plasma concentrations of the nonsteroidal anti-inflammatory drug tiaprofenic acid were determined following oral and intramuscular administration of a dose of 1 g to five fasted horses. Quantitation was performed by high-performance liquid chromatography (HPLC). The limit of quantitation (LOQ) was 0.1 μg/ml and 0.5 μg/ml in 2 ml plasma and 1 ml urine, respectively. Assay precision and extraction recovery were between acceptable values. Tiaprofenic acid pharmacokinetics were described by non-compartment analysis of the data. Absorption was faster after oral administration as maximum plasma concentrations (oral: 6.0±3.3 μg/ml; intramuscular: 6.6±2.5 μg/ml) were obtained after 1 h (oral) compared to 1.6±0.4 h (intramuscular) post dosage. Plasma binding (66±3%) was lower than measured in other species. Tiaprofenic acid was detected in urine for at least 24 h. The percentage of the parent drug excreted in the first 12 h after oral and intramuscular administration was 38±6% and 34±5%, respectively.

Keywords: Tiaprofenic acid

1. Introduction

Human drug preparations have been reported as illicit substances in body fluids of horses at racecourses and jumping events. In a Norwegian study of drug prescription in veterinary medicine it was found that 43% of the prescriptions were for human preparations and that the number of such prescriptions disposed for horses was 19% [1]. The detection of such substances in equine biofluids is facilitated by knowing how such drugs are excreted and metabolised in the horse. The present work is a continuation of a series of studies on the disposition and metabolism of human drug preparations in the horse [2] and describes the disposition of the orally and intramuscularly administered nonsteroidal anti-inflammatory drug (NSAID) tiaprofenic acid in the horse.

2. Experimental

2.1. Experimental animals

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent.

Five untrained standard-bred mares (mean 487±53 kg) were fasted 18 h before the start of the experiment. They received 1 g tiaprofenic acid orally and intramuscularly. These studies were performed

*Corresponding author.

0378-4347/97/$17.00
PII S0378-4347(97)00461-1

in a cross-over design. Five tablets of Artiflam® containing 200 mg of the active substance (Roussel, Brussels, Belgium) were administered by a stomach tube followed by 0.5 l water. Five ampoules of Artiflam® containing 200 mg of the active substance (Roussel) were dissolved in the supplied solvent and administered intramuscularly in the neck. The horses were allowed water ad libitum but did not have access to hay or straw during the experiment. Pelleted food was offered 8 h after the administration of tiaprofenic acid.

Heparinized blood samples (10 ml) were collected by direct venipuncture of the left jugular vein. Sampling times were 0 (control), 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24 and 36 h after administration. Blood samples were centrifuged at 1700 *g* for 10 min and plasma was removed and stored at −20°C until analysis. A balloon-tipped catheter was placed in the bladder of each mare. The total volume in the bladder was collected over a 12 h period following drug administration using the blood sample protocol from the 1 h sample onwards. After this 12 h period, aliquots of urine were taken every 12 h until 72 h after dosing. Urinary pH and volume were measured.

A portion was frozen (−20°C) to await analysis within 1–2 days of collection. The samples were analysed in duplicate and if necessary, dilutions were made with blank urine.

2.2. Reagents

Tiaprofenic acid and its two major human metabolites were obtained from Roussel. The internal standard prenazone was from Boehringer–Ingelheim (Brussels, Belgium). High-performance liquid chromatography (HPLC) grade water was obtained with the Milli-Q water purification system from Millipore (Brussels, Belgium). HPLC grade acetonitrile was from BDH Labs. (Poole, UK). The enzyme preparation β-glucuronidase (EC 3.2.1.31) *E. coli* was obtained from Boehringer Mannheim (Brussels, Belgium). All other chemicals were analytical grade.

2.3. Equipment

The HPLC system (TSP, Fremont, CA, USA) consisted of a P-4000 pump, Model AS 3000 autosampler and a Focus forward optical scanning detector set at 245 nm (internal standard) and 310 nm (tiaprofenic acid). A 100×3 mm I.D. reversed-phase column packed with octadecyl silica (5 μm Nucleosil Chrompack, Antwerp, Belgium) with an appropriate guard column was used. The loop volume was 20 μl. The mobile phase comprised 40% acetonitrile and 60% water–acetic acid (99:1, v/v). The flow-rate was 0.8 ml/min. The column was held at 35°C.

