

Pharmacokinetics of the Proton Pump Inhibitor CDRI-85/92 and its Ester Prodrug, A New H⁺/K⁺-ATPase Inhibitor with Anti-ulcer Activities, after Oral Doses in Rats

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Abstract

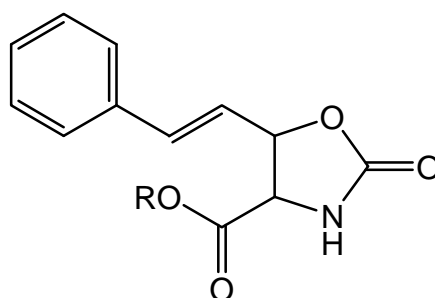
5-Styryl-4, 5-cis-1, 3-oxazole-2-one-4-carboxylic acid (CDRI-85/92) is a new proton pump inhibitor presently in advanced stage of preclinical trials as antiulcer pharmacophore. Since proton pump inhibitors are prodrugs requiring activation in acid environment, an ester prodrug of CDRI-85/92 was also synthesized. In view of the importance, pharmacokinetic study of CDRI-85/92 and its ester prodrug was generated after oral doses in rats. Following 20 mg/kg oral dose of CDRI-85/92, the compound was detectable in the serum samples up to 24 h with a maximum serum concentration (C_{max}) of 1838.40 ± 101.16 ng/ml at 1.5 h with elimination half-life of 4.96 h. Whereas, multiple C_{max} of CDRI-85/92 were observed with oral doses (equivalent to 20 mg/kg of CDRI-85/92) of the ester prodrug of the compound. All the three C_{max} of the compound were lower than that after oral dose of CDRI-85/92. The compound was eliminated slowly from serum with an elimination half-life of 5.14 h. Moreover, the systemic availability of CDRI-85/92 also decreased from 6111 ng.h/ml to 3463 ng.h/ml after the ester prodrug administration. The decrease in systemic availability of CDRI-85/92 could be due to its higher clearance after its ester prodrug administration.

Keywords: CDRI-85/92; HPLC; Pharmacokinetic; Rat; proton pump inhibitor, Prodrug

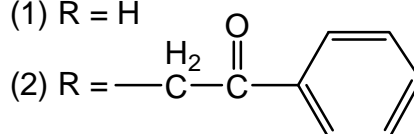
1. Introduction

Peptic and duodenal ulcers and gastro-esophageal reflux disease (GERD) result from excessive secretion of gastric acid and/or disruption of important defensive factor such as mucus by non-steroidal anti inflammatory drugs (NSAIDs), alcohol, heat, etc. Since 1976, proton-pump inhibitors (PPIs), the final common pathway of acid gastric secretion, have been the target of research in treating acid-related disorders. In such acid-related diseases, PPIs have dramatically improved the management options available for patients [1-3]. PPIs have a common chemical mechanism, namely inhibitor of H^+/K^+ -ATPase. Biochemical, pharmacological and toxicological properties can vary by the different side chains substituted at the core ring [4]. The advances in pathophysiology of peptic ulcer disease have been the driving forces for the development of new anti-acid peptic ulcer drug [5].

5-styryl-4, 5-cis-1, 3-oxazole-2-one-4-carboxylic acid (CDRI-85/92, Formula 1) was synthesized in house. The compound is an oxazole derivative and useful in the treatment of acid peptic disease [6,7]. It is an inhibitor of gastric $H^+,K(+)$ -ATPase, commonly referred to as the 'proton pump'. The compound also provides a selective and competitive inhibition of the gastric proton pump and does not inhibit the proton pumps of the kidney and bone. Percent inhibition in the free acidity fairly compares with that of Omeprazole; the latter is an irreversible proton pump inhibitor and therefore, increasing the concentration of ATP decreases the inhibition percentage of the acidity [4]. The compound has also shown good cytoprotective activity. It is currently in an advanced stage of preclinical trials. The compound shows almost equal, very low absorption rate with only 6.7-8.8% drug absorbed in 30 min at pH 2.6 and 7.4 and good metabolic stability [8,9].



