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ORIGINAL ARTICLE

Pharmacokinetic Study of Hepatoprotective Coumarinolignoids from *Cleome viscosa* in Mice Using Validated High-Performance Liquid Chromatography-Photodiode Array Method

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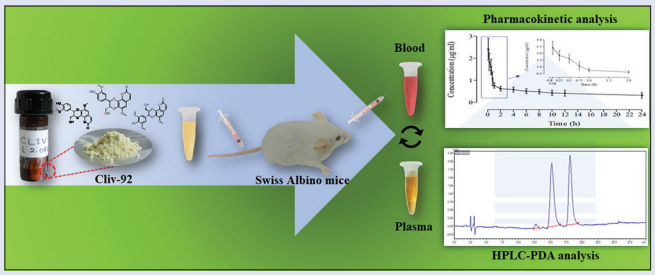
ABSTRACT

Background: Three key coumarinolignoids, cleomiscosins A, B, and C of *Cleome viscosa* seed origin in particular ratio, are a proven hepatoprotective agent known as Cliv-92. **Objectives:** However, the bioavailability and pharmacokinetics of Cliv-92 are still not clear. The present study is the first validated method which deals with the assay of Cliv-92 in mouse plasma. **Methods:** Single-step sample preparation meets the criteria of recovery (80%–93% with relative standard deviation [RSD] 2.14%–4.80%). Reverse-phase high-performance liquid chromatography with photodiode array detection has resulted into acceptable separation and sensitivity of three structurally similar cleomiscosins – A, B, and C of Cliv-92. The analysis involved a binary gradient of mobile phase and flow rate. Quantification was done at peak area at 326 nm using linear regression curve (r^2 > 0.999). The precision (0.46%–2.68% RSD) and accuracy (±2.09% bias) of Cliv-92 determination in plasma complied the criteria of the current international guidelines. We have also evaluated the matrix effect on sensitivities by spiking method. Limit of detection and limit of quantification in mouse plasma ranged between 0.13–0.24 µg/ml and 0.41–0.74 µg/ml. **Results:** Pharmacokinetic parameters were studied after intravenous bolus administration of Cliv-92 at 10 mg/kg dose in mice. Blood samples were collected at a predefined time up to 24 h post-injection. The Cliv-92 plasma half-life ($t_{1/2}$) was 2.77 h, and the clearance was estimated as 2.38 L/h/kg. **Conclusion:** The method is simple, sensitive, and accurate for the determination of plasma concentration of coumarinolignoids. The present preclinical pharmacokinetic study of coumarinolignoids has been anticipated in clinical studies with scaling techniques.

Key words: Bioavailability, Cliv-92, hepatoprotective, pharmacokinetic study, preclinical, plant bioactives

SUMMARY

- The present study deals in HPLC-PDA detection for quantitation of Cliv-92 (composition of three structurally similar coumarinolignoids, cleomiscosins A, B, and C) in mice plasma and its application to a preliminary pharmacokinetic study of Cliv-92 after intravenous bolus administration. The present research designates the rapid clearance of Cliv-92 from blood, thus enhanced bioavailability, either formula modifications or modified delivery system may be suggestive.



Abbreviations used: HPLC-PDA: High Performance Liquid Chromatography-Photodiode Array; HQC: High-Quality Control level; LOD: Limit of Detection; LOQ: Limit of Quantification; LQC: Low-Quality Control level; MQC: Medium-Quality Control level; QC: Quality Control; RT: Room Temperature.

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INTRODUCTION

The genus *Cleome* (family Capparaceae) is distributed in tropical and subtropical climate zones consisting of 206 accepted species. About 12 *Cleome* species are found in India. *Cleome viscosa* Linn., known as "Hurhur," has yellow flowers with penetrating odor and long slender pods containing seeds. The plant is used in traditional systems of medicine, i.e., Indian – *Ayurvedic* and Chinese – traditional Chinese medicine system. *Ayurvedic* practitioners use the seed of this plant for the management of various disorders such as bronchitis, inflammation, liver disorder, malarial fever, and skin diseases.[1,2] Additionally, different extracts of the plant have also been evaluated for numerous biological activities, namely analgesic,[3] anti-diarrheal,[4] antipyretic,[3] and hepatoprotective activities,[5,6] following the experimental pharmacological studies.[7]

