4.2 Individual In Vivo Study Reviews

4.2.1 ^{14}C-CS-747 (LY640315): Disposition after Oral Administration (H7T-LC-TAAB)

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Study Center: Lilly Laboratory for Clinical Research, Indiana University Hospital and Outpatient Center, Rm UH6134 550 North University Boulevard Indianapolis, Indiana 46202
Study Dates: May-June 2002
Phase 1

Objectives	To determine the disposition of CS-747 in healthy men after oral administration of a single dose of ^{14}C-CS-747. To determine the plasma pharmacokinetics of R-95913, R-106583 and R-119251, and radiocarbon after the administration of a single oral dose of ^{14}C -CS-747 to healthy male subjects. To assess radiocarbon mass balance by quantifying ^{14}C excretion in urine and feces. To identify major metabolites of CS-747 in urine and plasma.
Study Design	An open-label, single-center, mass-balance study.
Population	Five healthy male subjects
Investigational Drug	Unlabeled and radiolabeled CS-747 (dissolved in 170 mL of vehicle solution: degassed cola with 2% ethanol) to provide 15 mg CS-747 with approximately 100 μCi of radioactivity. Lot #: CT23001.
Dosage	The actual dose: 14.7 mg of CS-747 containing 90.03 (±3.59) μCi. The specific activity:13.6 dpm/ng. The nominal dose (15 mg; 100 μCi) was used in the report
Administration	A single oral dose of ^{14}C -CS-747 in 170 ml of vehicle solution Fasting 10 hours pre-dose and until 5 hours post-dose
Assay	Plasma concentrations of the one active and three inactive metabolites of CS-747: LC/MS/MS with tandem mass spectrometry methods. Plasma, whole blood, breath, platelet, urine, and feces concentrations of radioactivity: liquid scintillation counting.
PK Assessment	Noncompartmental methods (WinNonlin) for CS-747 metabolites and total radioactivity. Blood cell/plasma partition coefficients were calculated for radiolabelled material. Fraction of radiolabeled dose excreted in feces, urine, and in breath
Metabolites in Urine	-Solid phase extraction (SPE) prior to analysis. -Some samples were also hydrolyzed with ß-glucuronidase before SPE. -HPLC system microplate solid scintillation counting
Metabolites in fecal homogenates	Extracts of 0.5 to 0.7 g aliquots of the (various time points) of each subject were evaporated to dryness under nitrogen and reconstituted in 500 μL 0.2% formic acid/methanol/acetonitrile (7:1.5:1.5, v/v). -HPLC-profiles of radioactivity : microplate solid scintillation counting
Identification of Metabolites	Mass Spectrometric: LC/MS/MS, validated method

RESULTS

Demographics: Five of 6 subjects (except for #4508) completed the study according to the protocol.

Table 22. Subject Demographics

Subject Number	Subject ID Number	Age (yr)	Gender	Origin	Screening Height (cm)	Screening Weight (kg)
1	4508	24	M	CAUCASIAN	173	87.9
2	4463	31	M	CAUCASIAN	171	65.6
3	2440	60	M	CAUCASIAN	183	70.6
4	4486	43	M	BLACK	173	92.1
5	4503	34	M	BLACK	180	85.6
6	2949	45	M	CAUCASIAN	168	66.7

Assay:

The active and 3 inactive metabolites were measured in plasma

Table 23: Assay Characteristics of Inactive Metabolites in Plasma

Parameter	R119251		R106583		R95913	
Linearity	1 ng/mL to 500 ng/mL					
	Inter-batch	Intra-batch	Inter-batch	Intra-batch	Inter-batch	Intra-batch
Precision (CV %)	2.4 to 6.6	1.8 to 4.6	2.7 to 3.6	1.6 to5.2	14.5 to 6.2	1.6 to 8.0
Accuracy, %	-0.3 to -10.3	-10.7 to 0.1	–9.9 to 3.9	-9.9 to 7.9	0.1 to 12.5	4.3 to 15.3
LLOQ	1ng/mL					
Reviewer Comment	The assay characteristics and specificity are satisfactory, representative mass-chromatograms are shown					

