Chapter 5

Pharmacokinetic studies of osteogenic candidates K095, K054 and S006-1709

5.1. Experimental methods

5.1.1. Animals

Pharmacokinetic studies were performed in young and healthy female S.D. rats weighing 250±20 g. Animals were housed in well-ventilated cages and kept at room temperature on a regular 12 h light–dark cycle at Laboratory Animal Division of the institute. Animals were cared for in accordance with the guidelines laid by the institutional animal ethics committee (IAEC) for animal experimentation. All experimental animals were acclimatized for a minimum period of three days prior to the experiment. Standard pellet food and water were allowed freely. Animals in oral dose group were starved for 8-12h before dosing but allowed free access to water. Prior approval from IAEC was sought and the study protocols were approved before the commencement of the studies.

5.1.2. Drug formulation, administration and sampling schedule

Medicarpin (K095)

Oral pharmacokinetics study was carried out at the dose of 5mg/kg and the intravenous study at the dose of 1mg/kg. For oral administration to female S.D. rats, medicarpin (25mg) suspension was prepared in 10mL of 0.5% sodium methyl cellulose. The strength of the suspension was 2.5mg/mL so that the dosing volume (0.5mL) for each rat contained 1.25mg of K095. The intravenous formulation of K095 (5mg) was prepared in DMA (40%), Tween 20 (10%) and saline (q.s. to 5mL) at the concentration of 1mg/mL. The intravenous formulation was sterilized by filtration through 0.45μm filter. The dosing volume was 0.25mL, which contained 0.25mg of K095.

Animals were divided in to three groups with five animals in each group. Blood samples (0.5mL) were collected by cardiac puncture. Sparse sampling approach was followed so that not more than three samples were collected from each animal. The oral formulation was administered to rats by oral feeding needle after shaking well to ensure uniformity in content. Samples were collected by cardiac puncture in heparinised tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18 and 24h. Intravenous formulation was administered in tail vein after swabbing the tail region with alcohol. Samples were collected at 0.08, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18 and 24h.

Cladrin (K054)

Oral pharmacokinetics study was carried out at the dose of 10mg/kg and the intravenous study at the dose of 1mg/kg in female S.D. rats. For oral dosing, formulation of K054 (30mg) was prepared in 3mL of 0.5% sodium methyl cellulose suspension. The strength of the suspension was 10mg/mL so that the dosing volume of 0.25mL contained 2.5mg of K054. The intravenous formulation of K054 (1mg/mL) was prepared and sterilized similar to K095.

Animals were divided into three groups with three animals in each group. Blood samples (0.5mL) were collected by cardiac puncture and sparse sampling approach was followed. The oral formulation was administered to rats by oral feeding needle and samples were collected in heparinised tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24 and 30 hours. Intravenous formulation was administered in tail vein and samples were collected at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18 and 24h.

S006-1709

The oral and intravenous pharmacokinetic studies were performed at the dose of 10mg/kg and 1mg/kg respectively in female S.D. rats. The oral formulation (10mg/mL) and the intravenous formulation of S006-1709 (1mg/mL) were prepared similar to K054. The dosing volume for both oral and intravenous studies was 0.25mL.

Animals were divided in to three groups with three animals in each group. Blood samples (0.5mL) were collected by cardiac puncture and sparse sampling approach was followed. The samples for oral pharmacokinetics study were collected in heparinised tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24 and 30 hours. Samples for intravenous dosing were collected at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18 and 24h.

5.1.2. Sample clean-up and analysis

Blood samples were processed by LLE method using diethyl ether as described under the Chapter-4, Part I. 100µl of blood samples were processed similar to the processing of calibration standards. The dried residue obtained after evaporation of organic solvent was

reconstituted in 200µl of reconstitution solvent. 20µl of the processed sample was analyzed by the corresponding validated LC-MS/MS methods [1, 2].

5.1.3. Data analysis

The plasma concentration of K095, K054 and S006-1709 was plotted against sampling time to obtain the plasma concentration-time (c-t) profile. The pharmacokinetic parameters were derived by fitting the plasma concentration-time data using WinNonlin software (Ver 5.1).

5.2. Results and Discussion

5.2.1. Medicarpin (K095)

The plasma concentration-time profile of K095 after intravenous and oral dosing is shown in Fig-5.2. The pharmacokinetics parameters have been derived after non-compartmental analysis of its plasma c-t profile (Table-5.1).