To define the biological action of *C. viscosa*, various phytochemical investigations have been performed by various researchers.[8-10] The phytochemical cleomiscosins A, B, C, and D (coumarinolignoids) were firstly isolated from the seeds of *C. viscosa*. Out of these four

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coumarinolignoids, the maximum occurrence of cleomiscosin A was detected followed by that of cleomiscosin B. Cleomiscosins C and D were minor constituents.[8,11] Apart from *C. viscosa*, the said cleomiscosins were also isolated from different plant species, for example, cleomiscosins A and C from *Hibiscus taiwanensis*[12] and *Acer okamotoanum*,[13] respectively, cleomiscosins A and B from *Aesculus turbinata*,[14] and cleomiscosins A, C, and D from *Hibiscus syriacus*.[15]

Cliv-92 is a patented combination of three structurally similar coumarinolignoids namely cleomiscosins A, B, and C obtained from the seeds of *C. viscosa* as an hepatoprotective agent.[16,17] It has also proven to possess immunomodulatory[18] and anti-inflammatory activities.[19] However, individually, cleomiscosins A and C from *Acer okamotoamum* have been reported for their antioxidant potential.[13] The chemopreventive action of cleomiscosin A against lymphocytic leukemia has also been reported.[20] It is evident from the prior art that some pharmacological studies are reported from secondary metabolites of *C. viscosa*. However, some efforts were also made to define the quality of *C. viscosa* seed/leaves based on cleomiscosins A, B, and C using high-performance liquid chromatography (HPLC) and liquid chromatography with tandem mass spectrometry.[6,21-23] To the best of our knowledge, the pharmacokinetic studies of cleomiscosins belonging to coumarinolignoids class of compounds are still lacking.

This is the first report on the pharmacokinetic studies of coumarinolignoids in mice. In the present investigation, we propose an HPLC method for the estimation of Cliv-92 in mouse plasma for the first time as far as our knowledge is concerned. The present method is simple, accurate, sensitive, and reproducible. After validation, the method was successfully applied to study the pharmacokinetics of Cliv-92 in Swiss Albino mice.

EXPERIMENTAL

Chemicals and reagents

Cliv-92 [coumarinolignoids, structures of cleomiscosins A, B, and C as shown in Figure 1] was obtained from the Process Chemistry and Chemical Engineering Department of the institute. The detailed scale-up process of isolation and purity has been described elsewhere .[24] Methanol, diethyl ether, ethyl acetate, chloroform, acetonitrile, and acetic acid were procured from Merck India Pvt. Ltd. Chromatographic solvents of HPLC gradient grade were used in the study. Deionized water (18.2 MΩ/cm) was generated in-house using a Milli-Q System from Millipore (Bedford, MA, USA) and filtered with 0.22-µm nylon membrane before use.

Equipment

The HPLC system (Nexera-XR: LC-20AD XR, Shimadzu, Kyoto, Japan) was equipped with an LC-20AD single pump, DGU-20A5R degassing unit, CTO-10ASVP column oven, SIL-20AC autosampler, and SPD-M20A photodiode array (PDA) detector. Chromatographic data acquisition and processing were carried out using LabSolutions DB software (Shimadzu, Kyoto, Japan).

Chromatography

The optimum chromatographic separation was achieved according to previously reported in-house method[21] with minor modifications using a reverse-phase column (Water Symmetry® C_{18} column, 250 mm × 4.6 mm, 5.0 µm). The mobile phase comprised a mixture of (A) acetonitrile: methanol (1:2, v/v) and (B) water containing 0.5% acetic acid, filtered through a 0.22-µm membrane filter run in a gradient-elution mode. Initially, the gradient elution was started with 40% A with a flow rate of 1.0 ml/min which was reduced to 38% with 0.6 ml/min at 15.5 min for the next 7 min composition maintained with destined flow rate at 1.1 ml/min. In the next 2.5 min, the initial conditions were achieved. The column was re-equilibrated for 5 min between successive runs. A volume of 10 µL of each sample/standard was injected through autosampler. Data acquisition was carried out in the range of 20-400 nm, while quantitation was performed at 326 nm.

