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International Journal of Pharmaceutics 174 (1998) 117–124

international journal of pharmaceutics

Preliminary pharmacokinetics of a new anthelmintic agent, CDRI-81/470 in healthy subjects[1]

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Received 23 February 1998; received in revised form 29 June 1998; accepted 24 July 1998

Abstract

Single dose pharmacokinetic study of CDRI-81/470, a new broad spectrum anthelmintic agent, was carried out in 12 healthy human subjects after a single 375-mg oral dose. The serum, saliva and urine samples were analyzed by HPLC. The compound attained peak serum levels of 15.1 ± 4.6 μg/ml in 2.6 ± 1.1 h and could be measured up to 5 days. Mean serum AUC was 195 ± 69 μg per h/ml. The compound showed a mean apparent elimination half-life of 12.1 ± 4.5 h while mean residence time (MRT) was found to be 11.1 ± 1.7 h. Extent of urinary excretion of CDRI-81/470 (2.3 ± 0.7%) was less than that of its decarboxylate metabolite (5.3 ± 2.2%) up to 10 h post dose. In vivo protein binding in serum was 93.1 ± 1.2% and remained constant over in vivo concentration range. The salivary levels of CDRI-81/470 were higher than the corresponding unbound serum levels. There was a significant correlation between serum and salivary levels of CDRI-81/470, with a mean ratio of saliva to unbound serum levels of 2.04 ± 0.24, indicating the possibility of predicting serum concentrations of CDRI-81/470 from non-invasive salivary sampling technique. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Benzimidazole anthelmintic; CDRI-81/470; Human; Oral; Single-dose; Clinical pharmacokinetics

1. Introduction

CDRI-81/470 (methyl-*N*[5[[4-(2-pyridinyl)-1-piperazinyl]-carbonyl]-1*H*-benzimidazol-2-yl] carbamate; Fig. 1) is a new broad spectrum anthelmintic (Chatterjee et al., 1984) belonging to the benzimidazole class and is being developed at the Central Drug Research Institute (CDRI), for clinical and veterinary use. CDRI-81/470 has shown good efficacy against both intestinal and systemic parasites in laboratory experimental animals (Katiyar et al., 1984, 1987, 1988; Chatterjee et al., 1984; Srivastava et al., 1988), cattle, poultry

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[1] CDRI communication number 5759.

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PII S0378-5173(98)00251-8

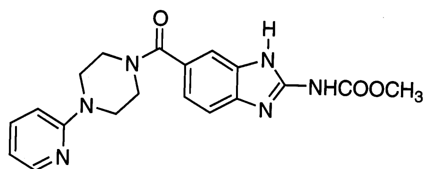

Fig. 1. CDRI compound CDRI-81/470.

and sheep (CDRI, 1997). Its pharmacokinetic behavior has been reported in rats with a mean elimination half-life of 4 h (Paliwal et al., 1989; Nagaraja et al., 1995a). In calf, biphasic absorption of CDRI-81/470 was observed from solution formulation while the suspension showed a delayed absorption with mean residence time (MRT) of more than 25 h (Nagaraja et al., 1998). It is in phase II clinical trials and has been given marketing permission for veterinary use in India. As part of the drug development programme, the present communication describes the single oral dose pharmacokinetics of CDRI-81/470 in healthy human subjects.

2. Materials and methods

2.1. Chemicals

The reference standard of CDRI-81/470 (99.7% pure) and capsules containing 375 mg of CDRI-81/470 were procured from the Pharmaceutics Division of this institute. The *N*-decarboxylate metabolite of CDRI-81/470 (DM) was synthesized in this division. Solvents like acetonitrile, ethylacetate, and isopropanol were of HPLC grade and procured from S.D. Fine Chemicals and Spectrochem, Mumbai (India). Anaesthetic grade diethyl ether I.P. (Industrial Solvents and Chemicals, Thane, India) was purified by distillation for extraction of biosamples. All other chemicals and reagents were of analytical grade and used without further purification. Serum was prepared from normal human blood, procured freshly from healthy volunteers through a local blood bank and pooled for spiking and using as control blank samples. Human serum albumin (HSA; fraction V) and protein assay reagent (solution of Coumassie Brilliant Blue) were purchased from Sigma, St. Louis, MO, USA) and Bio-Rad Laboratories, Hercules, CA, USA, respectively.