(1) R = H



Formula 1. Chemical structure of CDRI-85/92 (1) and its ester prodrug (2)

For many drug targets, synthetic strategies can be devised to balance the physicochemical properties required for high transcellular absorption and the SAR for the drug target. However, there are drug targets where the SAR requires properties at odds with good membrane permeability. These include a requirement for significant polarity and group that exhibit high hydrogen bonding potential such as carboxylic acids and alcohols. In such cases, prodrug strategies have been employed. The rationale behind the prodrug strategy is to introduce lipophilicity and mask hydrogen bonding groups of an active compound by the addition of another moiety, most commonly an ester [10]. Moreover, proton pump inhibitors are prodrugs requiring activation in acid environment. Therefore, an ester prodrug of CDRI-85/92 (Formula 1) was synthesized. Preclinical pharmacokinetic study of the compound is

important, so as to delineate/correlate its absorption, distribution, metabolism and excretion as well as its dose profile [11]. Till date, there is no pharmacokinetic data available for CDRI-85/92 and its ester prodrug. In the present study, the pharmacokinetic profile of CDRI-85/92 and its ester prodrug was generated after oral dose (equivalent to 20 mg/kg of CDRI-85/92) in rats. The pharmacokinetic parameters of CDRI-85/92 from the ester prodrug were then compared with that of CDRI-85/92.

2. Methods and Materials

2.1. Chemicals and solvents

Pure reference standard of CDRI-85/92 was obtained from the Medicinal Chemistry Division, Central Drug Research Institute, Lucknow. HPLC grade acetonitrile and HPLC grade n-hexane were obtained from Ranbaxy Laboratories Limited, SAS Nagar, India. Analytical grade ammonium acetate was obtained from E.Merck (India) Ltd., Bombay. Glacial acetic acid, HPLC grade, was obtained from J.T. Baker, Phillisburg, NJ, USA. MilliQ Millipore water was used during the study as and when required. Parafilm (Parafilm 'M' Lab film, American can company, CT, USA) was used to seal containers during storage and processing of samples.

Serum obtained from male Sprague Dawley rats (250 ± 25 g) was used as the biological matrix. Blood was collected in clean test tubes from inferior vena cava of male Sprague Dawley rats, allowed to clot for 45 min, centrifuged at 2000 rpm for 10 min to separate the serum. The serum thus separated was pooled and stored at -60°C .

2.2. Animals

The studies were carried out in young, healthy, male Sprague Dawley rats weighing 250 ± 25 g. They were obtained from Laboratory animal division of the institute, housed in plastic cages and were given rodent food and water *ad libitum*. The rats were acclimatized at least for one day before the commencement of the study. All experiments in rats, euthanasia and disposal of carcasses were carried out as per the guidelines laid down by Local Ethics Committee for animal experimentation. Care was taken to minimize the trauma due to pain during all surgical procedures and blood sampling by the use of ether anesthesia, taking suitable pre- and post-operative care.

2.3. Pharmacokinetic study

A suspension formulation containing 5 mg/ml of CDRI-85/92 was prepared by triturating the compound and 5 mg carboxymethyl cellulose, by geometric addition, per each ml of triple distilled water added drop wise in a mortar and pestle for dose of 20 mg/kg respectively. The suspension formulation containing 7.5 mg/ml of ester prodrug (equivalent to 5 mg/ml of CDRI-85/92) was prepared as described above. The suspensions were freshly prepared just before dosing and required quantities were accurately weighed as per the rats to be dosed.