Intravenous pharmacokinetics:

The volume of distribution at steady state (Vss) was 12.27 L/kg, which was greater than the total volume of body water (0.7L/Kg). This indicates that the tissue distribution of K095 is high. The half life was 3.08h and its clearance (Cl=2.76 L/h/Kg) was lesser than the total hepatic clearance (4L/h/Kg) [3]. K095 could be detected in plasma till 18h. Thus, K095 also shows high tissue distribution with low clearance, which is similar to other isoflavones. The $AUC_{0-\infty}$ was 362.35ng.h/mL, which is 8 times greater than $AUC_{0-\infty}$ of K095 (42.12 ng.h/mL) obtained after i.v. dosing of F147. This difference could be due to less content of K095 in F147, which is equivalent to the dose of 0.2mg/kg.

Oral pharmacokinetics:

The parameters T_{max} (0.75h), $t_{1/2}$ (5.33h) were comparable to that obtained in oral pharmacokinetics study of F147 (Chapter-3, Table-3.2). K095 was detected in plasma till 24h after oral dosing. The $AUC_{0-\infty}$ and % F were 412.81ng.h/mL and 22.34% respectively. The bioavailability of K095 (22.34%) at 5mg/kg is similar to the reported value of genistein at 6.25mg/kg (21.9%) [4]. The plasma concentration profile shows the

presence of multiple peaks at 4h and 8h, which is indicative of entero hepatic circulation of K095. The conjugation and subsequent absorption after hydrolysis of conjugated from is characteristics of isoflavones. As pterocarpan is also a class of isoflavone, K095 may also undergo entero hepatic circulation, giving rise to multiple peaks in plasma concentration profile.

(A)