Table 24 Assay Characteristics of an Active Metabolite in Plasma

Parameter	R138727
Linearity	0.5 ng/mL to 250 ng/mL
	Inter-batch
Precision (CV %)	0.98 to 3.39
Accuracy, %	-7.0 to -5.98
LLOQ	0.5ng/mL
Reviewer Comment	The assay characteristics and specificity are satisfactory, representative mass-chromatograms are shown

Radioactivity was measured in plasma, whole blood, urine, feces, and an expired air. Radioactivity in platelets was too low to permit a meaningful interpretation of the data.

Urine and fecal excretion

Table 25. Mean (±SEM) Cumulative Percentages of a Single 15-mg (100 μCi) Oral Dose of [^{14}C]CS-747 Excreted in Human Urine and Feces

Time	Feces	Urine	Total
0-6[a]	---	32.12 ± 1.74	32.12 ± 1.74
0-12	---	52.52 ± 4.16	52.52 ± 4.16
0-24	9.64[b]	61.26 ± 3.64	65.12 ± 6.45
0-48	21.00 ± 2.68	66.32 ± 3.23	87.32 ± 4.88
0-72	24.63 ± 1.07	67.48 ± 3.20	92.11 ± 3.09
0-96	25.84 ± 0.70	67.76 ± 3.24	93.60 ± 2.87
0-120	26.45 ± 0.90	67.93 ± 3.24	94.37 ± 2.86
0-144	26.67 ± 0.84	68.06 ± 3.24	94.73 ± 2.89
0-168	26.84 ± 0.91	68.14 ± 3.24	94.97 ± 2.89
0-192	26.90 ± 0.89	68.20 ± 3.24	95.10 ± 2.91
0-216	26.96 ± 0.88	68.24 ± 3.23	95.20 ± 2.89
0-240	27.00 ± 0.86	68.26 ± 3.23	95.25 ± 2.91

a) Feces samples collected on the first day are reported under the 0-24 row.
b) N=2, no SEM is reported.

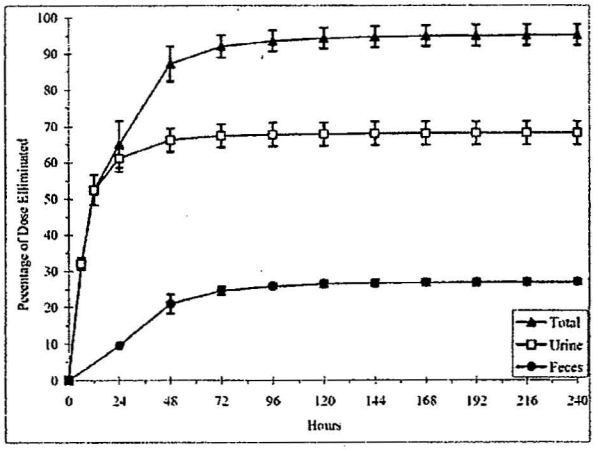


Figure 29. Mean (±SEM) of the percentages of ^{14}C dose eliminated in humans following a single oral 15-mg (100 μCi) dose of ^{14}C -CS-747.

Metabolites in Plasma

CS-747, the prodrug, is metabolized rapidly and was not detected in plasma collected from the 5 subjects following the [^{14}C]LY640315 (CS-747) dose. Subsequent interpretation of radiochemical profiles and mass spectral data confirmed the presence of R-95913, R-138727, R-106583, R-119251, and 12 additional metabolites in plasma collected over the first 12 hours. Radiochromatographic profiling was performed with both derivatized and non-derivatized plasma samples. A representative radiochromatogram of the 0.5-hour underivatized plasma obtained from Subject 4503 is shown below