Thirty animals were divided into two equal groups. All animals were fasted overnight before oral drug administration. Animals were provided with rodent food and water *ad libitum* after 2 h of oral dose. To the first group of rats, suspension formulation containing CDRI-85/92 was administered and the other group of rats were administered the suspension formulation containing ester prodrug of CDRI-85/92. The rats were fed orally using rat feeding needle at doses of 20 mg/kg to conscious rats in a volume of approximately 1 ml/250 gm rat and the time of dosing was recorded. Blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, and 24 h post dose. Two blood samples were withdrawn from each animal, from both the groups. An initial 1 ml blood sample was withdrawn by cardiac puncture under light anesthesia followed by sample from inferior venacava (terminal sample), from the dosed rats

using 24G needle and a syringe in a clean and dry test tube. Total volume of blood collected from each rat was not more than 3% of the total body volume. The blood was allowed to clot, by keeping the tube on a slant, approximately for 45 min. This was centrifuged at 2000 rpm for 10 min. The serum was separated into clean and neatly labeled tubes and stored at -60°C pending analysis.

2.4. Instrumentation and chromatographic conditions

The HPLC system consisted of LC-10AD pumps (Shimadzu Corporation, Kyoto, Japan) equipped with a model 7725 injector (Rheodyne, Berkeley, USA) fitted with a fixed 100- μ l sample loop. Separations were performed on a C-18 column (5 μ m, 220 \times 4.6 mm, i. d.) coupled with a guard column packed with the same material (5 μ m, 30 \times 4.6 mm, i. d.) (E. Merck, Darmstadt FR Germany) maintained at ambient temperature. Column eluents were monitored using a model SPD-10AV UV-Visible detector (Shimadzu Corporation, Kyoto, Japan) set at wavelength of 250 nm. Chromatographic responses were integrated using Chromatopac C-R5A integrator (Shimadzu, Kyoto, Japan). The HPLC system was equilibrated for approximately 30 min at a flow rate of 1 ml/min prior to commencement of the study.

Vortex-mixer (Maxi Mix, Thermolyne, USA) was used to vortex-mix the samples during the course of the study. Sonicator (Branson Cleaning Co., Shelton, USA) was used to sonicate the mobile phase before analysis to remove dissolved gases and ensure proper mixing. Model SVC-220H Speed vacuum concentrator (Savant Instruments Inc., USA) was used to concentrate the samples after extraction. Model BHG Hermle (Germany) centrifuge was used to centrifuge the samples as and when necessary. Ultra Freeze (U41085, -80°C) Ultra low Freezer (New Brunswick Scientific) was used to store the samples pending analysis. Besides these, Electronic weighing balances Mettler AE 163, Mettler PE 1600 and APX 175E/C Control dynamic pH meter (CD instrumentation Pvt. Ltd., Bangalore) was used during weighing and pH adjustment, respectively.

2.5. Preparation of mobile phase and reconstitution solution

Mobile phase A was prepared by mixing methanol and ammonium acetate buffer (10 mM, pH 5.0) in 30:70 % (v/v) ratio and degassed in an ultrasonic bath for 15 min just before chromatography. Mobile phase B was prepared by mixing acetonitrile and buffer in 80:20 (% v/v) ratio and degassed in an ultrasonic bath for 15 min just before chromatography. Mobile phases were pumped under isocratic conditions, through pumps A and B at 0.05 and 0.95 ml/min, respectively. The reconstitution solution was prepared by mixing mobile phase A and mobile phase B in 5:95 (%v/v).

2.6. Stock and standard solutions

The stock solution of CDRI-85/92 was prepared in acetonitrile at 100 μ g/ml, labeled and stored at 4°C. Working stock solutions (WSS) containing 0.2, 0.4, 0.8, 2, 4, 8, 20, 40 and 80 μ g/ml of CDRI-85/92 was prepared in acetonitrile from the stock solution. The method of individual dilution was followed. Seven analytical standards in mobile phase containing 25, 50, 100, 250, 500, 1000 and 2500 ng/ml concentrations of compound CDRI-85/92 were prepared from WSS. 1.25 ml of each WSS was taken in different volumetric flasks (10 ml) and the volume was made up to 10 ml with mobile phase. These were vortex-mixed for 1 min, labeled and stored at 4°C.

Calibration standards of CDRI-85/92 were prepared by individually spiking rat serum. The WSS were used to prepare calibration standards in rat serum containing 10, 20, 40, 100, 200, 400 and 1000 ng/ml of CDRI-85/92. 25 μ l of each WSS was transferred to different test tubes and diluted with 0.5 ml normal rat serum.