Preparation of standard solution

The stock solution (1.0 mg/ml) of Cliv-92 was prepared by dissolving requisite amount in methanol and stored at −20°C in a glass vial. Independent stock solutions were prepared for calibration and quality controls (QCs). Individual stock solution of Cliv-92 constituents, i.e., cleomiscosins – A, B, and C (1.0 mg/ml), was diluted with methanol to prepare working solutions (0.01–2.5 µg/ml) for sensitivity determination. To evaluate the matrix effect on quantitation, the standards were used to construct calibration curves for the quantitation of Cliv-92 components at plasma concentrations (0.1–5.0 µg/ml). The calibration standards and QC samples were prepared by spiking drug-free mouse plasma with the working solution (5% of the total plasma volume). For each validation and assay run, the calibration curve standards were prepared freshly from the working solutions. Standard stock and working solutions used for spiking were stored at 4°C, whereas the calibration curve and QC samples in plasma were stored at −20°C until use.

Preparation of calibration standards and quality control samples

QC samples were independently prepared by spiking blank plasma with various amounts of Cliv-92 to prepare concentrations corresponding to the low QC (LQC; 50 µg/ml), medium QC (MQC; 150 µg/ml), and high QC (HQC; 300 µg/ml) levels of the calibration curves. The QC samples were prepared to determine the limit of quantitation, the intra- and inter-assay precision, accuracy, and stability of analyte stored under different conditions. Aliquots of the QC samples were stored at −20°C until analysis and thawed to room temperature (RT) before analysis together with the mouse plasma samples.

Selection of extraction solvent and plasma extraction procedure

The plasma samples were prepared using protein precipitation method and liquid–liquid extraction method for extracting Cliv-92 from mouse plasma. All the plasma samples and standard working solutions were thawed at RT before use. For preparing calibration standards and QC

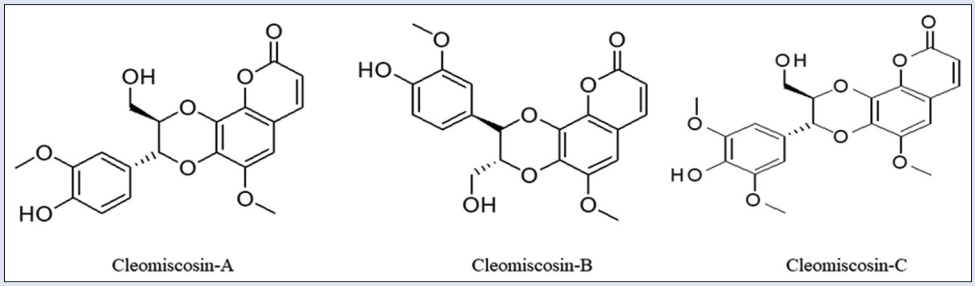

Figure 1: Chemical structures of different coumarinolignoids present in Cliv-92

dilutions, aliquots of 200-µl mouse plasma were transferred to a 2.0-ml microcentrifuge tube. The required amount of standard working solution was added to plasma to make the calibration standards in the range of 10–500 µg/ml. Five hundred microliters of respective organic solvents, namely, acetonitrile, chloroform, dichloromethane, diethyl ether, ethyl acetate, methanol, and t-butyl methyl ether, was added to precipitate the plasma protein and optimum recovery of the analytes. The tubes were capped and vortex mixed for 2 min and then subjected to centrifuge at 20,817 g for 15 min at 4°C (Eppendorf Model-5430R) to remove any precipitated material. A 200-µl aliquot of the organic layer was transferred to a fresh tube and evaporated to dryness under a nitrogen stream. This dried residue was reconstituted in 200-µl methanol and filtered with 0.22-µm nylon disc before HPLC-PDA analysis.