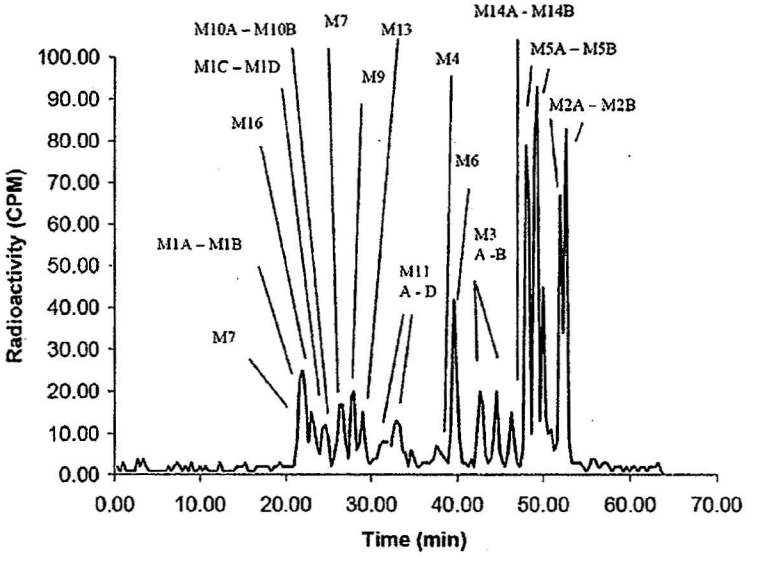
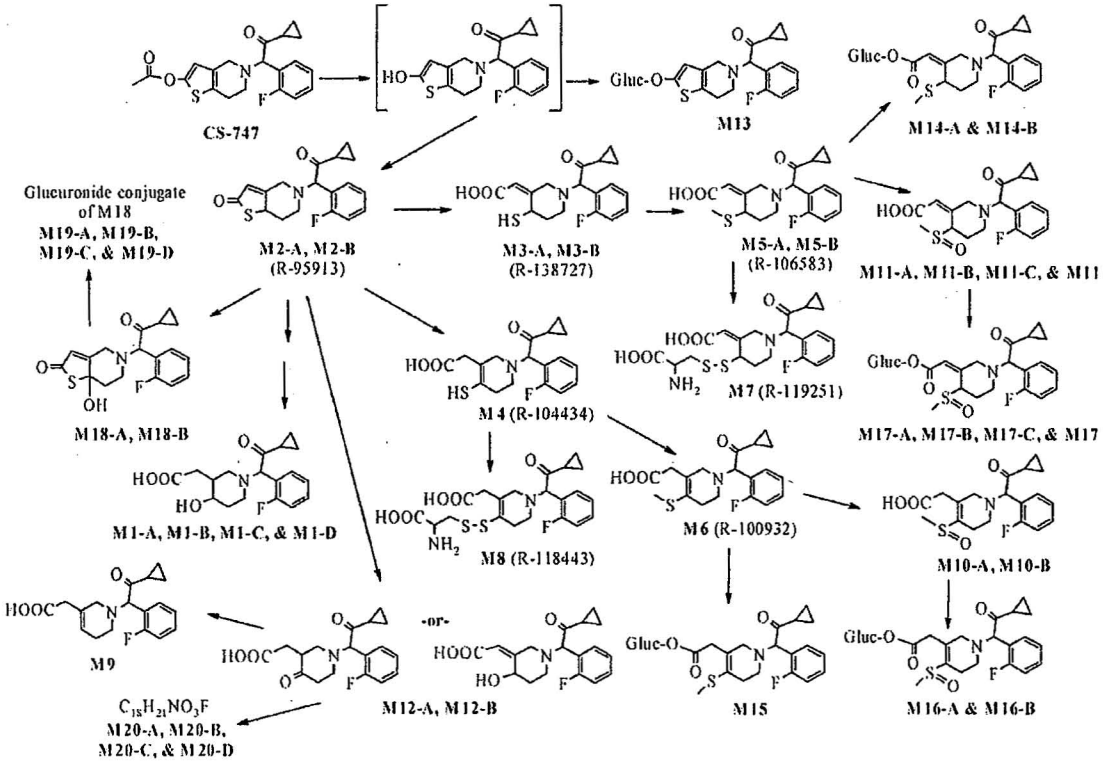