Quality control samples of low (10 ng/ml), medium (100 ng/ml) and high (1000 ng/ml) concentrations of CDRI-85/92 were prepared by spiking 25 µl of appropriate WSS to 0.5 ml normal rat serum taken in clean and dry tubes, while vortexing.

2.7. Sample preparation

To 0.5 ml serum (blank, spiked or test) in a test tube, 1.5 ml of precipitating solvent, acetonitrile was added and vortex-mixed for 10 sec. These were allowed to stand for 30 min at 4°C for complete precipitation and then vortex-mixed for 1 min and centrifuged at 2000 rpm for 10 min at 5°C. The supernatant (1.5 ml) was transferred into a clean conical tube by pipette without disturbing the lower precipitated pellet. To this, 2.0 ml of extraction solvent, n-hexane was added and vortex-mixed for 1 min. The hexane layer was removed by decanting after snap freezing the acetonitrile layer in liquid nitrogen and the acetonitrile layer was evaporated to dryness in speed vac concentrator without applying any heat. After vacuum drying, the residue was reconstituted in 200 µl of mobile phase, vortex-mixed for 1 min and centrifuged at 2000 rpm for 10 min. The clear solution was injected onto HPLC system for analysis.

2.8. Method validation

The HPLC assay method was validated in terms of linearity, accuracy, recovery, and precision. A protocol was prepared which contained the complete details of the compound, chemicals and instrumentation, stocks and standard solutions, serum requirements and the plan of validation.

2.8.1. Calibration curve and linearity

For data analysis, a calibration curve was plotted using peak heights of the calibration standards (CS) obtained, against their corresponding concentrations. In the present study, triplicates of each calibration standard were assayed. The spiked concentration and their respective peak heights were subjected to least-squares regression (with and without intercepts) and weighted least-squares regression (1/x and 1/x²) using Microsoft Excel (Version 5.0) and suitable method was adopted.

2.8.2. Recovery

For calculation of recovery of the compound, spiked control samples were prepared at low (10 ng/ml), medium (100 ng/ml) and high (1000 ng/ml) concentrations for serum. The samples were processed as mentioned above and the concentration of CDRI-85/92 was determined from the regression of the analytical standard calibration curve. Recovery was calculated by comparing the observed concentrations with the spiked concentrations using the formula:

$$\% \text{ Absolute recovery} = \frac{\text{Mean observed concentration}}{\text{Nominal concentration}} \times 100$$

2.8.3. Sensitivity

The detection limit of the method (LOD) was the drug quantity detected in the biomatrix after the sample clean-up corresponding to three times the baseline noise (S/N > 3). The limit of quantitation (LOQ) was defined as the concentration of the sample, which was quantified, with less than 20% deviation in precision.

2.8.4. Accuracy and Precision

To determine accuracy and precision, replicates of the spiked control samples at low, medium, and high concentrations were prepared. One set of the samples consisting of low,

medium and high concentrations of the compound was assayed on the day of preparation. The concentrations of the compound in the samples were determined from the calibration curve constructed from the calibration standards, prepared by spiking CDRI-85/92 in corresponding matrix, to their true or normal value. The assay was repeated with another set of samples spiked at the three concentrations on three more occasions. Intra- and inter-batch accuracy was determined by calculating the %bias from the theoretical concentration.

$$\% \text{ Bias} = \frac{\text{Observed concentration} - \text{Nominal concentration}}{\text{Nominal concentration}} \times 100$$

Precision was obtained by subjecting the data to one-way analysis of variance (ANOVA) as within and between percent relative standard deviation (%RSD). RSD was calculated using the mean square value. An acceptance limit of 20% was employed for the low concentrations and 15% was applied for medium and high samples [12].