2.4. Analytical procedures

2.4.1. Plasma

Samples were prepared in duplicate by pipetting 2.0 ml into a 15 ml screw-capped tube, followed by the addition of 50 μl of internal standard (I.S.) (prenazone in methanol, 50 μg/ml), 250 μl of 1 *M* HCl and 5 ml of diethyl ether. After centrifugation (1100 *g*, 10 min), the organic layer was transferred to a clean screw-capped tube and evaporated under nitrogen at 40°C. The extraction step was repeated and, after evaporation of the combined organic extracts, the residue was redissolved in 200 μl of the mobile phase, briefly vortexed and 20 μl were injected.

2.4.2. Urine without hydrolysis

Duplicate samples (1 ml) were acidified with 250 μl of 1 *M* HCl and 50 μl I.S. added. Extraction was performed by rolling with 5 ml diethyl ether (15 min). The organic layer was removed into another tube and washed by vortexing (1 min) with 1 ml of a freshly prepared $NaHCO_3$ solution (1%). The diethyl ether layer was separated and subsequently treated as described for plasma.

2.4.3. Enzymatic hydrolysis of urine

Urine samples (1 ml) were buffered with 100 μl of 0.2 *M* phosphate buffer pH 7 and 50 μl β-glucuronidase added. Hydrolysis was performed at 56°C for 3 h. After cooling the hydrolysate was extracted as described above.

2.4.4. Alkaline hydrolysis

Urine (1 ml) was treated with 50 μl of 1 *M* NaOH for 30 min at room temperature. The hydrolysate was

then acidified with 300 μl of 1 *M* HCl, the I.S. added and subsequently extracted.

2.4.5. Quantitative determination

Standard curves were obtained by subjecting spiked plasma and urine to the appropriate extraction method in triplicate at each concentration. The prepared tiaprofenic acid concentrations were 0.10, 0.25, 0.5, 1, 2.5 and 5 μg/ml for plasma and 0.5, 1, 2.5, 5, 7.5 and 10 μg/ml for urine. The precision of the assay was measured at three different concentrations 2.5, 0.50 and 0.10 μg/ml in plasma and 7.5, 5.0 and 0.5 μg/ml in urine.

2.4.6. Extraction recovery

The recovery of tiaprofenic acid was estimated from the changes in peak-height ratios when the drug was added to the plasma (urine) and the I.S. was added to the final extract, compared with the peak-height ratios when both the drug and I.S. were dissolved in the mobile phase.

2.4.7. Stability of tiaprofenic acid solutions

The decomposition of tiaprofenic acid in different solutions (methanol, HPLC solvent mixture and 0.01 *M* NaOH) was measured by analysing aliquots (100 μl) over a period of four days. The solutions were stored either at room temperature, in the freezer (−20°C) or at room temperature but protected from light. A 1 ml volume of distilled water was added to the aliquot and quantitation performed as for urine samples.

2.4.8. Plasma protein binding in vitro

The binding of tiaprofenic acid to horse plasma protein was determined by ultrafiltration. The plasma was obtained from a fresh pooled heparinised blood sample. Solutions of tiaprofenic acid (1 μg/ml and 2 μg/ml) were prepared with plasma and allowed to equilibrate for 30 min. Quadruplicate samples (1 ml) were centrifuged in the micropartition apparatus (Model MPS-I, Amicon) at 1500 *g* for 20 min. Tiaprofenic acid concentrations were measured by the HPLC method.

2.5. Pharmacokinetic analysis

Non-compartmental pharmacokinetic parameters [3] including area under the plasma concentration versus time curve (AUC), area under first moment curve (AUMC), mean residence time (MRT), elimination constant (k_e) and effective half-life ($t_{1/2}$) were determined using appropriate equations. The AUC and the AUMC were calculated using the trapezoidal rule and were extrapolated to the *x*-intercept using final plasma concentration and terminal slope determined from the terminal portion of each curve. The time at which peak concentration occurred (t_{max}) and the peak concentration itself (C_{max}) were estimated from the experimental data points. The renal clearance (Cl_{ren}), extraction ratio and percentage of the dose eliminated after 12 h were determined from the urinary data.

2.6. Statistical analysis

Differences between values were compared using Student's *t*-test for paired data, and were considered to be significant at the 95% probability level.