Figure 30. Radiochromatogram of the 0.5-hour underivatized plasma (Subject 4503)

The identified metabolites are shown below:



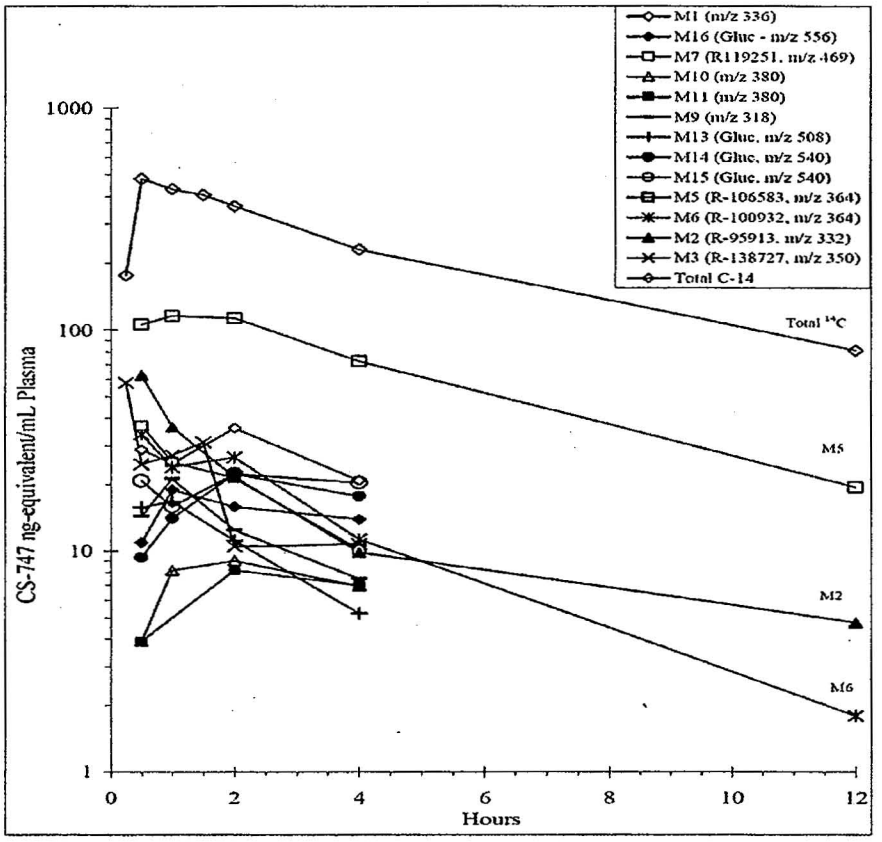


Figure 31. Mean plasma concentrations of LY640315 metabolites in plasma following a 15-mg oral dose of [^{14}C]LY640315, (N = 5).

The results show that R-106583 is the major metabolite in human plasma, followed by R-95913 and R-138727.

R-106583 (M5) represented 21.6% ± 5.0% (0.5 hour, mean± SD; N = 5), 27.5% ± 5.6% (1 hour), 31.4% ± 4.7% (2 hours), 30.5% ± 5.6% (4 hours), and 19.1% ± 11.8% (12 hours).

The pharmacologically active metabolite, M3 (R-138727), and M4 (R-104434) were observed as minor metabolites in the underivatized plasma due to their instability as underivatized compounds in a biological matrix. In plasma samples with derivatizing agent (3-methoxy-phenacyl bromide) added to the whole blood, R-138727 was observed in relatively higher concentrations in plasma. The extraction efficiency of radioactivity from plasma was approximately 96% in the samples collected between 0.5 and 4 hours. The recovery of radioactivity from the 12-hour plasma samples following extraction was 70.0%. Treatment of the 12-hour plasma samples with dithiothreotol (DTT) resulted in an increase of the percentage of radioactivity extracted to 82%. This suggests disulfide binding between metabolites of LY640315 (containing a sulfhydryl moiety) and plasma proteins.