2.9. Data analysis

Concentrations of CDRI-85/92 were calculated by comparing the detector responses with the calibration curves drawn with the same batch. A non-compartmental approach was adopted to explain the data using WinNonlin program (Standard Version 1.5, SCI Software Inc., Lexington, KY). Statistical analyses like ANOVA, linear regression, etc. were performed using Microsoft Excel (Version 5.0, Microsoft Corporation, USA). The area under the serum concentration-time curve from time zero to time of last sample with quantifiable analyte concentration (AUC_t) was calculated using linear trapezoidal rule. The area under the serum concentration-time profile from time zero to infinity ($AUC_{0-\infty}$) was calculated from (AUC_{0-t}) adding the extrapolated area determined by dividing the observed concentration at the time of last serum concentration ($>LLOQ$) by the slope (K) of the terminal log-linear phase. The terminal rate constant (K_e) was calculated by linear regression using least square method, which fitted a straight line to terminal phase of semi logarithmic plot of serum-concentration data. Half-life of elimination ($t_{1/2}$) was obtained from the formula $0.693/K_e$. The values of total body clearance (Cl/F) and those of apparent volume of distribution (V_d/F) were calculated as $\text{Dose}/AUC_{0-\infty}$ and $\text{Dose}/(K_e \cdot AUC_{0-\infty})$ respectively. The mean residence time (MRT) was calculated from $MRT = AUMC/AUC_{0-\infty}$.

3. Results

3.1. Method validation

The HPLC assay method was successfully validated for three days for the quantitation of CDRI-85/92 in rat serum. The assay provided reproducible estimates of the compound with sensitivity up to 10 ng/ml. The endogenous impurities did not interfere with the elution of the compound indicating that the method was selective. With the HPLC conditions, the compound eluted at an optimum retention time (8.5 ± 0.2 min). Representative chromatograms of (a) Mobile phase standard containing 250 ng/mL CDRI 85/92 (b) Drug-free rat serum, (c) Calibration standard containing 100 ng/mL CDRI 85/92, (d) Test serum sample taken after 2h after 20 mg/kg oral dose of CDRI 85/92 in rat are shown in Figure 1.

Calibration curves were derived from three injections of seven concentrations of calibration standards. The % error was $\leq 20\%$ at low and $\leq 15\%$ at all other concentrations showing that the calibration curve was linear. A typical calibration curve had the regression equation of $Y = (109.90) X + 259.05$ ($r = 0.9999$). Recoveries from the serum were always greater than 75% at all the concentrations of the compound in QC samples as summarized in Table 1. As shown in Table 2, the overall mean precision, defined by the relative standard deviations, ranged from -9.17 to 9.43%. Analytical accuracy, expressed as the percent difference of the mean observed values compared with known concentration varied from -9.17 to 10.59%.

3.2. Pharmacokinetic studies

The animals tolerated the treatments as no peculiarities in the animals' behaviour were observed. The course of serum concentration-time curves obtained after oral dose of the compound and its ester prodrug are shown in Figure 2. Following 20 mg/kg oral dose of CDRI-85/92, serum level of the compound could be estimated up to 24 h with a C_{max} of 1838.40 ± 101.16 ng/ml which occurred at 1.5 h post dose. The elimination half-life, clearance (Cl/F) and volume of distribution (V_d/F) were 4.96 h, 3.27 L/h/kg and 23.42 L/kg, respectively (Table 3).

Following oral dose of ester prodrug of the compound, CDRI-85/92 could be estimated up to 24 h but the concentration-time profile was discontinuous and showed secondary peaks (Figure 2). The elimination half-life, clearance (Cl/F) and volume of distribution (V_d/F) were 5.14 h, 5.54 L/h/kg and 41.07 L/kg, respectively (Table 3).

4. Discussion

During optimization of the mobile phase composition, the best results in terms of resolution, sensitivity and run time were obtained using solvent A [30% methanol in ammonium acetate buffer (10 mM, pH 5.0)] in pump A and solvent B [80% acetonitrile in ammonium acetate buffer (10 mM, pH 5.0)] in pump B at a flow rate of 1 ml/min with C-18 column. Isocratic elution, with pumps A and B delivering 5 and 95% of solvents A and B, respectively, was employed to facilitate resolution of the analyte. Acetonitrile was chosen as the organic solvent in mobile phase due to its good elution strength and less viscosity. Moreover, strongly acidic mobile phase can shorten column life. The extraction procedure and the chromatographic conditions were suitable for the quantitative analysis of the analyte.