Method validation

The present HPLC-PDA method was validated for selectivity, sensitivity, linearity, accuracy, precision, recovery, and stability following the Food and Drug Administration (FDA) guidelines for bioanalytical method.[25]

Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of endogenous compounds in the sample matrix. Six randomly selected blank plasma samples were analyzed under optimized chromatographic conditions. In order to evaluate interference at the retention times of the analytes, the separation of Cliv-92 and endogenous compounds from plasma was checked by comparing the chromatograms of QC samples with blank plasma.

Limit of detection and limit of quantification

For determination of the limit of detection (LOD) and limit of quantification (LOQ) for the assay, a blank plasma sample was extracted (n = 6) and analyzed using optimized HPLC conditions. Afterward, the spiked plasma samples were processed and analyzed. The LOD could be defined as the lowest concentration of each analyte in the spiked plasma, producing a peak with a signal-to-baseline noise (S/N) ratio of ≥3, whereas LOQ could be defined as the lowest concentration on the calibration curve exhibiting a peak with a S/N ratio ≥10.

Linearity of calibration curves

Calibration curve for Cliv-92 was constructed using four data points in triplicate within the concentration range of 0.01–2.5 and 0.1–5.0 µg/ml in analytical standard solution and spiked plasma, respectively. The linear relationship in plasma was expected to demonstrate the analyst bias and efficiency and their impact on method sensitivities. The replicate calibration curves were constructed on separate days by analyzing freshly prepared samples. Analyte peak area was plotted against the corresponding concentrations of Cliv-92. Least-square linear regression analysis was performed using the equation $Y = a + bX$. The linearity of a calibration curve was calculated from the slope, intercept, and correlation coefficient (r^2) of the curve. Unknown analyte concentration was evaluated by interpolation using the linear regression equation. The correlation coefficient (r^2) of the calibration curves was set at least 0.99 or better.

Precision and accuracy

The precision and accuracy of the method were estimated by analyzing three different concentrations of QC samples. For the intra-day assay, plasma aliquots of each QC sample were analyzed on the same day, whereas for interassay determinations, the QC samples were analyzed on three different days in triplicates for calculating the precision and accuracy of the method. The precision was expressed as the relative standard deviation (RSD%) of the measured concentrations, and accuracy was expressed as the bias% for each QC level.

Recovery of Cliv-92 components

For extraction recovery of Cliv-92 from plasma, the aliquots of blank plasma samples were analyzed after spiking with QC concentrations. Simultaneously, equivalent Cliv-92 concentrations were also spiked into the solvent and analyzed. The samples were extracted following the procedure described in earlier section. For determination of absolute matrix effect, procedure reported elsewhere[26] was adopted. The extraction recovery was determined by comparing the peak area ratio from a given concentration of Cliv-92 spiked in drug-free plasma with the peak area ratio obtained for the same concentration of the Cliv-92 extracted and analyzed using Equation 1.

$$\text{Recovery \%} = \frac{\text{Peak area of Cliv-92 extracted from plasma}}{\text{Peak area of Cliv-92 spiked into plasma}} \times 100 \quad (1)$$

Pharmacokinetic study in mice

Animal experiments

Animal experiments were conducted using 10-week-old Swiss Albino mice of either sex after 1-week acclimatization in the experimental room of "Jeevanika" – an *in vivo* testing facility of the institute. All the animal protocols were duly approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CIMAP/IAEC/2016-19/20-ICMR) and performed as per the Institutional Animal Ethical Committee guidelines. A total of 72 mice were randomly divided into 12 groups (having an equal number of both sexes) respective to predetermined time points, namely, 0.08, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, and 24 h and supplied with *ad libitum* food and water. Each group (n = 6) received 10 mg/kg of Cliv-92 solution prepared in normal saline (0.9% NaCl) with 0.5% Kolliphor® EL (Sigma-Aldrich, India) intravenously (i.v.) through tail vein. After that, mice were ether anesthetized by open-drop method and blood was collected in heparinized tubes by retro-orbital plexus at the predetermined time point. Finally, the mice were sacrificed by cervical dislocation. The plasma was separated from the blood by centrifugation at 10,600 rpm for 5 min at 4°C and stored at −20°C for further analysis.