Metabolites in Urine

A representative radio-HPLC profile of a pooled 0- to 24-hour urine sample is shown below.

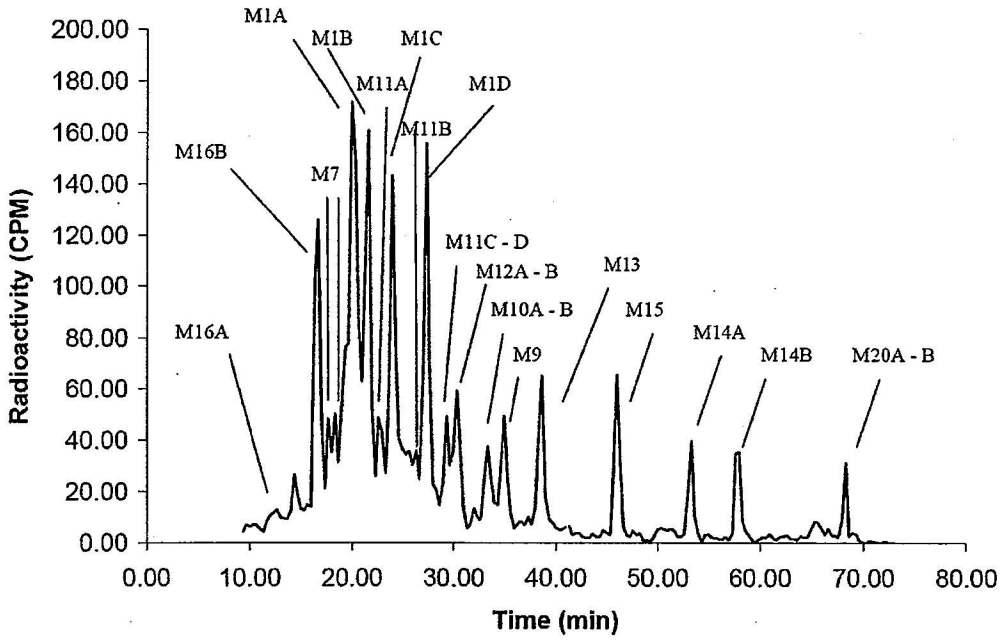


Figure 32 Radiochromatogram of the pooled 0-24 hour urine of Subject 4503

The radioactivity in the 0- to 24-hour pooled urine samples represented approximately 90% of the radioactivity excreted in the urine over the entire 240-hour collection period and 61% of the administered radioactive dose. Approximately 86% of the urinary radioactivity (approximately 52% of the LY640315 dose) was putatively identified. The major metabolites in urine were the diastereomers of M1, which collectively accounted for 34.6% ± 5.2% (mean ± SD; N=5) of the urinary radioactivity (21.3% ± 5.1% of the dose). Total of thirteen metabolites were identified in urine.

Table 26. Mean Percentages Metabolites in Pooled 0-24 hours Human Urine

Metabolite Peak ID	Mean ± SD [a]	Percent of Dose ± SD
M1 (A - D) [d]	34.6 ± 5.2	21.3 ± 5.1
M16 (A - B) [c]	9.9 ± 1.1	6.0 ± 1.0
M7 (R-119251)	3.3 ± 0.7	2.0 ± 0.5
M10 (A - B) [c]	3.2 ± 6.4	2.3 ± 3.7
M11 (A - D) [d]	10.4 ± 4.1	6.0 ± 2.4
M9	4.0 ± 1.5	2.5 ± 0.9
M12 (A - B) [c]	4.6 ± 1.9	2.7 ± 1.0
M13	5.5 ± 1.4	3.3± 0.8
M14 (A - B) [c]	4.3 ± 0.4	2.6 ± 0.4
M15	4.7 ± 0.9	2.8 ± 0.4
M20 (A -D) [d]	1.4 ± 0.3	0.8 ± 0.1
Total Identified Metabolites	**85.8 ± 5.1**	**52.4 ± 6.2**
Amount Excreted [b]	**NA**	**61.3 ± 3.6[c]**

Metabolites in Feces

Approximately 27% of the ^{14}C dose was eliminated in feces, 91% of which was recovered within the first 72 hours after administration of [^{14}C]LY640315 (CS-747). Six metabolites were detected in feces, which were also observed in plasma. A representative radiochromatogram of a 24-hour fecal extract is shown below.