3. Results and discussion

Several analytical methods including HPLC [4,5] and capillary zone electrophoresis [6] are available for quantitative analysis of tiaprofenic acid in human biological specimens. However, the observed detection limits make their application to the quantitation of tiaprofenic acid in horse plasma less appropriate.

Tiaprofenic acid which is suspected to cause phototoxic effects in humans [7] has been shown to be a photo-unstable compound with various degradation products of known structures [8]. This photodegradation could complicate the assay procedure. Geisslinger et al. [9] found that at pH 7.4 and under normal laboratory light conditions more than 50% of the drug was degraded after 2 h. It was therefore important to investigate the photostability when developing this assay. As indicated in Fig. 1 a solution containing tiaprofenic acid is rapidly degraded when unprotected from light. The decomposition of tiaprofenic acid by laboratory light is even

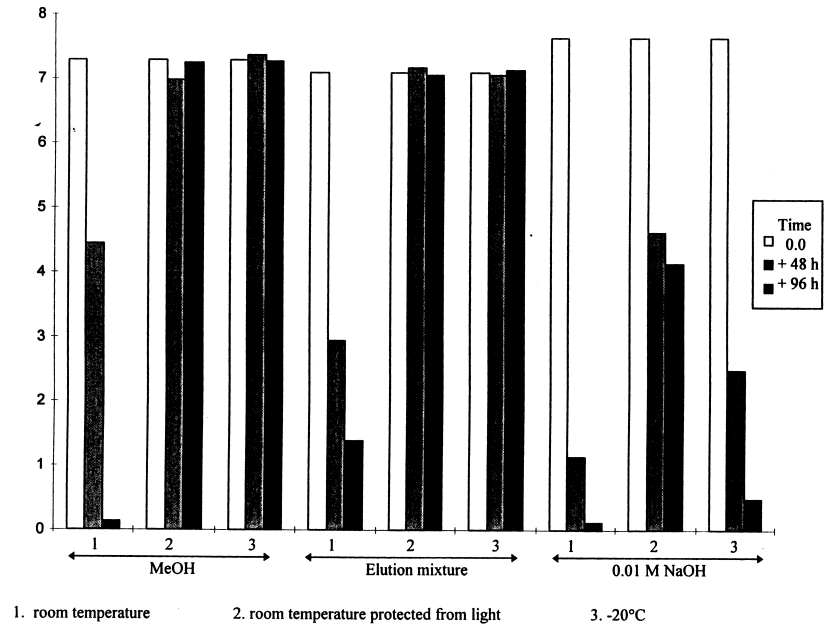

Fig. 1. Stability of tiaprofenic acid (50 μg/ml) in different solutions under variable conditions (expressed as peak-height ratio tiaprofenic acid: I.S.).

more pronounced at lower concentrations. These observations are in contrast with a recent report [10] suggesting that under normal laboratory light tiaprofenic acid concentrations were slowly decreased with apparent $t_{1/2}$ values of 13 days. When the methanolic or HPLC tiaprofenic acid solutions were protected from light or stored in the freezer no noticeable degradation was observed. However, tiaprofenic acid is unstable under strong alkaline conditions even when stored at −20°C. As a result of the previous observations the collected plasma and urine samples were stored at −20°C, while the number of samples extracted and processed by HPLC was limited to six in one batch.

Under the described HPLC conditions, the peaks corresponding to tiaprofenic acid and prenazone were well resolved, sharp and symmetrical. No endogenous compound extracted at the same time interfered with these peaks. The retention times for tiaprofenic acid and prenazone were 2.7 min and 8.3 min, respectively (Fig. 2). For both urine and plasma assays no interferences were found with other NSAIDs mainly used in veterinary practice including naproxen, flunixin, phenylbytazone and oxyphenbutazone or with the human drugs flurbiprofen, diclofenac, alclofenac, piroxicam, fenoprofen, ibuprofen and indomethacin.

Linear calibration graphs were obtained in the ranges 0–5 μg/ml for plasma ($y=0.268x-0.030$) and 0–10 μg/ml for urine ($y=0.589x+0.070$), respectively. The respective correlation coefficients were 0.998 and 0.995. At a signal-to-noise ratio of 3, the limits of quantitation (LOQs) in equine plasma and urine were 0.10 μg/ml and 0.50 μg/ml, respectively.