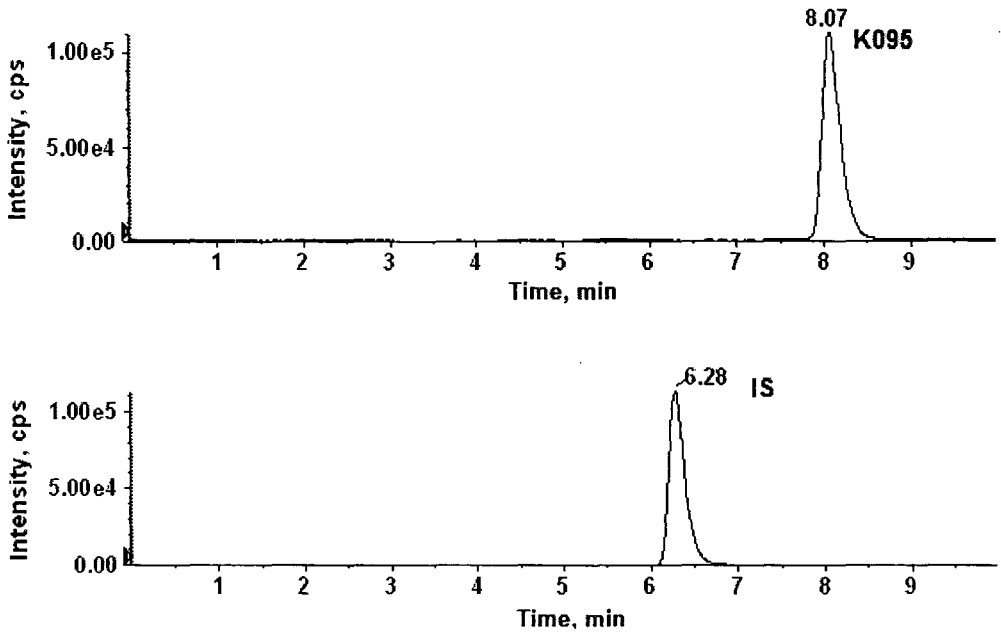


Fig-5.1.1: LC-MS/MS chromatogram of K095 after i.v. dosing in rats

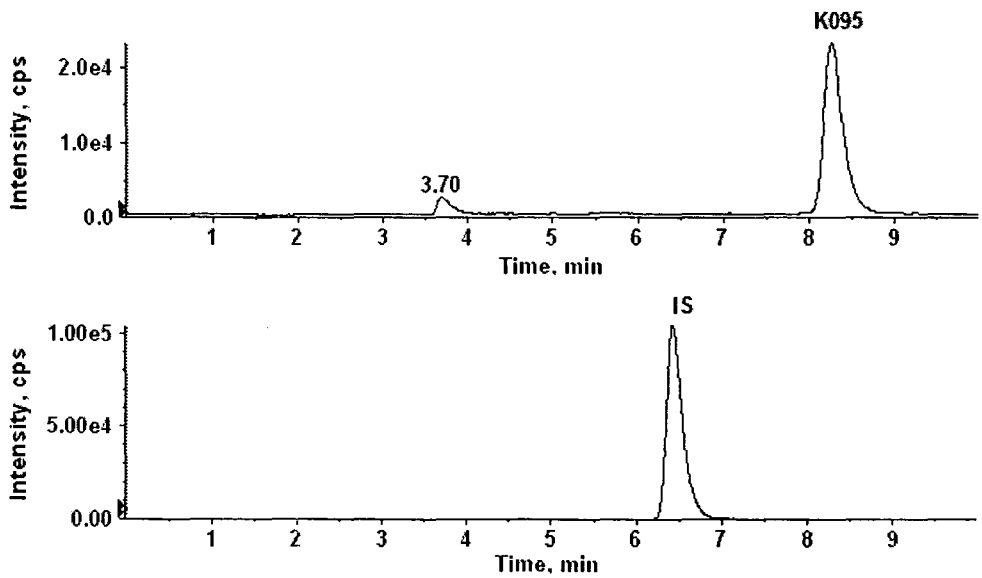


Fig-5.1.2: LC-MS/MS chromatogram of K095 after oral dosing in rats

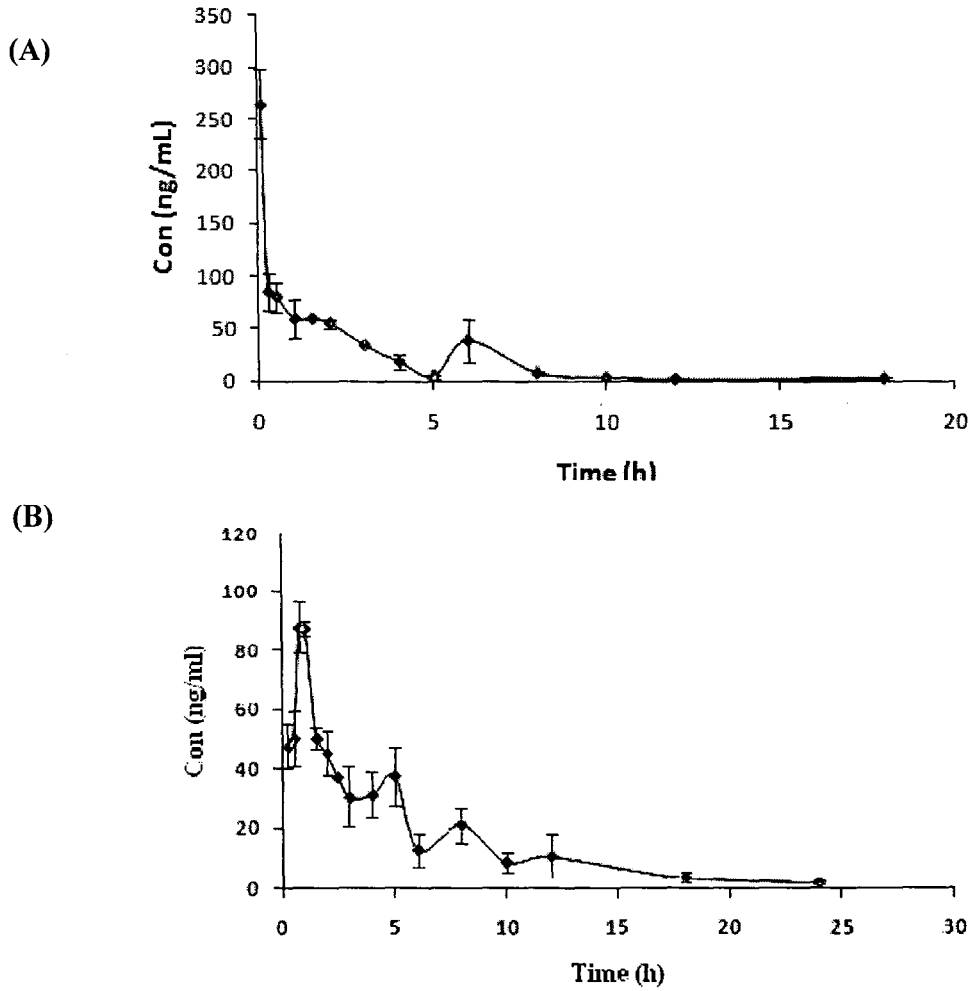


Fig-5.2: Plasma c-t profile of K095 after (A) i.v and (B) oral dosing in rats

Table-5.1: Pharmacokinetic parameters of K095 after i.v and oral dosing in female S.D. rats

Pharmacokinetic parameters	**Intravenous (1mg/kg)**	**Oral (5mg/kg)**
C_{max} (ng/mL)	--	87.9
T_{max} (h)	--	0.75
C_0 (ng/mL)	489.60	--
$AUC_{0-\infty}$ (ng.h/mL)	362.35	412.81
$T_{1/2}$ (h)	3.08	5.33
K_a (1/h)	0.22	0.13
MRT (h)	2.90	5.95
Cl (L/h/kg)	2.76	2.71
V_{ss} (L/kg)	12.27	20.80
% F	--	22.34

5.2.2. Cladrin (K054)

The plasma c-t profile of cladrin after intravenous and oral dosing is shown in Fig-5.4. The pharmacokinetic parameters were determined after fitting the data by noncompartmental analysis using WinNonlin software (Table-5.2).