Pharmacokinetic analysis

HPLC analysis was performed using the blood plasma collected at different time points. The concentration versus time plot of Cliv-92 was prepared using GraphPad® Prism software (Version 5.04, San Diego, CA, USA). The pharmacokinetic parameters were obtained using PKSolver 2.0 with one-compartment analysis after i.v. bolus input.[27]

RESULTS AND DISCUSSION

Optimization of sample preparation

Detection of drugs and their metabolite in plasma is a tedious task due to a complex biological milieu of plasma.[28] Prior to HPLC analysis, it is necessary to eliminate plasma proteins and potential interfering endogenous compounds. To fulfill this requirement, the sample clean-up procedure was adopted for optimum Cliv-92 recovery. The clean-up procedures often influence the sensitivity and selectivity of the method. Therefore, it is advisable to curtail clean-up steps in sample preparation procedure to increase the recovery of an analyte as well as to avoid loss of targeted analyte. Fast sample clean-up methods commonly involve protein precipitation by an organic solvent(s) and/or acids which in turn also disrupt the protein–drug binding.[29] In the present study, different organic solvents, for example, methanol (CH_3OH), diethyl ether [$(CH_3CH_2)_2O$], ethyl acetate ($CH_3COOC_2H_5$), tert-Butyl methyl ether [$(CH_3)_3COCH_3$], chloroform ($CHCl_3$), acetonitrile (CH_3CN), and dichloromethane (CH_2Cl_2) were attempted for protein precipitation, and

the corresponding extraction recoveries (mean ± SD) were calculated as 28.45% ± 8.94%, 19.56% ± 2.88%, 28.26% ± 5.32%, 30.75% ± 5.94%, 82.29% ± 5.83%, 88.46% ± 4.02%, and 75.35% ± 10.52%, respectively. The highest extraction recovery (75%–89%) of Cliv-92 was estimated in acetonitrile, dichloromethane, and chloroform ($P \geq 0.05$) with liquid–liquid extraction. Dichloromethane was not selected due to large variability (poor reproducibility) along with clean endogenous environment relative to other solvents. Finally, the choice of acetonitrile as a protein precipitant over chloroform was based on its relatively better reproducibility and nonchlorinated nature [Figure S1]. The present extraction procedure for Cliv-92 has a single step and is less time consuming (including evaporation under N_2 current and reconstitution in methanol), so it has been found appropriate for high-throughput pharmacokinetic analysis.

Optimization of chromatographic parameters

The experiment was designed to analyze Cliv-92 in mouse plasma using HPLC with PDA detector. Chromatographic separation and resolution of Cliv-92 (all the three cleomiscosins A, B, and C in the peak area ratio of 49:50:01) from endogenous plasma compounds were achieved using Water Symmetry® C_{18} column with acidified mobile-phase conditions (as mentioned earlier). Optimum separation efficiency could be achieved with Symmetry® C_{18} column within 20 min with acceptable resolution. However, the equivalent column (USP-L1) from Waters was also tested but could not produce satisfactory separation (data not included). Even different mobile-phase compositions (acetonitrile, methanol, and water with additive acetic acid) were also tried in isocratic as well as gradient programs to achieve the efficient separation of the analyte. The optimal mobile phase was established as a combination of acetonitrile-methanol (1:2, v/v) and water containing 0.5% acetic acid in gradient elution (as mentioned previously). Under the optimized analysis conditions, the total run time of this assay was 30 min, and cleomiscosin C, B, and A were retained at 13.73, 16.38, and 19.03 min, respectively, with acceptable reproducibility. This optimized HPLC-PDA conditions offered good baseline separation with acceptable peak homogeneity (peak purity index of 1.00 as checked by LabSolution DB software) of all the three components of Cliv-92 as shown in Figure 2.