Generally the separation of NSAIDs from biofluids using solvent extraction is relatively difficult and not precise. However, the use of a double extraction step in the plasma assay resulted in fairly good recoveries and reproducibilities (Table 1). Due to the washing step with $NaHCO_3$ slightly lower recoveries were noticed in the urine assay (Table 1).

The chiral propionic acid drug tiaprofenic acid is a potent analgesic in animals and humans. The pharmacokinetics have been studied in adults [11], neonates [12] and in several animals including mice, rats, rabbits and dogs [13].

Typical plasma log concentration–time curves

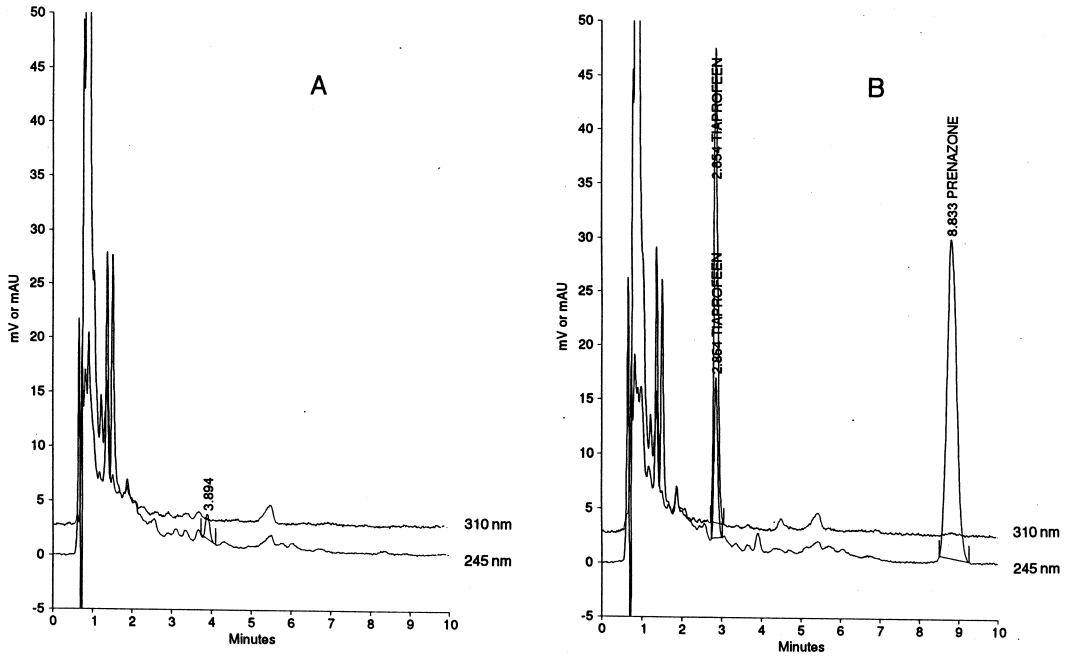

Fig. 2. HPLC chromatograms (245 nm and 310 nm) of (A) blank plasma extract and (B) plasma extract 6 h after the oral administration of 1 g tiaprofenic acid to horse 1.

after oral and intramuscular administration of 1 g tiaprofenic acid to one horse are depicted in Figs. 3 and 4. The mean results for the five horses are illustrated in Figs. 5 and 6. With a LOQ of 0.1 μg/ml tiaprofenic acid could be detected in plasma for periods up to 9–12 h after administration. The compartment independent individual and mean pharmacokinetic parameters are presented in Tables 2 and 3. From these results it seems that the oral administration of tiaprofenic acid produced a rapid absorption rate similar to other species [12,13]. It is likely that following oral administration absorption occurs mainly by passive diffusion of the undissociated drug in the first part of the gastrointestinal (GI) tract at low pH. Drug absorption from the injection site after intramuscular administration was rapid (1.6 h) but significantly ($p<0.05$) slower than after an oral dose (1.0 h). The maximum plasma

Table 1
Extraction recovery and precision of the tiaprofenic acid assay

	Extraction recovery ($n=5$)		Precision ($n=5$)		
	Concentration added (μg/ml)	Recovery (%±S.D.)	Concentration added (μg/ml)	Concentration found (μg/ml)	C.V. (%)
Plasma					
	0.5	95.8±3.3	0.1	0.08	4.2
	1.0	93.3±3.4	0.5	0.46	3.2
	2.5	98.2±3.3	2.5	2.61	3.1
Urine					
	1	90.2±3.1	0.5	0.48	2.7
	2	90.5±3.1	5.0	4.63	3.3
	5	91.2±2.4	7.5	7.54	2.6