2.2. Subjects

After approval of the study protocol by the Ethics Committee constituted in accordance with the regulations of the Drug Controller of India, 12 healthy volunteers aged between 20 and 45 years (35 ± 9 years) and weighing between 40 and 69 kg (52 ± 9 kg) were recruited. A complete medical check-up including routine physical and haematological tests and urinalysis was carried out before the initiation of the study, was repeated 24 h after dosing and again at the end of the study to monitor any adverse effects of CDRI-81/470. All the subjects were non-smokers and were not taking any known enzyme-inducing or inhibiting agent within 30 days of the study. Further, the subjects abstained from alcohol, tobacco and concurrent medication for 3 days prior to and during the study. However there was no restriction on tea or coffee prior to and 10 h after the dose.

2.3. Formulation and dosing

The dose for preliminary clinical pharmacokinetic studies was calculated from efficacy studies in experimental animals. The volunteers were admitted to the Clinical and Experimental Division of CDRI and fasted overnight with free intake of water. Each subject received a capsule in the morning with 200 ml water. Standard breakfast was given 2 h after the dosing followed by vegetarian lunch, evening tea, and vegetarian dinner. The subjects were discharged from the clinic after collecting the 10-h samples and were allowed their normal food during the remaining period of the study.

2.4. Sample collection

After dosing, serial blood samples (5 ml) were obtained at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 48, 72, 96, and 120 h. Initial samples up to

10 h were collected from a cephalic vein via an indwelling catheter and later by direct venipuncture. Blood was allowed to clot at 37°C for 30 min and serum was harvested. Urine was completely voided just before dosing in only four volunteers and then at frequent intervals (1–2 h) up to 10 h after dosing. Volume of urine voided at each interval was measured and an aliquot was stored pending analysis. Saliva (0.5–1 ml) could be collected in only two volunteers at 0, 1.5, 2, 3, 4, 6, 8, 10, and 24 h post dose simultaneously with blood sampling. Sodium chloride was placed in the mouth for enhancing salivation. All the samples were stored at −30°C in glass tubes until analysis (15–45 days).

2.5. Sample analysis

2.5.1. Serum and saliva

Serum and saliva were analysed by HPLC method reported earlier (Nagaraja et al., 1995b) with minor modifications. Serum or saliva (0.2 ml) was extracted twice with 3-ml diethyl ether in a glass tube. The combined organic phase was evaporated to dryness and the residue was reconstituted in the mobile phase for chromatography. The recoveries of CDRI-81/470 from serum at 5 and 10 μg/ml in validation samples ($n = 5$) were found to be over 85% and were comparable to the reported values (Nagaraja et al., 1995b). Recovery of CDRI-81/470 from saliva was also over 85% and consistent over the calibration range. Variations in accuracy and precision at low, medium, and high concentrations in saliva were within acceptable limits (Shah et al., 1992). Calibration standards and quality control (QC) samples at low, medium, and high concentrations in both serum and saliva were processed and analysed with each batch of samples.

2.5.2. Urine

Urine samples were analysed by a simultaneous HPLC estimation method for CDRI-81/470 and its metabolite DM in rat urine, developed in this laboratory (unpublished). Briefly, the urine was acidified and extracted with ethyl acetate for the selective removal of polar impurities. Urine layer was then basified to pH 8 and extracted with a solvent mixture containing diethyl ether:isopropanol (90:10, v/v). The organic layer was evaporated to dryness before reconstitution in the mobile phase for HPLC analysis. Calibration standards and QC samples were analysed as described for serum and saliva. The validation of the assay method was done in terms of recovery, accuracy and precision at 10, 100, and 1000 ng/ml for CDRI-81/470 and at 25, 100, and 1000 ng/ml for DM. The inter- and intra-batch percent relative standard deviation (%RSD) for CDRI-81/470 and DM were less than 12 and 16%, respectively. The %bias from the nominal spiked concentration for both the compounds was less than 11%. The recovery of CDRI-81/470 and DM from fortified urine samples was from 90 to 109% and from 75 to 83%, respectively.