Assay selectivity

Six different batches of blank mouse plasma were analyzed and compared for assay selectivity. The representative chromatograms are shown in Figure 2. The retention times of cleomiscosin C, B, and A were about 13.73, 16.38, and 19.03 min, respectively. The selectivity of the method was evaluated by checking the peak purity of targeted

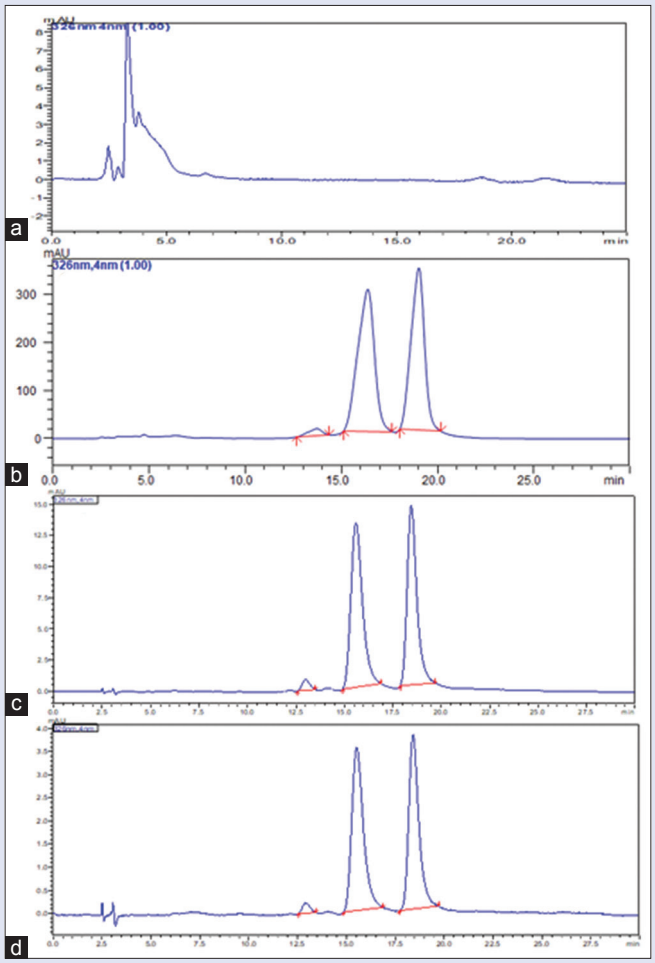

Figure 2: Representative chromatograms of: (a) blank plasma; (b) Cliv-92, 500 µg/ml; (c) blank plasma spiked with 100 µg/ml Cliv-92; (d) plasma sample collected from mice 6 h post intravenous bolus administration of Cliv-92 (10 mg/kg). Rt: 13.73 min, cleomiscosin-C; Rt: 16.38 min, cleomiscosin-B; and Rt: 19.03 min, cleomiscosin-A

Table 1: Linear regression data for the calibration curve of Cliv-92 constituents in pure solution and sample matrix, i.e., mouse plasma

Parameter	A	B	C
Analyte concentration–peak area relationship in solution			
Regression	Linear	Linear	Linear
Concentration range (µg/ml)	0.01–2.5	0.01–2.5	0.01–2.5
Y-intercept	−277.3±1184	5317±667	8791±2621
Slope	74,740±879	69,680±495	198,500±1945
Goodness of fit (r^2)	0.9997	0.9999	0.9998
LOD (µg/ml)	0.08	0.05	0.06
LOQ (µg/ml)	0.24	0.14	0.20
Analyte concentration–peak area relationship in mouse plasma			
Regression	Linear	Linear	Linear
Concentration range (µg/ml)	0.1–5.0	0.1–5.0	0.1–5.0
Y-intercept	5900±4040	1538±4275	8394±6211
Slope	79,120±1423	75,310±1505	198,400±2187
Goodness of fit (r^2)	0.9994	0.9992	0.9998
LOD (µg/ml)	0.22	0.24	0.13
LOQ (µg/ml)	0.67	0.74	0.41

LOD: Limit of detection; LOQ: Limit of quantification; A: Cleomiscosin-A; B: Cleomiscosin-B; C: Cleomiscosin-C

cleomiscosins in sample matrix chromatogram. The chromatographic peaks were well resolved to baseline, and no endogenous interference was noticed in chromatographic separation at the retention times of the analytes. The purity index of respective cleomiscosin had reached the values >0.999 for all plasma samples collected at a different time interval.