Intravenous pharmacokinetics:

A two compartment model was applied to derive the pharmacokinetic parameters as the plasma concentration profile of K054 showed a bi-exponential decline of drug. Non-compartmental analysis was done due to higher values of *Akaike's information criterion* (AIC) and *Schwarz criterion* (SC).

The volume of distribution at steady state (Vss) was 6.72 L/kg, while the clearance was 2.25 L/h/Kg. K054 showed high tissue distribution and low clearance, similar to K095. The half-life of cladrin was calculated to be 3.4h and MRT was found to be 2.98h. K054 was detected in plasma till 12h. The AUC was 443.42ng.h/mL, which shows that the systemic exposure of K054 after intravenous dosing of 1mg/kg was similar to K095.

Oral pharmacokinetics:

The absorption of K054 was also rapid and the compound appeared in the plasma in the first sample collected at 15 minutes. It reached the maximum concentration (C_{max}) of 44.8ng/mL at 0.5h. Multiple peaks were also observed in the plasma concentration data at 1.5h and 4h. The half-life was calculated to be 4.1h and the MRT was 9.93h. After oral dosing, K054 was also detected in plasma till 24h. The $AUC_{0-\infty}$ of K054 was 608.72ng.h/mL at the dose of 10mg/kg and the absolute bioavailability was found to be 13.7 %. This shows that the systemic exposure of K054 was lower when compared to that of genistein at similar dose. Genistein has been reported to be 21.9% and 33.5% bioavailable at the dose of 6.25mg/kg and 12.5mg/kg respectively in rats [4]. Though the bioavailability of K054 was less, it has better efficacy than other isoflavones [5].