2.5.3. Chromatographic conditions

Mobile phase (Nagaraja et al., 1995b) consisted of phosphate buffer (50 mM, pH 6) and acetonitrile in the ratio of 65:35, v/v for the analysis of serum or saliva and 75:25 for the analysis of urine and was pumped at 1 ml/min by an HPLC pump (Kontron, Model 600, Zurich, Switzerland). A fluorescence HPLC monitor (Shimadzu, Model RF-535, Kyoto, Japan), set at excitation and emission wavelengths of 295 and 375 nm, respectively, coupled with a Philips computing integrator (Pye Unicam, Model PU4811, Cambridge, UK) was used to monitor the compound. Separations were achieved on a reversed-phase C_{18} column (Spheri-5, 5 μm, 220 × 4.6 mm i.d.) preceded by a guard column (30 × 4.6 mm i.d.) (Pierce Chemical, Rockford, IL, USA). Samples were injected through a fixed 50-μl loop injector (Rheodyne, Model 7125, Cotati, CA, USA). Retention times for CDRI-81/470 were 5.0 ± 0.2 min in serum and saliva, and 11.0 ± 2.0 min in urine and that of DM in urine was 7.0 ± 0.2 min. There was no interference from the endogenous components of the matrices or metabolites in the region of interest as indicated by the clean chromatograms. The peaks of drug and metabolites were sufficiently resolved for quantitation. The calibration curves were linear in serum (10–10000 ng/ml) and saliva (10–2000 ng/ml) for CDRI-81/470, and 10–1000 ng/ml for CDRI-81/470 and

25–1000 ng/ml for DM in urine (coefficients of correlation, $r > 0.999$ in serum, saliva, and urine).

2.6. Protein binding

Representative serum samples collected up to 24 h post dose from all the volunteers ($n = 36$) were used to assess the protein binding of CDRI-81/470. Bound and unbound fractions of the compound were separated by ultrafiltration technique. A 1-ml aliquot was introduced into disposable Centrifree™ Amicon Micropartition System (Amicon, Beverly, MA, USA) which utilizes a YMT ultrafiltration membrane (molecular weight cut-off, ≈ 30000), and the serum sample was centrifuged at $1500 \times g$ in a fixed angle rotor for 10 min at 37°C to collect approximate 10% (100 μl) of the original volume of serum as ultrafiltrate. Ultrafiltrates were analysed for CDRI-81/470 content by mixing with mobile phase (1:1, v/v) and directly injecting onto HPLC system. Preliminary mass balance studies indicated that CDRI-81/470 is not adsorbed to the membrane or ultrafiltration device and that leakage of serum proteins was less than 0.005%, as determined by Bradford method (Bradford, 1976).

2.7. Data analysis

Pharmacokinetic parameters were estimated by fitting the concentration-time data of individual subjects with PCNONLIN software (Version 4, Statistical Consultants, Lexington, USA). The apparent elimination rate constant was calculated using terminal data points of the log-linear curve. MRT was determined as AUMC/AUC. Total serum clearance (Cl/F) and volume of distribution (V/F) were calculated from the AUCs using CL/F = dose/AUC and V/F = dose/(AUC $\cdot$ k), where k is calculated by linear regression of the terminal 4–5 declining concentrations. C_{max} and T_{max} are the mean observed values. In addition, AUCs up to last sampling time of saliva and unbound serum concentrations were calculated by combination of log- and linear-trapezoidal rule. Unbound CDRI-81/470 concentrations in serum of each subject was determined by using the equation:

$$\%\text{unbound} = (C_f/C_t) \times 100$$

where C_f = concentration of drug in ultrafiltrate and C_t = concentration of drug in serum sample before ultrafiltration.

Statistical analysis was performed using Microsoft Excel software (Version 5, Microsoft Corporation, USA), with a confidence level of 95%.

3. Results

After administration of CDRI-81/470 each subject was closely monitored. Pulse rate and blood pressure were recorded hourly for the first 10 h. None of the subjects complained of any undesirable experience or untoward effects. Haematological, biochemical and urinalysis tests did not reveal any significant change between pre- and 24 h and 5 day post-dose parameters.