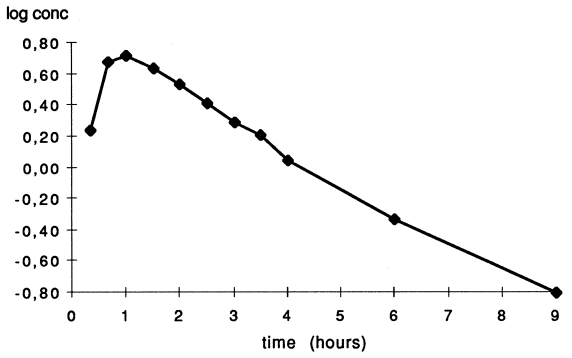

Fig. 3. Plasma log concentration–time curve of tiaprofenic acid in horse 4 after oral administration of 1 g tiaprofenic acid.

concentrations (C_{max}) in humans and horses were reached at the same t_{max}, while the values of C_{max} after the administration of comparable doses were much higher in humans [14], respectively 23 μg/ml and 18 μg/ml following oral and intramuscular dosing.

Significant inter-individual differences in absorption as reported for other NSAIDs in the horse [15] were not noticed for tiaprofenic acid.

As the same dose was used for both experiments and since the AUC after intramuscular administration was significantly higher than after oral administration, it is likely that intramuscular absorption is more complete.

Elimination of tiaprofenic acid was relatively fast after all administrations as reflected by the short plasma half-lives from Tables 2 and 3. Two orally dosed horses had a longer $t_{1/2}$ possibly resulting from a slow absorption process.

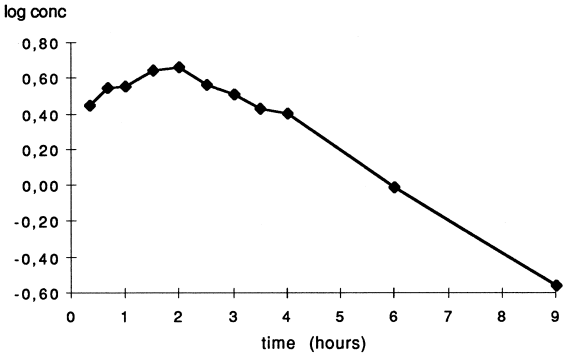

Fig. 4. Plasma log concentration–time curve of tiaprofenic acid in horse 4 after intramuscular administration of 1 g tiaprofenic acid.

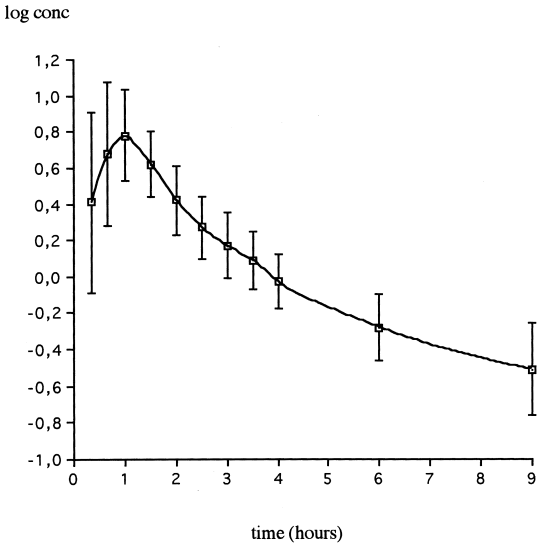

Fig. 5. Mean plasma log concentration–time curve of tiaprofenic after oral administration of 1 g tiaprofenic acid to five horses.