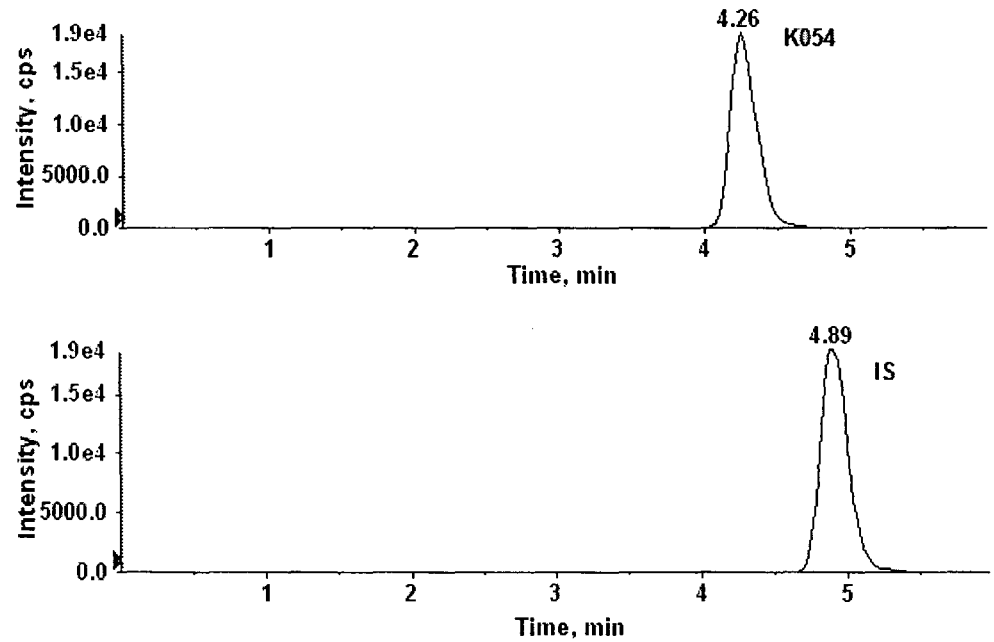


Fig-5.3.1: LC-MS/MS chromatogram of K054 after i.v. dosing in rats

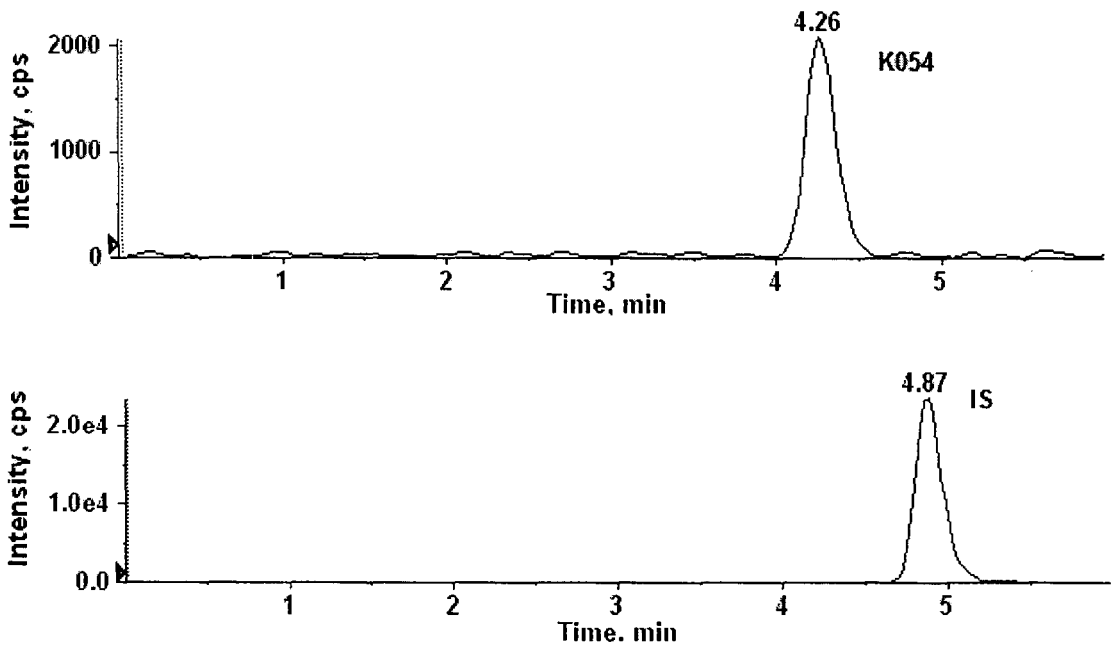
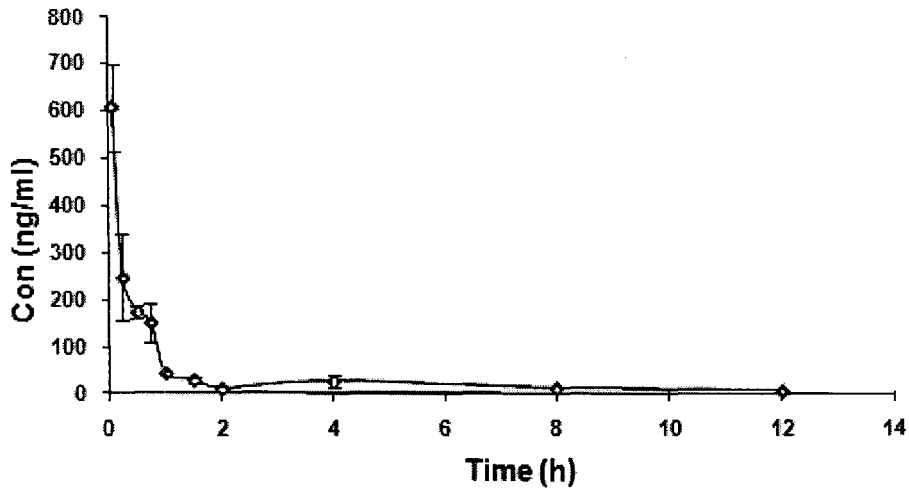


Fig-5.3.2: LC-MS/MS chromatogram of K054 after oral dosing in rats

(A)



(B)