3.1. Pharmacokinetics in serum

The parent compound could be measured in the serum of all 12 subjects from 0.5 h (first sampling time) up to 72 h in two, 96 h in seven, and 120 h in three subjects. The concentrations in subject 1 were significantly lower than the other eleven subjects and no absorption phase was observed in subject 1. This data set could not be fitted to an appropriate pharmacokinetic model with extravascular dosing. Hence the parameters were estimated by non-compartmental method (Table 1). Mean serum concentration-time profile ($n =$

Table 1
Pharmacokinetic parameters of CDRI-81/470 in serum of human volunteers

Parameter	Mean ± S.D. ($n = 11$)	Subject 1[a]
C_{max} (μg/ml)	15.1 ± 4.60	1.75
T_{max} (h)	2.64 ± 1.10	0.50
$AUC_{0-\infty}$ (μg · h/ml)	195 ± 69.10	12.10
$t_{1/2}$ (h)	12.1 ± 4.50	28.80
MRT (h)	11.1 ± 1.70	12.90
CL/F (l/h)	2.23 ± 1.00	31.10
V/F (l)	36.3 ± 13.90	1289.23

[a] Outlier.

11) is presented in Fig. 2 with the inset giving the profile in subject 1. Peak serum concentrations (15.1 ± 4.6 μg/ml, $n = 11$) were achieved at 2.64 ± 1.1 h. The apparent elimination half-life was 12.1 ± 4.5 h and was comparable with MRT (11.1 ± 1.7 h). There were no secondary peaks in the elimination phase of serum concentration-time curves of CDRI-81/470 in human volunteers, indicating the absence of any significant enterohepatic recirculation of CDRI-81/470.

3.2. Binding of CDRI-81/470 in in vivo serum samples

Extent of binding of CDRI-81/470 in serum samples ($n = 36$) collected between 0.5 and 24 h post dose from 12 volunteers was studied. To determine the effect of concentration of drug these samples were arbitrarily divided into three groups: low, medium and high. Three serum samples from each volunteer, representing low (less than 2 μg/ml), medium (2–7 μg/ml), and high (10–27 μg/ml) in vivo levels of CDRI-81/470 (0–24 h) were analysed. The binding of CDRI-81/470 was linear and non-saturable in the therapeutic range as shown in the scatter plot in Fig. 3 and ranged from 90 to 94% in all the subjects. The %binding at low, medium and high was 92.55 ± 1.24, 93.26 ± 1.11 and 93.18 ± 1.23, respectively.

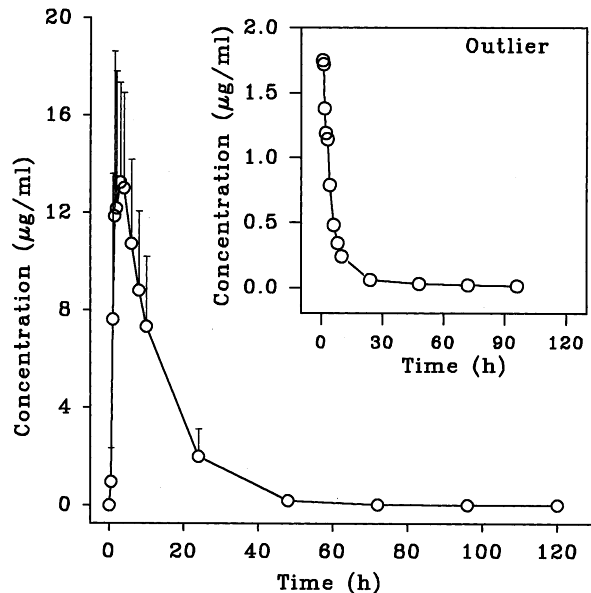

Fig. 2. Concentration-time profile of CDRI-81/470 in serum of human volunteers (mean $\pm$ S.D., $n = 11$). Inset shows the profile in the outlier.

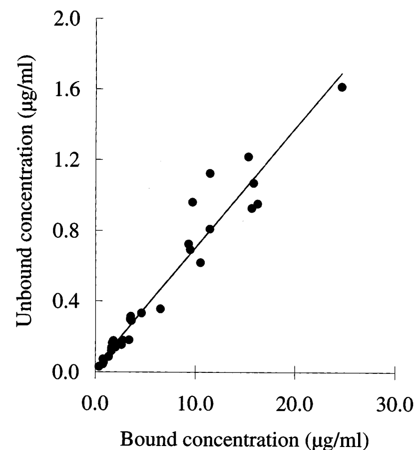

Fig. 3. Linear relationship between bound and unbound concentration of CDRI-81/470 in serum of 12 healthy subjects receiving single 375-mg oral dose.