Plasma binding of tiaprofenic acid was 63.8±1.8% and 67.7±2.9%, respectively at the 1 μg/ml and 2 μg/ml level. The mean value

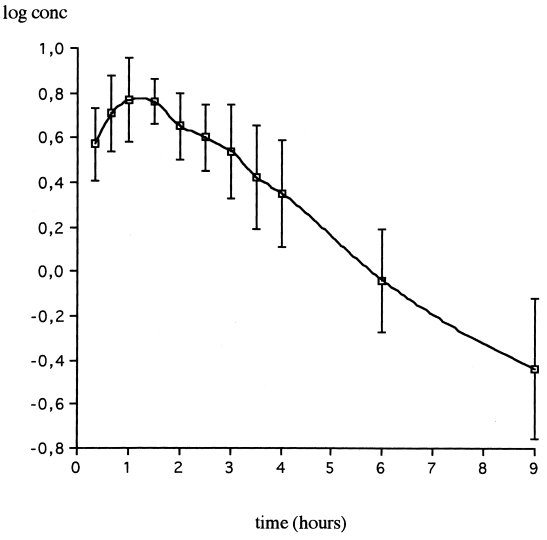

Fig. 6. Mean plasma log concentration–time curve of tiaprofenic after intramuscular administration of 1 g tiaprofenic acid to five horses.

Table 2
Pharmacokinetic parameters of tiaprofenic acid in five horses after oral administration of 1 g tiaprofenic acid

Parameter	H 1	H 2	H 3	H 4	H 5	Mean±S.D.
t_{max} (h)	1.0	1.0	1.0	1.0	1.0	1.0±0.0
C_{max} (μg/ml)	3.0	10.7	7.9	5.2	3.0	6.0±3.3
AUC (μg h/ml)	12	17	17	14	12	14±2.5
AUMC (μg h^2/ml)	109	25	36	32	109	62±42.9
MRT (h)	9.3	1.5	2.1	2.3	9.3	4.9±4.0
$t_{1/2}$ (h)	6.5	1.0	1.5	1.6	6.4	1.9[a]
k_e (l/h)	0.1	0.7	0.5	0.4	0.1	0.4±0.3
Cl_{ren} (ml/min)	455	464	334	310	262	365±90.1

[a] Harmonic mean.

Table 3
Pharmacokinetic parameters of tiaprofenic acid in five horses after intramuscular administration of 1 g tiaprofenic acid

Parameter	H 1	H 2	H 3	H 4	H 5	Mean±S.D.
t_{max} (h)	1.5	1.0	2.0	2.0	1.5	1.6±0.42
C_{max} (μg/ml)	6.4	10.9	4.9	4.6	6.2	6.6±2.5
AUC (μg h/ml)	19	19	17	18	25	20±3.1
AUMC (μg h^2/ml)	52	35	47	52	68	51±11.9
MRT (h)	2.8	1.8	2.8	2.9	2.7	2.6±0.5
$t_{1/2}$ (h)	1.9	1.3	1.9	2.0	1.9	1.8[a]
k_e (l/h)	0.4	0.6	0.4	0.4	0.4	0.4±0.1
Cl_{ren} (ml/min)	236	352	207	171	575	308±163.8

[a] Harmonic mean.

(65.8±3.0%) is much lower than obtained in other species, respectively 98% in rats [13] and 97% in humans [13,16]. As a result of this low plasma binding, a larger portion of the free fraction of tiaprofenic acid is available at the receptor site and for distribution and elimination processes.

From the values in Table 4 it seems that maximum tiaprofenic acid excretion rates occurred between 2–4 h after dosage. With a LOQ of 0.5 μg/ml, no parent drug could be detected 72 h after administration.

Formation of acyl glucuronides has been described in different species for several NSAIDs. In the horse, however, phase II metabolism additionally includes glycine and taurine conjugation for this type of drug [17]. Therefore detection may be improved by enzymatic or alkaline hydrolysis of the urine. For tiaprofenic acid, however no significant differences

Table 4
Excretion rate (μg/h) after oral and intramuscular administration of 1 g tiaprofenic acid to five horses

Time (h)	Oral					Intramuscular				
	H 1	H 2	H 3	H 4	H 5	H 1	H 2	H 3	H 4	H 5
1	–	161	–	–	–	351	541	–	315	13
2	1540	1139	134	19	315	613	480	22	1101	112
3	624	666	–	39	305	492	68	478	168	–
4	–	360	375	126	223	62	105	–	–	276
6	17	154	108	43	264	354	103	367	59	168
9	49	76	25	13	231	102	68	229	99	260
12	114	67	33	5	69	29	166	53	33	32

in urinary concentration were obtained after hydrolysis of equine urine.