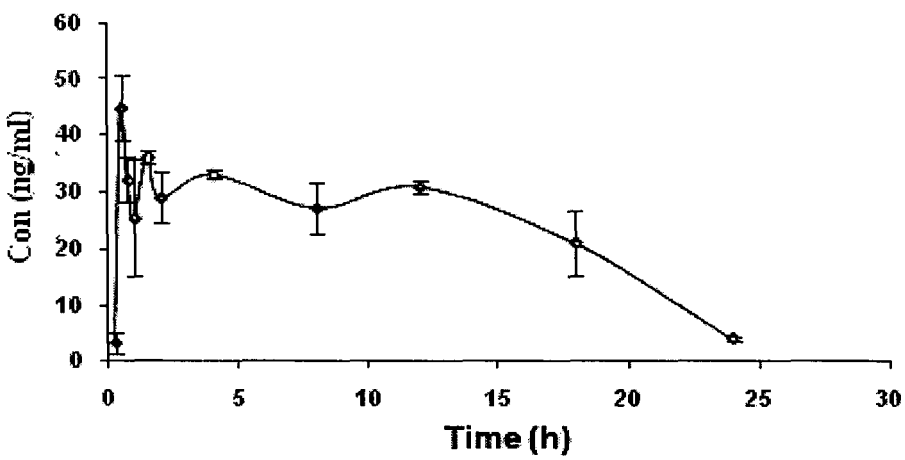


Fig-5.4: Plasma c-t profile of K054 after (A) i.v and (B) oral dosing in rats

Table-5.2: Pharmacokinetic parameters of K054 after i.v and oral dosing in female S.D. rats

Pharmacokinetic parameters	**Intravenous (1mg/kg)**	**Oral (10mg/kg)**
C_{max} (ng/mL)	--	44.83
T_{max} (h)	--	0.5
C_0 (ng/mL)	928.88	--
$AUC_{0-\infty}$ (ng.h/mL)	443.42	608.73
$T_{1/2}$ (h)	3.4	4.1
K_a (1/h)	0.20	0.17
MRT (h)	2.14	9.93
Cl (L/h/kg)	2.25	2.25
V_{ss} (L/kg)	6.72	13.35
% F	--	13.7

7.2.3. S006-1709

The plasma c-t profile of S006-1709 after intravenous and oral dosing is shown in Fig-5.6. The pharmacokinetic parameters have been obtained after non-compartmental analysis of its plasma c-t profile (Table-5.3)

Intravenous pharmacokinetics:

The volume of distribution at steady state (Vss) was 17.93 L/kg, which shows that the tissue distribution of S006-1709 was extensive. The value of clearance (7.19 L/h/kg) was greater than the total hepatic clearance (4 L/h/kg). The systemic exposure ($AUC_{0-\infty}$= 141.12 ng/h/mL) was lower, when compared to K095 and K054 at 1mg/kg. The low value of $AUC_{0-\infty}$ could be due to increased clearance of S006-1709 from the body. S006-1709 is also a pterocarpan and shows large tissue distribution similar to medicarpin but it differs in its clearance parameter, which is usually low for isoflavones.

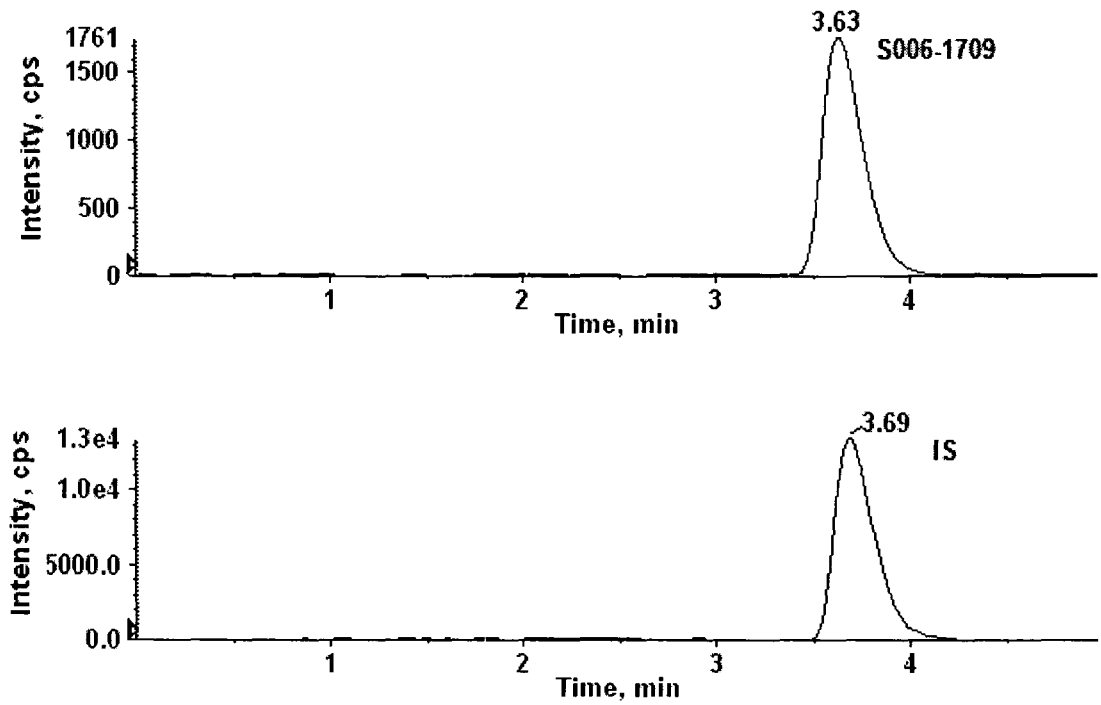


Fig-5.5.1: LC-MS/MS chromatogram of S006-1709 after i.v. dosing in rats

Oral pharmacokinetics:

The absorption of S006-1709 from GI tract was rapid as it could be detected in plasma within 15mins. The compound reached maximum concentration of 30.6ng/mL (C_{max}) at 0.5 h (T_{max}). The half life and mean residence time were 4.05h and 4.53h respectively. These values are similar to the reported values of other soy isoflavones [6] and also its natural analogue K095. S006-1709 could be detected in plasma up to 18h indicating its prolonged exposure. The plasma c-t profile shows the appearance of second maxima at 1.5h (Fig-5.9). The plasma concentration of S006-1709 at 1h and 1.5h were 17.3ng/mL and 19.03ng/mL respectively. This difference in concentration between these two time points was quite low, which could be due to the sparse sampling approach followed in this study.

The value of area under curve ($AUC_{0-\infty}$) was determined as 162.95ng.h/mL. The % bioavailability was 11.53% at the dose of 10mg/kg. This value was comparable to that of K054 but lesser than that of genistein. But this compound also shows potent osteogenic activity despite its lower bioavailability.

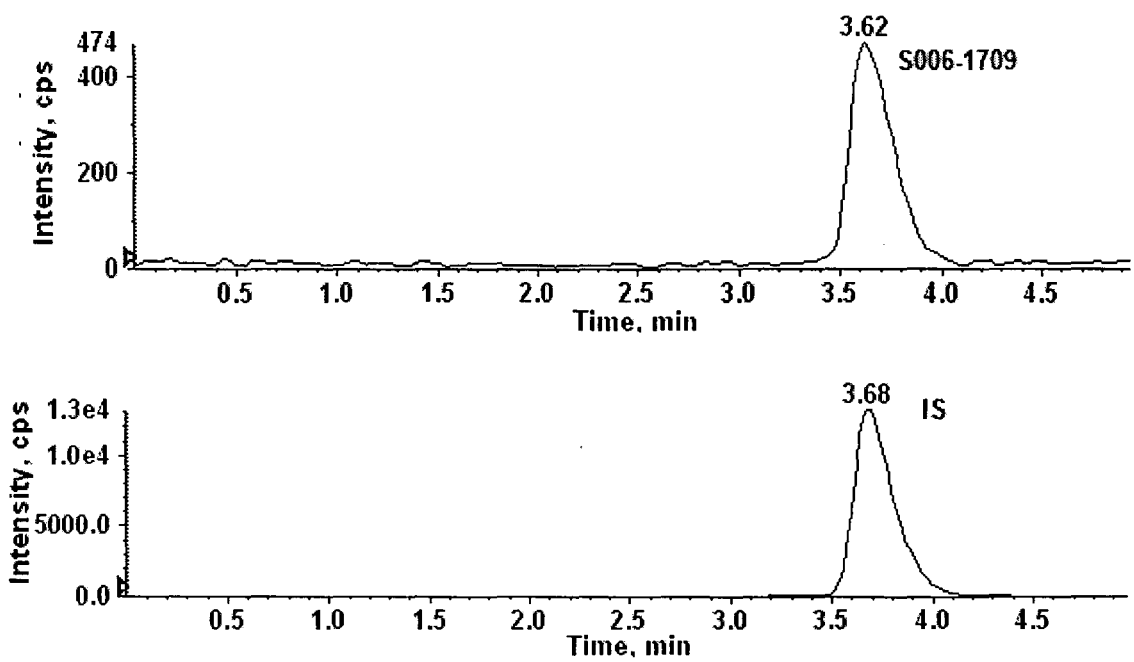


Fig-5.5.2: LC-MS/MS chromatogram of S006-1709 after oral dosing in rats

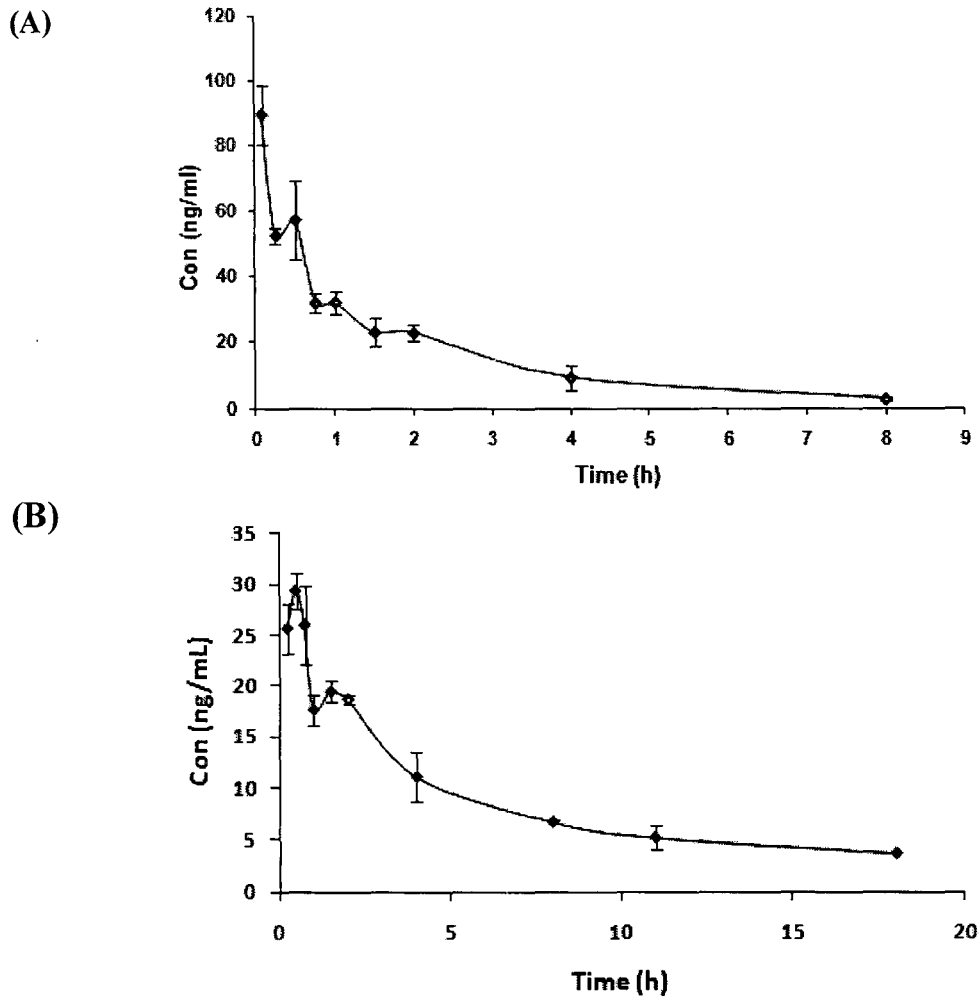


Fig-5.6: Plasma c-t profile of S006-1709 after (A) i.v. and (B) oral dosing in rats

Table-5.3: Pharmacokinetic parameters of S006-1709 after i.v and oral dosing in female S.D. rats

Pharmacokinetic parameters	**Intravenous (1mg/kg)**	**Oral (10mg/kg)**
C_{max} (ng/mL)	--	30.6
T_{max} (h)	--	0.5
C_0 (ng/mL)	115.24	--
$AUC_{0-\infty}$ (ng.h/mL)	141.12	162.95
$t_{1/2}$ (h)	2.15	4.05