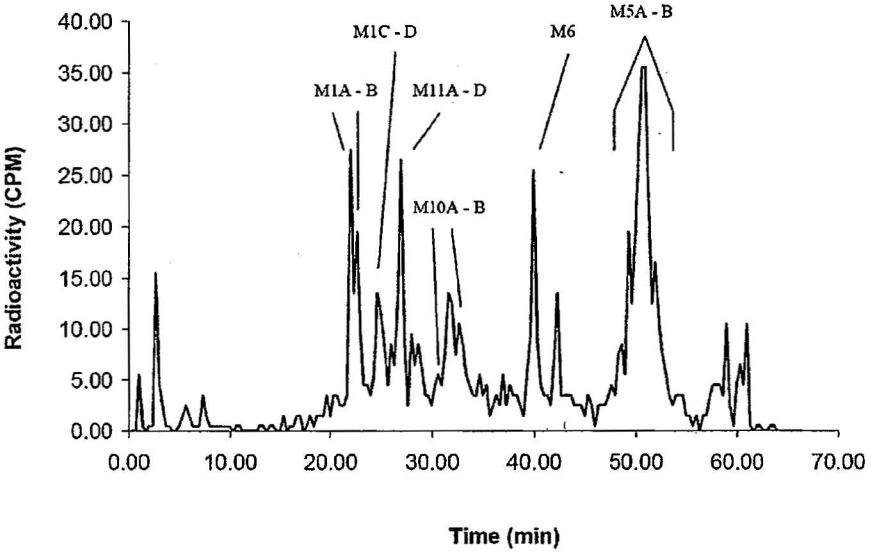


Figure 33 Radiochromatogram of a 0-24 hour fecal extract of Subject 2949

The major metabolites were the diastereomers of M5 (R-106583) and M1 (m/z 336). Overall, 8.3% of the dose in feces was identified.

Metabolite ID	Percent of Dose	
	Mean	**SD**
M1 (A - D) [d]	2.6	0.3
M9	0.5	0.3
M10 (A - B) [e]	0.8	0.6
M11 (A - D) [d]	0.5	0.4
M5 (A-B) [e] (R-106583)	3.4	1.4
M6 (R-100932)	0.6	0.4
Total Mean % of Dose	8.3	1.7
Amount Excreted	24.6	1.1

Pharmacokinetics

PK analysis was conducted on plasma concentrations of R95913, R138727, R119251, and R106583, for total radioactivity in plasma (expressed in ^{14}C -CS-747 ng-eq/mL) and in whole blood (expressed in ^{14}C -CS-747 ng-eq/g). The mean concentration-time profiles are shown below.

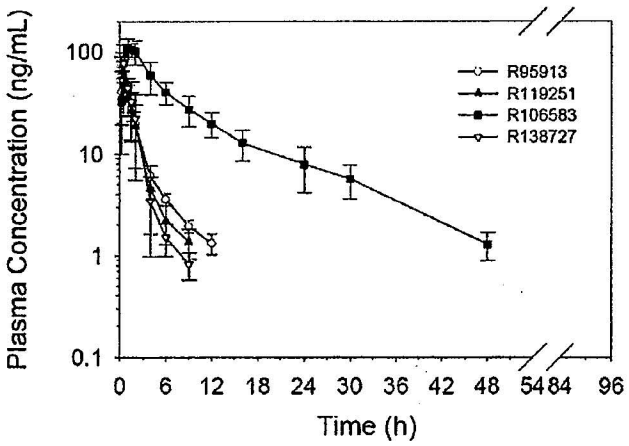


Figure 34. R-95913, R-119251, R-106583, and R-138727 arithmetic mean (±SD) plasma concentration versus time profiles after a single oral dose of 15 mg ^{14}C -CS-747

After the dose of drug, plasma concentrations of R95913 and R119251 were quantifiable 24 hours, of R138727 - 16 hours, and of R106583 - 72 hours. PK Parameters were estimated for these metabolites.