3.3. Excretion in saliva

The parent compound could be measured in all the collected saliva samples. Ratios of CDRI-81/470 salivary concentration to unbound serum concentration were calculated at each time point and the mean ratios were found to be 2.06 ± 0.31 and 2.04 ± 0.24 in two subjects. The salivary to unbound serum concentration ratios based on AUC were 1.99 and 2.03 in the two subjects, respectively. An excellent correlation was observed between serum and salivary concentrations of CDRI-81/470 (Fig. 4; $r = 0.9444$, $p < 0.05$).

3.4. Excretion in urine

Intact CDRI-81/470 and its metabolite, DM, were excreted in urine. Extent of urinary excretion of parent compound (2.32 ± 0.72% of dose) was less than DM metabolite (5.26 ± 2.25%, expressed as equivalent to CDRI-81/470), up to 10 h (Table 2).

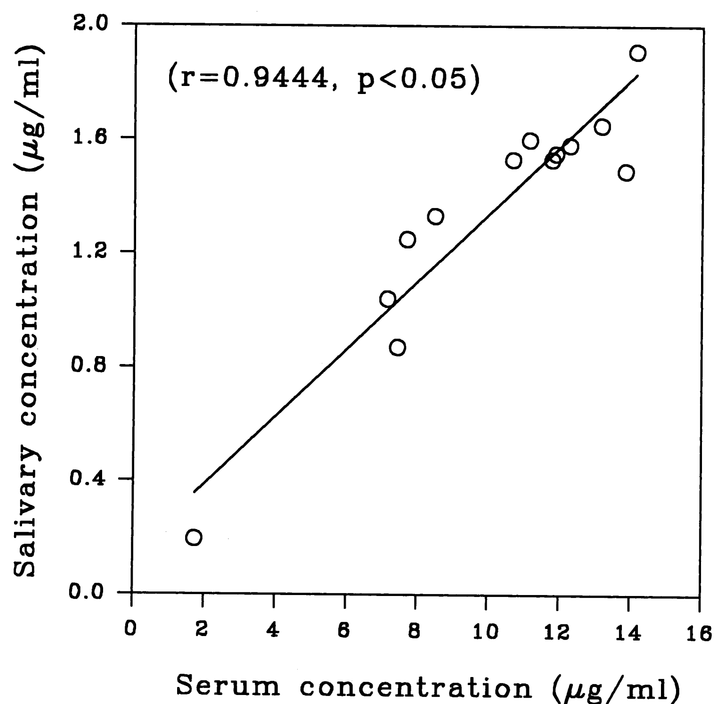

Fig. 4. Linear relationship between serum and salivary concentrations of CDRI-81/470 in serum of 12 healthy subjects receiving single 375-mg oral dose.

4. Discussion

Benzimidazoles have limited solubility in water; consequently, minor differences in solubility tend to have a major effect on absorption. Benzimidazole group of anthelmintics, except thiabendazole, show poor absorption (Morris et al., 1983; Katz, 1986). Mebendazole shows only 5–10% absorption by oral route with extensive first-pass metabolism (Goodman et al., 1996). In patients receiving 26–161 mg/kg per day mebendazole for prolonged periods, 10–300-ng/ml concentrations have been observed (Luder et al., 1985, 1986). Another study reported serum mebendazole concentrations of about 30 ng/ml after a 1.5-g oral dose (Bekhti et al., 1986). With a 400-mg single dose of albendazole, peak serum concentrations of its active sulphoxide metabolite were about 1 μg/ml (Morris et al., 1983; Marriner et al., 1986) and AUC of 2 μg·h/ml. In the present study, following a single oral dose of 7.2 mg/kg (average of 52 kg body weight), CDRI-81/470 gave higher peak serum levels of 15.1 ± 4.6 μg/ml and AUC of 195 ± 69 μg·h/ml. When viewed with this perspective, it appears that CDRI-81/470 shows higher bioavailability of parent compound than either mebendazole or albendazole. CDRI-81/470 like thiabendazole is rapidly absorbed after oral ingestion and reaches peak levels in ≈ 2–3 h. In contrast, tablet formulations of mebendazole are

Table 2
Excretion of CDRI-81/470 in human urine up to 10 h post oral dose

Volunteer	% Administered dose excreted	
	CDRI-81/470	DM[a]
3	2.93	8.41
4	2.73	4.22
5	1.32	3.21
6	2.29	5.2
Mean ± S.D.	2.32 ± 0.72	5.26 ± 2.25

[a] Expressed as equivalent to CDRI-81/470.

poorly and erratically absorbed, and concentrations in plasma are low and do not reflect the dosage taken (Witassek et al., 1981). After a 400-mg oral dose, albendazole is also erratically absorbed and cannot be detected in plasma due to extensive first pass effect (Marriner et al., 1986). Unlike other benzimidazoles, no metabolites of CDRI-81/470 were detected in serum.