A validation protocol was prepared which contained the complete details of the compound, chemicals and instrumentation, stocks and standards, serum requirements, spiking procedure for the HPLC method and the plan of validation and was followed. The HPLC assay method was validated in terms of reproducibility, linearity, recovery and accuracy and precision of the compound in spiked control samples. Calibration curves were derived from three injections of seven concentrations of calibration standards. The % error was ≤ 20 at low and ≤ 15 at all other concentrations showing that the calibration curve was linear. A typical calibration curve had the regression equation of $Y = (109.90) X + 259.05$ ($r = 0.9999$). After examining the residuals and % error, least square regression (with intercept) was finally chosen for data analysis. The analysis of validation samples in three batches also confirmed the suitability of the model. Recoveries from the serum were always $>75\%$ with a coefficient of variation of $<13\%$ of the QC samples (Table 1). It was demonstrated that the HPLC method for the determination of CDRI-85/92 was reliable and reproducible since both % relative standard deviations and %bias were $\leq 11\%$ for all estimated concentrations of the compound (Table 2). The various validation parameters like accuracy, precision and recovery were within the acceptable limit of variation [12]. The intra-assay and inter-assay accuracy and precision were thus found to be acceptable for the analysis of serum sample in support of pharmacokinetic studies.

Following oral dose of the compound and its ester prodrug, serum levels of CDRI-85/92 was detected up to 24 h (Figure 2). Oral dose of the parent compound showed a C_{max} which occurred at 1.5 h post dose. Whereas, treatment with ester prodrug of the compound at oral dose equivalent to CDRI-85/92 resulted in multiple C_{max} of CDRI-85/92 at 0.5, 2 and 8 h. All the three C_{max} of CDRI-85/92 were lower than that after oral dose of CDRI-85/92. Moreover, the systemic availability of CDRI-85/92 also decreased from 6111 ng.h/ml to 3463 ng.h/ml after ester prodrug ingestion. The multiple peak phenomena have been widely observed with various classes of drugs including cimetidine [13], ranitidine [14], furosemide

[15], penicillamine [16], acebutolol [17], veralipride [18] and alprazolam [19]. Several possible mechanisms also include variable absorption rates along the gastro-intestinal tract due to motility cycle [20-22], storage and subsequent release from a post absorptive depot site (possibly liver parenchymal cells). Bile and food related independent erratic absorption might be responsible for multiple peaks [17]. There can be a possibility of intestinal metabolism or saturable absorption [21]. Another reason for the appearance of secondary peaks could be that the drug undergoes entero-hepatic recirculation thus leading to an intermittent rises and falls in the concentration [21]. In the present study, the double-peak phenomenon may be the result of poor aqueous solubility of the ester prodrug of the compound that might have resulted in the precipitation of the drug in the intestine where slow dissolution of the ester prodrug in the absorptive regions could have led to discontinuous profile. Probably, the carboxylic acid moiety makes the molecule too hydrophilic for membrane permeability necessary for favorable oral bioavailability of parent compound. Esterases in the intestine appear to contribute to the less than bioavailability of aspirin after oral administration [23]. These enzymes may also be important for the bioactivation of drugs that are given in the form of prodrugs.

Primary parameters V_d/F and Cl/F values after ester prodrug treatment with a dose equivalent to 20 mg/kg dose of CDRI-85/92 were calculated to be 41.07 L/Kg and 5.54 L/h/kg. The clearance of CDRI-85/92 was higher than the hepatic blood flow (2.9 L/h/kg, [24]) of the rat, suggesting a significant amount of extrahepatic elimination of this compound. The V_d/F was found to be higher than that after parent compound indicating better distribution. Moreover, the volume of distribution is much larger than the volume of the rat body fluid indicating extensive distribution of the compound into highly perfused organs [24]. The higher clearance resulted in lower systemic availability of CDRI-85/92 after the ester prodrug administration.