Plasma vs Whole blood

The mean radioactivity concentration time-profiles in plasma and whole blood are shown below

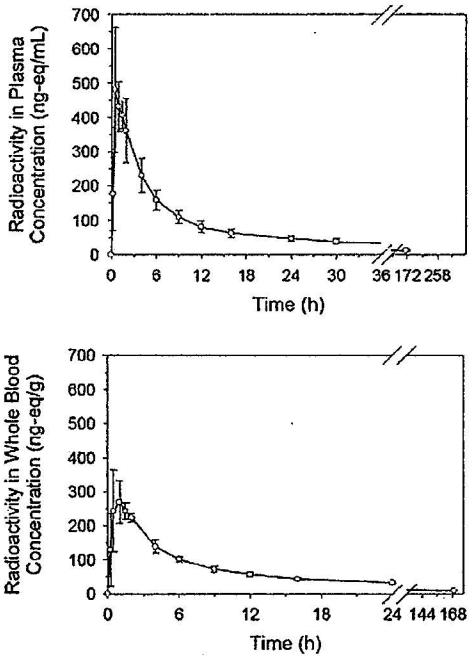


Figure 35. Plasma (upper panel) and whole blood (lower panel) radioactivity arithmetic mean (±SD) concentration versus time profiles after a single oral dose of 15 mg ^{14}C -CS-747

Figure below shows the individual plasma to whole blood radioactivity ratios over time, assuming that the density of plasma and whole blood are equivalent.

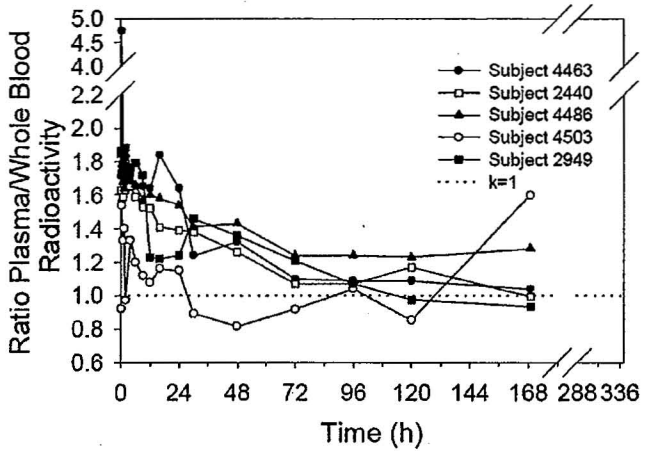


Figure 36. Ratio of radioactivity in plasma and whole blood after a single oral dose of 15 mg ^{14}C -CS-747.

Since for the majority of observations this ratio is greater than one, the concentration of drug-related material is lower in the blood cell fraction than the plasma fraction.

The sponsor proposed the simplified scheme of metabolism of CS-747.