Linearity of calibration curve

The different concentrations of Cliv-92 over the range of 0.1–5.0 µg/ml were evaluated for calibration curve in mouse blank plasma. The calibration curves were found to be linear for analytes over a selected concentration range constructed by linear least-squares regression. A linear correlation was the best-fit model in the selected concentration range with an acceptable correlation coefficient [Table 1]. The fitness of the curve was also confirmed by the back-calculated calibrator concentrations.[30]

Limit of detection and limit of quantification

The least squares regression data were also used for calculating the LOD and LOQ. The LOD of the method was found to be 0.08, 0.05, and 0.06 µg/ml for cleomiscosins A, B, and C, respectively, and LOQ of the method was calculated as 0.24, 0.14, and 0.20 µg/ml for cleomiscosins A, B, and C, respectively, as shown in Table 1.

Precision and accuracy

Results of intra- and inter-day precision and accuracy incurred at different concentrations (LQC, 50 µg/ml; MQC, 150 µg/ml; and HQC, 300 µg/ml) of QC plasma samples for Cliv-92 with six determinations per concentration on the same day and 3 consecutive days are summarized in Table 2. The concentrations were calculated by the calibration curve. The intra- and inter-day variations in the assay of plasma with graduated Cliv-92 plasma concentration at low-to-high concentrations were found to be within 5% and 2%, respectively. Results met the criteria of the US FDA's requirements for bioanalytical method validation and demonstrated the assay flexibility.

Recovery of Cliv-92 components

The results of the recovery of the three components of Cliv-92 are shown in Table S1. The recoveries of analyte were calculated by comparing peak areas of pre- and post-extraction from spiked samples.[31] The average recovery was ranged 80%–93% with RSD 2.14%–4.80% (n = 6) at targeted concentrations (50, 150, and 300 µg/ml) for Cliv-92. The results of the recovery experiments further confirm method suitability for the bioanalysis of Cliv-92.

Stability

Short-term storage stability study was explored to cover the time spent for sample preparation to affirm the stability of Cliv-92 in plasma at RT. The QC samples were exposed to ambient laboratory conditions for 2 h to simulate these conditions. Freshly prepared samples were analyzed to evaluate the stability of determination at short term (2 h, at RT), long term (1 month, at −20°C), three freeze-thaw cycles (−20°C), and post preparative (24 h, at RT) at three concentration levels, namely 50, 150, and 300 µg/ml, respectively. All stability QC samples were analyzed in six replicates. These results suggested the acceptable stability of Cliv-92 in plasma [Table S2].

Pharmacokinetic study

Bioavailability assessment of Cliv-92 in mice has demonstrated the present method's applicability in preclinical experiments and bioanalysis. The sensitivity and specificity of the assay were adequate to determine the plasma concentrations of Cliv-92 in mice following i.v. bolus administration. Each mouse was administered a dose of 10 mg/kg of Cliv-92. The selection of the dose was based on the protective potential of Cliv-92 in CCl_4-induced hepatotoxicity in Swiss Albino mice using parallel dose comparison method (data not shown). The typical chromatogram of the plasma sample from dosed mice is presented in Figure 2. The mean plasma concentration–time profile of Cliv-92 in plasma is presented in Figure 3. The one-compartmental model analysis was employed to calculate the pharmacokinetic parameters

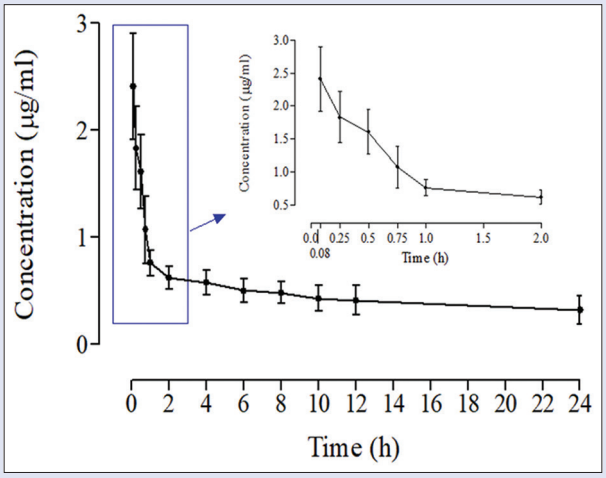

Figure 3: Mean plasma concentration–time curve of Cliv-92 (10 mg/kg; intravenous bolus) in mouse plasma

