Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 819 (2005) 181–184
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of deracoxib in feline plasma samples using high performance liquid chromatography

Sherry K. Cox[a,*], Jacob Roark[b], Adam Gassel[b], Karen Tobias[b]

[a] *Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37966, USA*
[b] *Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37966, USA*

Received 9 December 2004; accepted 24 January 2005
Available online 6 February 2005

Abstract

A new HPLC procedure for the determination of deracoxib, a selective cyclooxygenase-2 inhibitor, has been developed and validated. Following a liquid–liquid extraction using isopropyl alcohol and chloroform, samples were separated by isocratic reversed-phase HPLC on an Atlantis C_{18} column and quantified using UV detection at 252 nm. The mobile phase was a mixture of 10 mM potassium phosphate (pH 4.5) and acetonitrile, with a flow-rate of 1.0 ml/min. The procedure produced a linear curve over the concentration range 10–1500 ng/ml. The development of the assay allowed the determination of pharmacokinetic parameters after oral administration of deracoxib in cats and would be suitable for other pharmacokinetic studies.

Keywords: COX-2 inhibitors; Deracoxib; HPLC

1. Introduction

Deracoxib (deramaxx) is a non-steroidal, non-narcotic drug that is approved for the treatment of pain and inflammation associated with osteoarthritis and orthopedic surgery in canines [1]. It is a 4-[5-(3-difluoro-4-methoxyphenyl)-(difluoromethyl)-1H-pyrazole-1-yl] benzesulfonamide. Deracoxib is a cyclooxygenase-2 (COX-2) inhibitor developed to reduce the serious side effects of non-steroidal anti-inflammatory (NSAIDs) drugs associated with the inhibition of cyclooxygenase-1 (COX-1). COX-1 is an important constitutive enzyme that is essential for the production of beneficial prostaglandins, most notably those produced in the stomach, platelets and kidney. Inhibition of COX-1 results in decreased production of beneficial prostaglandins and is thought to be associated with the development of side effects such as gastric ulcers, decreased renal blood flow and disorders of primary hemostasis. COX-2 production is induced during active inflammation and results in production of prostaglandins that amplify pain, create edema, and promote the production of other inflammatory mediators [1].

The use of traditional and selective NSAIDs to treat inflammation and pain in the dog and cat is widely accepted. Basic pharmacological and toxicological data for traditional and selective NSAIDs has been determined in a variety of species with limited information available in cats [2–7]. Compared to other species, cats tend to be more susceptible to the toxic side effects of NSAIDs, due to a relative deficiency in the transferring enzyme glucuronyl transferase. Oral formulations of deracoxib have been licensed for use in the dog for postoperative pain and inflammation associated with orthopedic surgery and osteoarthritis. To our knowledge no other studies have been reported on deracoxib pharmacokinetics in the cat. Because of the therapeutic potential of this drug in cats, pharmacologic and toxicologic parameters should be investigated. Based on pharmacokinetic and toxicologic studies, efficacious and safe dosage schedules could be established for convenient use of this product in the cat.

* Corresponding author. Tel.: +1 615 974 5576; fax: +1 865 974 5640.
E-mail address: scox6@utk.edu (S.K. Cox).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.01.025

A literature search revealed that there were no analytical procedures for the quantification of deracoxib but there were several methods of analysis [8–12] for celecoxib which is structurally similar to deracoxib. Therefore, we developed and validated a new method for determination of deracoxib in feline plasma that would be suitable for pharmacokinetic studies. We chose a reverse-phase HPLC system, with UV detection for the analysis.

2. Experimental

2.1. Reagents and chemicals

Acetonitrile, chloroform and isopropyl alcohol (IPA) were HPLC grade while potassium phosphate monobasic and phosphoric acid were reagent grade (Fisher Scientific, Pittsburg, PA, USA). Tolbutamide (100% purity), the internal standard (Fig. 1) was purchased from Sigma (St. Louis, MO, USA). Deracoxib (100% purity) (Fig. 1) was a gift from Novartis Animal Health (Greensboro, NC, USA).

2.2. Chromatography

The analytical system consisted of a 626 solvent delivery system, 717 plus autosampler and 996 photodiode array detector (Waters, Milford, MA, USA). The compounds were separated on an Atlantis DC_{18} (4.6 mm × 150 mm, 5 μm) column with an Atlantis DC_{18} guard column (Waters). Empower software (Waters) was used for data acquisition and processing. The mobile phase was an isocratic mixture of 10 mM potassium phosphate buffer (pH 4.5) and acetonitrile (52:48, v/v). The phosphate buffer was prepared fresh daily with double-distilled deionized water. All solutions were filtered through a 0.22 μm membrane and degassed before use. The flow-rate was 1 ml/min and the column temperature was 30 °C. UV absorbance was measured at 252 nm.