Mean renal clearance of tiaprofenic acid after oral administration to horses was 0.73 ml/min/kg (Table 2). Based on creatinine clearance glomerular filtration rate in horses is approximately 1.92 ml/min/kg [18]. Since approximately 34% of plasma tiaprofenic acid was not bound to plasma proteins, the renal clearance of unchanged tiaprofenic acid by glomerular filtration should be approximately 0.65 ml/min/kg. Thus, the estimated renal clearance of tiaprofenic acid is of the same magnitude as the measured renal clearance, indicating that unchanged tiaprofenic acid is likely to be excreted mainly by glomerular filtration.

Approximately 38±6% and 34±5% of the dose was excreted unchanged in the urine after 12 h following respectively, oral and intramuscular dosing. These percentages are higher than the values found after the administration of other human drug preparations containing NSAIDs to the horse [15,19,20] including alclofenac (0.7%), indomethacin (12%) and fenoprofen (13.0%). Two metabolites of tiaprofenic acid are formed in different species (humans, dogs, mice and rabbits), one resulting from the reduction of the ketone group to an alcohol and the other from oxidation generating a phenol in the benzene ring in *para*-position to the ketone group [13]. Although the present analytical procedure allows for their detection, these metabolites were not found in hydrolysed equine urine.

Acknowledgements

The technical assistance of Mr. K. Roels and Mr. T. Claeys is gratefully acknowledged. Thanks are due to Mrs. E. Raulo-Roelens for typing the manuscript.

References

[1] K. Grave, M. Bangen, M. Engelstad, N.E. Soli, J. Vet. Pharmacol. Ther. 15 (1992) 45.
[2] F.T. Delbeke and M. Debackere, in P. Kallings, U. Bondesson and E. Houghton (Editors), Proceedings of the 10th International Conference of Racing Analysts and Veterinarians, Stockholm, 1994, R and W Publications, Newmarket, 1995, pp. 100–104.
[3] M. Gibaldi and D. Perrier, in J. Sevarbick (Editor), Drugs and the Pharmaceutical Sciences, Vol. 1, Pharmacokinetics, Marcel Dekker, New York, 1975.
[4] F. Jamali, A.S. Russell, B.W. Berry, C. Lehmann, J. Chromatogr. 23 (1984) 327.
[5] W. Rieck, D. Platt, J. Chromatogr. 421 (1987) 206.
[6] I. Bechet, M. Fillet, P. Hubert, J. Crommen, J. Pharm. Biomed. Anal. 13 (1995) 497.
[7] J.V. Castell, M.J. Gomez-Lechon, D. Hernandez, L.A. Martinez, M.A. Miranda, Photochem. Photobiol. 60 (1994) 586.
[8] F. Bosca, M.A. Miranda, F. Vargas, J. Pharm. Sci. 81 (1992) 181.
[9] G. Geisslinger, S. Menzel, K. Brune, J. Chromatogr. B 675 (1996) 77.
[10] M. Vakily, F. Jamali, J. Pharm. Sci. 85 (1996) 638.
[11] M. Vakily, F. Jamali, J. Pharm. Sci. 83 (1994) 495.
[12] A. Pariente-Khayat, M.C. Dubois, F. Vanzelle-Kervroëdan, E. Roy, P. D'Athis, M. Bouvier D'Yvoire, C. Saint Maurice, G. Olive, G. Pons, Int. J. Clin. Pharmacol. Ther. 34 (1996) 342.
[13] J. Pottier, D. Berlin, J.P. Raynaud, J. Pharm. Sci. 66 (1977) 1030.
[14] P. Claessens (Editor), Compendium 95 AVGI, Brepols, Turnhout, 1995.
[15] F.T. Delbeke, J. Landuyt, M. Debackere, J. Pharm. Biomed. Anal. 13 (1995) 1041.
[16] O.G. Nilsen, A. Storhaug, O. Dale, Arzneim. Forsch. 35 (1985) 984.
[17] M.V. Marsh, J. Caldwell, R.L. Smith, M.W. Horner, E. Houghton, M.S. Moss, Xenobiotica 11 (1981) 655.
[18] C.W. Kohn, S.L. Strasser, Am. J. Vet. Res. 47 (1986) 1332.
[19] F.T. Delbeke, J. Landuyt, M. Debackere, J. Vet. Pharmacol. Ther. 17 (1994) 353.
[20] F.T. Delbeke, M. Debackere, L. Vynckier, J. Vet. Pharmacol. Ther. 14 (1991) 145.