K_a (1/h)	0.32	0.17
MRT (h)	1.93	4.53
Cl (L/h/kg)	7.19	7.13
V_{ss} (L/kg)	17.93	42.12
% F	--	11.53

5.3. Conclusion

Single dose oral pharmacokinetic studies were performed for medicarpin (K095) at 5mg/kg, cladrin (K054) and S006-1709 at 10mg/kg each, in female S.D. rats. The intravenous pharmacokinetic studies were also performed at the single dose of 1mg/kg for each compound. K095 showed rapid absorption, large tissue distribution (12.27 L/kg) and low clearance (2.76 L/h/kg). This pharmacokinetic behaviour of K095 compound, when administered separately, was similar to that observed in the pharmacokinetics study of herbal fraction F147. The absorption of K054 was also rapid with large tissue distribution (6.72 L/kg) and low clearance (2.25 L/h/kg). For, S006-1709, the tissue distribution (17.93 L/kg) and clearance (7.19 L/h/kg) were greater.

The half-life of all the three compounds was between 4h to 5h and the T_{max} values between 0.5h to 0.75h, after oral dosing. The bioavailability of K095 was highest (22.34%) while the bioavailability of other two compounds K054 and S006-1709 were comparable and greater than 10%. The single dose pharmacokinetic studies performed for K095, K054 and S006-1709 indicates that these compounds have favourable pharmacokinetic parameters and correlate well with their activity *in vivo,* thereby establishing the PK-PD correlation. Thus, the three active compounds promise to be potential candidate for treatment and management of osteoporosis, even when they are used as an individual compound.

References

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