It has been recently reported that prolonged use of proton pump inhibitors can lead to pneumonia, osteoporosis and enteric infection [25]. Further there is involvement of CYP3A4 and CYP2C19 in the metabolism of proton pump inhibitors [26], thus having alarm for polymorphisms and or drug-drug interactions. However, since CDRI-85/92 is a selective gastric proton pump inhibitor and does not inhibit the bone proton pump inhibitors, there is remote possibility for osteoporosis. Moreover, there is least metabolism involving Phase-I and the compound undergoes cis-trans conversion by the aid of glutathione-S-transferase [9]. Therefore, CDRI-85/92 seems to be at par with the existing proton pump inhibitors in terms of enhanced elimination half-life and negligible metabolism from Phase-I drug metabolism enzymes [9], thus having low probability of drug-drug interactions and polymorphisms issue [8] and also low potential for side-effects.

The preclinical animal data show that rats dosed with CDRI-85/92 had relatively good systemic availability of CDRI-85/92. However, the ester prodrug could not yield higher circulating concentrations of the active component post absorption than that after the oral dose of the active component. The relatively higher systemic availability is likely related to the good absorption of the parent compound as compared to its ester prodrug.

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Table 1. Recovery of CDRI-85/92 from spiked control samples on different days

Concentration (ng/ml)	Absolute recovery (mean \pm S.D.)	CV (%)
Low 10 ng/ml	88.88 \pm 10.33	11.62
Medium 100 ng/ml	92.78 \pm 11.74	12.66
High 1000 ng/ml	98.33 \pm 9.63	9.79

Table 2. Intra- and inter-day accuracy (% bias) and precision [% relative standard deviation (RSD)] of the HPLC method for the determination of CDRI-85/92

Concentration (ng/ml)	Spiked sample		
	Low	Medium	High
Theoretical	10 ng/ml	100 ng/ml	1000 ng/ml
Observed (Mean \pm SD)	10.49 \pm 0.96	99.42 \pm 16.48	1033.19 \pm 101.24
%Bias _{intra-assay}	6.36	-9.17	3.10
%Bias _{inter-assay}	10.59	-4.87	2.50
RSD _{intra-assay} (%)	9.43	0.77	0.07
RSD _{inter-assay} (%)	8.59	2.49	0.24

Table 3. Pharmacokinetic parameters of CDRI-85/92 obtained after oral administration of CDRI-85/92 and its ester prodrug in rats

Parameters		CDRI-85/92	Ester Prodrug of CDRI-85/92
C_{\max} (ng/ml)	1	1838.40 ± 101.16	604.25 ± 26.55
	2	-	410.44 ± 38.24
	3	-	264.10 ± 15.11
t_{\max} (h)	1	1.5	0.5
	2	-	2
	3	-	8
Elim. $t_{1/2}$ (h)		4.96	5.14
MRT (h)		4.74	7.48
V_d/F (L/kg)		23.42	41.07
Cl/F (L/h)		3.27	5.54
$AUC_{0-\infty}$ (ng.h/ml)		6111	3463
$AUC_{\text{Ester Prodrug}} / AUC_{\text{CDRI-85/92}}$			0.57

Values of C_{\max} are mean ± SEM;

Abbreviations: $AUC_{0-\infty}$ = area under the serum concentration-time curve upto time infinity, Cl/F = clearance, C_{\max} = serum peak concentration, Elim. $t_{1/2}$ = elimination half-life, MRT = mean residence time, t_{\max} = time to C_{\max} , V_d/F = volume of distribution

Caption for the figures:

Figure 1. Chromatograms of (A) standard containing 250 ng/ml; (B) drug-free serum; (C) serum containing 400 ng/ml; (D) rat serum sample taken 0.5 h post 200 mg/kg oral dose of the ester prodrug of CDRI-85/92 in rats

Figure 2. Serum concentration-time profile of CDRI-85/92 obtained after oral administration of CDRI-85/92 and its ester prodrug in rats, Values are Mean ± SEM