Table 2: Intra- and inter-day accuracy and precision of Cliv-92 in mouse plasma

Cnom (µg/ml)	Cdet (µg/ml)			Accuracy (bias %)			Precision (RSD %)		
	Cleomiscosins			Cleomiscosins			Cleomiscosins		
	A	B	C	A	B	C	A	B	C
Intraday									
50	50.55±0.67	49.75±0.94	49.94±0.82	1.09	−0.51	−0.12	1.32	1.89	1.64
150	151.08±0.71	152.09±0.93	149.72±1.84	0.72	1.39	−0.19	0.46	0.61	1.23
300	297.91±5.01	299.70±3.13	304.33±2.89	−0.70	−0.10	1.44	1.68	1.04	0.95
Interday									
50	50.51±0.75	49.70±0.78	49.09±1.11	1.01	−0.60	−1.82	1.49	1.56	2.25
150	150.92±0.80	153.13±0.70	149.00±1.67	0.62	2.09	−0.67	0.53	0.46	1.12
300	293.74±3.63	305.77±8.18	299.15±2.88	−2.09	1.92	−0.28	1.24	2.68	0.96

Values are expressed as mean±SD, (n=6). C_{nom}: Nominal concentration; C_{det}: Detected concentration; A: Cleomiscosin-A; B: Cleomiscosin-B; C: Cleomiscosin-C; SD: Standard deviation; RSD: Relative SD

Table 3: Pharmacokinetic parameters of Cliv-92 (10 mg/kg) after intravenous bolus administration in Swiss Albino mice

Parameter	Unit	Value
$t_{1/2}$	h	2.77±0.16
AUC_{0-t}	μg × h/ml	6.17±0.34
$AUC_{0-\infty}$	μg × h/ml	6.42±0.37
AUMC	μg × h^2/ml	39.40±2.01
MRT	h	4.00±0.17
V	L/kg	5.27±0.31
CL	L/h/kg	2.38±0.13

Values are expressed as mean±SD; (n=6). $t_{1/2}$: Plasma half-life; AUC: Area under the plasma concentration-time curve; AUMC: Area under the moment curve; MRT: Mean resident time; V: The volume of distribution; CL: Clearance; SD: Standard deviation

of Cliv-92 which are listed in Table 3. After administration of Cliv-92, pharmacokinetic parameter values of half-life ($t_{1/2}$), area under the curve ($AUC_{0-\infty}$), mean residence time, the volume of distribution (V), and systemic clearance were found to be 2.77 ± 0.16 h, 6.42 ± 0.37 μg/ml/h, 4.00 ± 0.17 h, 5.27±0.31 mg/(μg/ml), and 2.38 ± 0.13 mg/(μg/ml)/h, respectively.

CONCLUSION

This is the first validated method for sensitive, accurate, and precise HPLC-PDA determination of Cliv-92 in mouse plasma. Single-step extraction and protein precipitation with acetonitrile is suitable for the quantification of Cliv-92 in pharmacokinetic research with time-saving and cost-effective purification. The concentration of Cliv-92 was 2.04 μg/ml at 5 min after i.v. bolus administration and quickly went down to about 68% at 1 h. The kinetic properties of Cliv-92 are best fit to the one-compartment model. From the current experiment, it is reasonable to conclude that administration of Cliv-92 via i.v. injection is very appropriate, but for enhanced bioavailability, either formula modifications or modified delivery system is essential. This method can also be applied for detailed pharmacodynamics investigation of the same analyte.

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Conflicts of interest

There are no conflicts of interest.

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