The absorption rate of CDRI-81/470 in humans was comparable with rats which also showed T_{max} of 1–2 h (Nagaraja et al., 1995a). These results were in contrast with those observed earlier in calf (Nagaraja et al., 1998), wherein, solution formulation showed rapid absorption with secondary peaks while suspension showed delayed absorption ($T_{max} > 15$ h) without secondary peaks. These differences in absorption of CDRI-81/470 between humans and calf are attributable to the differences in anatomical and physiological features between monogastric and ruminant species (Lin, 1995).

The serum concentration-time data of CDRI-81/470 could be fitted to one compartment model in most of the subjects. A biexponential decay was observed only in the subjects showing measurable concentrations of CDRI-81/470 beyond 96 h ($n = 3$). However, the terminal phase started as late as 72 h with very low concentrations and could not be fitted by two compartment model satisfactorily. Hence, all the 11 data sets were analysed by one compartment model. The mebendazole plasma concentration-time profiles differed considerably between patients and the elimination half-lives ranged from 2.8 to 9.0 h (Braithwaite et al., 1982). Plasma half life of albendazole sulphoxide, the active metabolite of albendazole, were 8–9 h (Marriner et al., 1986).

There was a significant difference in the apparent elimination half-life of CDRI-81/470 between humans (12 h) and rats (4.3 h) ($p < 0.05$), while MRT was comparable in these two species (11.2 h in humans and 8 h in rats). However, MRT in either human or rat was significantly different from calf (25 h). These results clearly highlight the differences in the disposition of CDRI-81/470 between monogastric and ruminant species.

Extent of binding of CDRI-81/470 in the in vivo serum samples of the volunteers was determined taking into consideration different time points after dosing and different concentrations of the compound. The binding results in all the volunteers were of the same order (90–94%) and were comparable with in vitro binding data in human serum (unpublished results). As the binding of CDRI-81/470 remained constant over the three in vivo concentrations (1–27 μg/ml) and over different time points (0–24 h), it could be concluded that the binding of CDRI-81/470 is independent of sampling time and drug concentrations in the range studied. The binding of CDRI-81/470 was comparable to structurally similar drug, mebendazole, showing 91% binding in serum (Luder et al., 1985), but was different from sulfoxide metabolite of albendazole (70% bound; Marriner et al., 1986).

Urine of four volunteers could be collected only up to 10 h post dose and analysed for CDRI-81/470 and DM levels. Both parent compound and its decarboxylate metabolite were present in urine samples. This was in contrast with the results in rats where DM was found only in trace levels in a few urine samples, though the assay method was capable of quantitating as low as 25 ng/ml of this metabolite. In humans, extent of excretion of this metabolite up to 10 h was more than that of parent compound.

The parent compound is excreted through saliva also. The salivary concentrations of CDRI-81/470 were higher than the corresponding unbound serum concentrations. A good correlation ($r = 0.9444$, $p < 0.05$) was observed between total serum concentrations and salivary concentrations (Fig. 4). These results indicate the possibility of predicting serum concentrations of CDRI-81/470 from salivary data. CDRI-81/470 is a weak base having $pK_a = 2.08$ and $\log P = 1.07$. It will remain largely in unionized form at physiological pH, unaffected by the fluctuations of salivary pH. Hence excretion of CDRI-81/470 in saliva will not be affected by variations in the pH of saliva or blood. Thus salivary sampling will be of immense utility in future therapeutic drug monitoring and comparative bioavailability studies of CDRI-81/470. However, this aspect requires extensive experimentation before adopting this non-invasive sampling technique.

Acknowledgements

The authors thank Dr C.M. Gupta, Director and Dr V.P. Kamboj, former Director, CDRI, for providing the necessary facilities and encouragement. N.V. Nagaraja thanks the Council for Scientific and Industrial Research, New Delhi, India, for providing him with a Senior Research Fellowship.

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