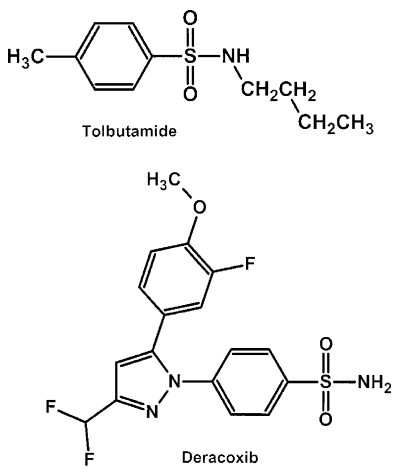

Fig. 1. Structures of deracoxib and tolbutamide.

2.3. Preparation of stock solutions and calibration standards

A stock solution of deracoxib was prepared by dissolving 5 mg in methanol in a 50 ml volumetric flask. The internal standard was prepared by dissolving 10 mg in methanol in a 50 ml volumetric flask. The stock solutions were aliquoted into 1 ml vials to prevent evaporation and cross contamination. All solutions were stored at 4 °C. For preparation of calibration standards and quality control samples, appropriate aliquots of the stock solutions were evaporated to dryness and reconstituted in blank feline plasma. The final concentrations were 10, 25, 50, 100, 250, 500, 800, 1000, and 1500 ng/ml for the calibration standards and 30, 300, and 900 ng/ml for quality control samples. Linearity was assessed by linear regression analysis. The calibration curve had to have a correlation coefficient of 0.99 or better. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value except lower limit of quantification (LLOQ) which was set at 20%.

2.4. Sample treatment

Previously frozen plasma samples were vortexed and 1 ml placed in 15 ml round bottom centrifuge tubes followed by 100 μl of tolbutamide (internal standard, 200 μg/ml) and 6 ml of IPA:chloroform (80:20). Tubes were placed on a tube rocker for 15 min then centrifuged at 1000 × *g* for 15 min. Supernatants were transferred to a clean tube and the organic phase evaporated at 30 °C with nitrogen. Samples were reconstituted in 1 ml of mobile phase and a 100 μl injection was analyzed.

3. Results

Endogenous plasma components did not interfere with the elution of the compounds of interest. Blank plasma samples for specificity testing were prepared in the same way as study samples. Seven different blank plasma samples were used in the pre-validation process and a blank plasma sample of each study subject was included in the analysis. Fig. 2 shows chromatograms of a blank plasma sample (A), a plasma standard (B) and a feline plasma sample after a 1 mg/kg dose of deracoxib. Retention times were 6.15 min for tolbutamide and 9.92 min for deracoxib. Commonly used pain medications were assessed for potential interferences. Phenacetin, ketoprofen, ibuprofen, butorphanol, acetaminophen, piroxicam and sulindac did not interfere with the compounds of interest.

The plasma peak area ratio (area of deracoxib divided by internal standard area) versus concentration was plotted which produced a linear curve for the concentration range used (10–1500 ng/ml) with the correlation coefficients ranging from 0.998 to 0.999. The mean slopes, intercepts and r^2 values are reported in Table 1. Intra-assay R.S.D. for plasma

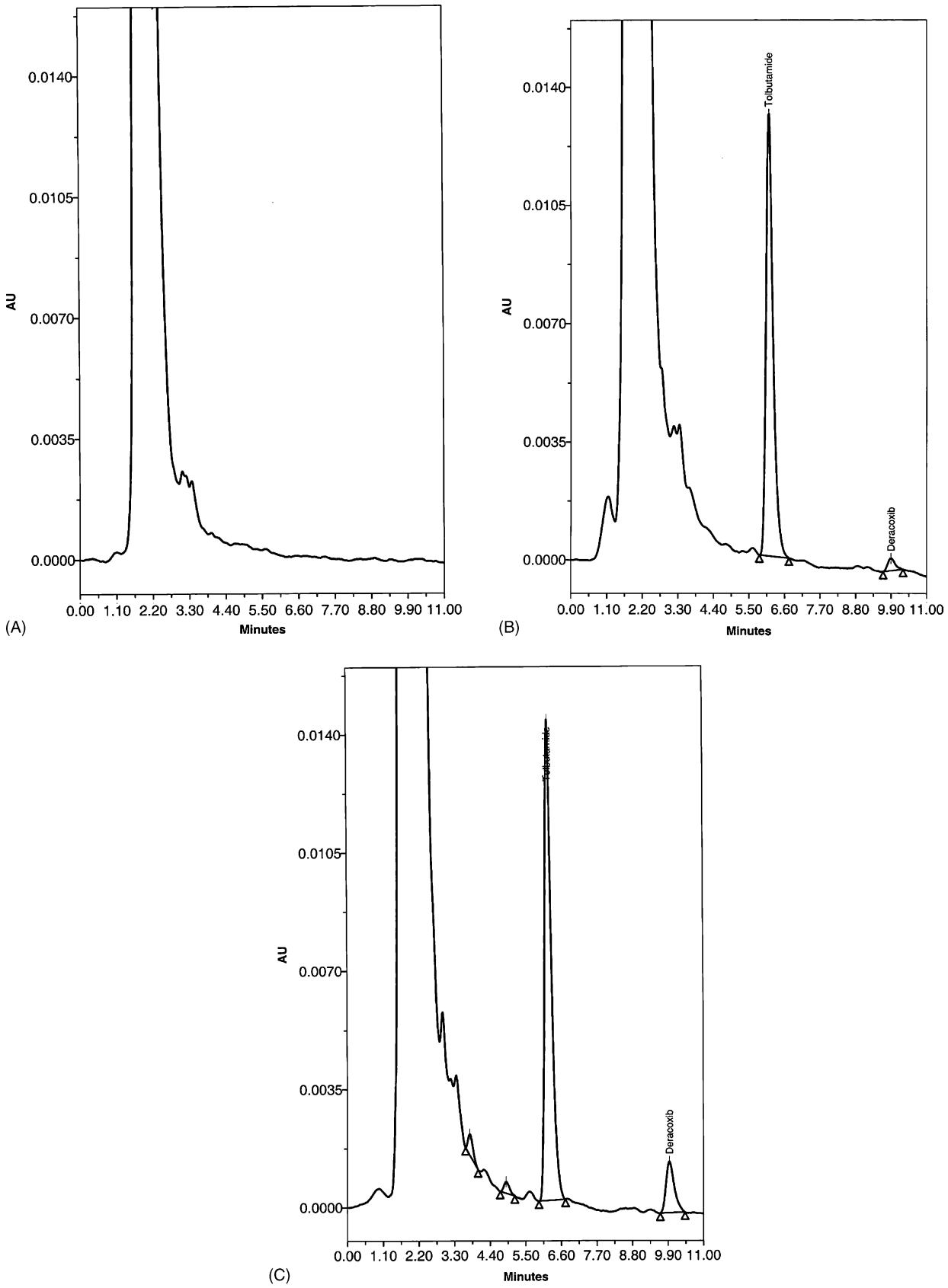

Fig. 2. Chromatograms for deracoxib and tolbutamide (IS). (A) Blank plasma sample, (B) plasma standard (10 ng/ml), (C) plasma sample 4 h post-administration of 1 mg deracoxib (98 ng/ml).

Table 1
Intra-assay accuracy, precision and assay linearity for deracoxib in plasma

Concentration added (ng/ml)	Concentration measured (ng/ml) (mean ± S.D.)	R.S.D. (%)
Intra-assay variability ($n = 6$)		
10	10 ± 0.9	8.5
30	33 ± 1	3.4
300	300 ± 9	3.0
900	912 ± 11	1.2
Assay linearity ($n = 6$)		
Intercept	−0.002615 ± 0.0030	11.6
Slope	0.00095 ± 0.00004	4.0
r^2	0.99914 ± 0.00007	0.07

S.D.: standard deviation, *n*: number of samples.

Table 2
Inter-assay variability and recovery for deracoxib in plasma ($n = 6$)

Concentration added (ng/ml)	Concentration measured (ng/ml)	R.S.D. (%)	Recovery (%)
10	9	11.1	95
25	23	9.1	92
50	50	10.0	100
100	84	10.0	85
250	220	7.1	90
500	481	7.7	92
800	779	5.8	94
1000	955	6.8	88
1500	1351	6.5	89

n: number of days.

spiked with specific concentrations of deracoxib ranged from 1.2 to 3.4% (Table 1). The LLOQ was 10 ng/ml and its R.S.D. was 8.5% (Table 1). The inter-assay R.S.D. ranged from 5.8 to 11.1%. The individual values are reported in Table 2. The recovery of deracoxib from spiked feline plasma was compared with the directly injected analyte at concentrations of 10, 25, 50, 100, 250, 500, 800, 1000, and 1500 ng/ml. Values ranged from 85 to 100% (Table 2). The recovery of the internal standard, tolbutamide was 94% at the concentration used in the assay (200 μg/ml). The limit of detection for deracoxib in plasma was 5 ng/ml. This represents a peak approximately three times baseline noise. Testing of autosampler and short-term stability of standards for 24 h indicated that deracoxib is stable. Values for 25 and 1000 ng/ml standards after 24 h in the autosampler were 23 and 999 ng/ml and 26 and 1001 ng/ml for short-term 24 h storage.

4. Discussion

To be useful for pharmacokinetic investigations a method must be simple as well as sensitive and reproducible. Deracoxib was quantified in feline plasma by combining a liquid extraction with isocratic reverse-phase HPLC analysis. Several solvent combinations were tested in the extraction process (methylene chloride, hexane, ethyl acetate, chloroform) as well as a solid phase extraction cartridges (Oasis HLB).

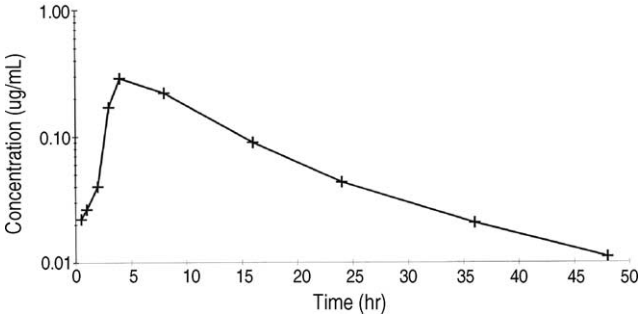

Fig. 3. Plasma concentration–time profile for deracoxib, following oral administration of 1 mg/kg to a feline.

However, the combination of chloroform and isopropyl alcohol produced the greatest recovery and optimum peak shape. The retention times of tolbutamide and deracoxib remained unaffected by changes in pH of the mobile phase (pH range 4–5.5). The use of tolbutamide as an internal standard corrects for intra- and inter-assay variability in the extraction. The stability studies indicate that samples are stable for 24 h after extraction. Therefore, if there was an equipment malfunction, samples could be reanalyzed. Samples in our study were thawed one time and analyzed therefore, freeze–thaw studies were not conducted. The limit of detection and recovery are more than adequate for use in pharmacokinetic studies.

In conclusion, the newly developed method is simple, sensitive, specific and allowed the analysis of deracoxib in feline plasma samples. The method was found to be suitable for the generation of plasma concentration–time curves (Fig. 3) and the determination of pharmacokinetic parameters in studies conducted at this facility.

References

[1] D.L. Millis, J.P. Weigel, T. Moyers, F.C. Buonomo, Compend. Contin. Educ. Pract. Vet. 3 (2002) 7.
[2] C.E. Akesson, P.E. Linero, Am. J. Vet. Res. 43 (1982) 1471.
[3] D.G. Allen, I.B. Johnstone, S. Crane, Am. J. Vet. Res. 46 (1985) 660.
[4] L.E. Davis, J. Am. Vet. Med. Assoc. 176 (1980) 65.
[5] E.J. Larson, J. Am. Vet. Med. Assoc. 143 (1963) 837.
[6] P. Lees, P.M. Taylor, Br. Vet. J. 147 (1991) 298.
[7] R.A. Yeary, W. Swanson, J. Am. Vet. Med. Assoc. 163 (1973) 1177.
[8] E. Stromer, S. Bauer, J. Kirchheiner, J. Brockmoller, I. Roots, J. Chromatogr. B 783 (2003) 207.
[9] H.H.S. Chow, N. Anavy, D. Salazar, D.H. Frank, D.S. Alberts, J. Pharm. Biomed. Anal. 34 (2004) 167.
[10] M.J. Rose, E.J. Woolf, B.K. Matuszewski, J. Chromatogr. B 738 (2000) 377.
[11] G. Jayasagar, M.K. Kumar, K. Chandrasekhar, P.S. Prasad, Y.M. Rao, Pharmazie 57 (2002) 619.
[12] M.S. Guirguis, S. Sattari, F. Jamali, J. Pharm. Pharmaceut. Sci. 4 (2001) 1.