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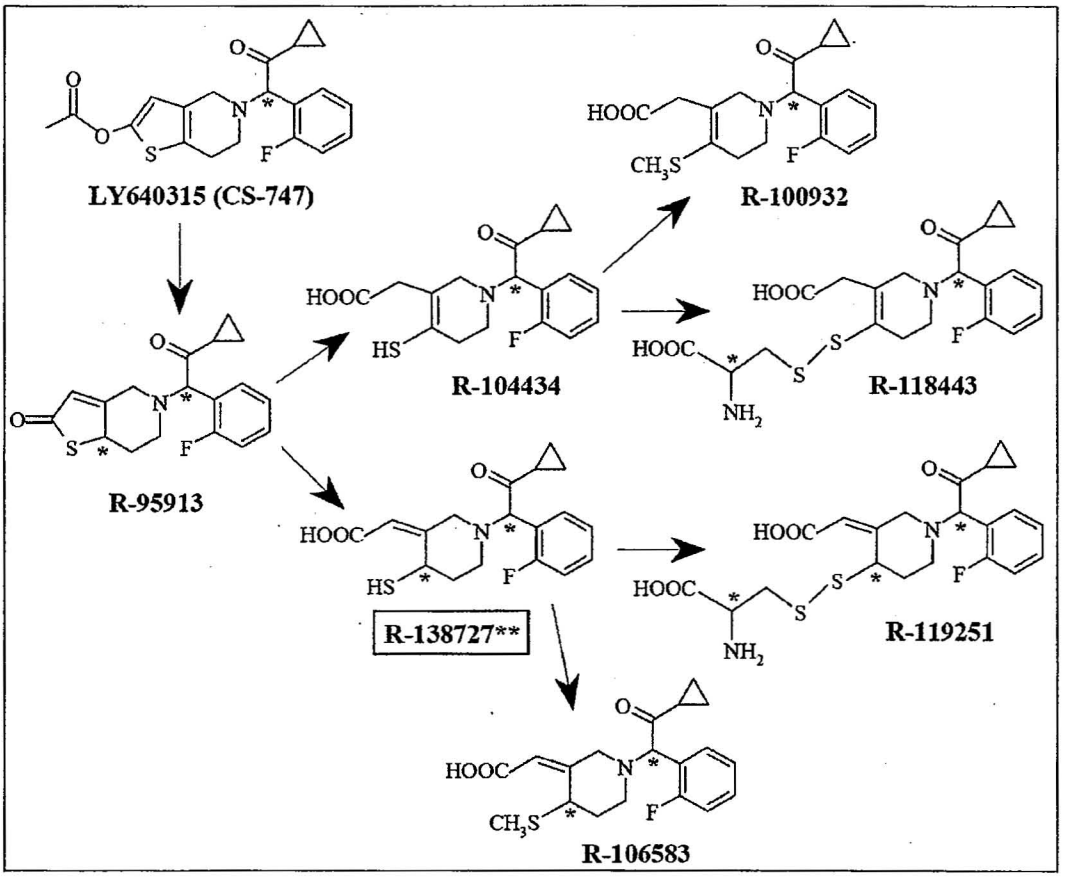
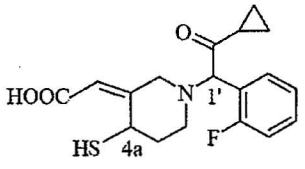


(*) Chiral centers.
(**) Pharmacologically active compounds (enantiomers shown below)



Compound	4a	1'
R-125687	S	S
R-125688	S	R
R-125689	R	R
R-125690	R	S

Sponsor's Conclusions:

1. Humans eliminated 61.3% of the CS-747 dose in the urine within 24 hours of dosing, increasing to 68.3% of the dose within 10 days. The elimination in the feces accounted for 27% of the dose. The results show that CS-747 is rapidly absorbed and extensively metabolized and that renal excretion is the major route for elimination of CS-747 metabolites in humans.
2. R-106583 is the major metabolite in human plasma, followed by R-95913 and R-138727.

3. The major metabolites observed in the urine were the diasteromers of M1 (m/z 336). The isolation of the four diastereomeric peaks resulted in further confirmation of the postulated structure for this metabolite. Radiochromatographic profiling and mass spectrometry showed that metabolites M1-A and M1-B were inter-convertible, and metabolites M1-C and M1-D were inter-convertible.
4. After a single oral dose of 15 mg (100 μCi) of ^{14}C -CS-747 in solution, plasma pharmacokinetics for the inactive metabolites were similar to those observed after administration of solid dosage forms. Of total plasma radioactivity, the measured metabolites contributed to a median 14.7% (range 11.0%-20.6%) of AUC(0-8). The terminal half-life of plasma radioactivity (median 188 hours, range 68.9– 228 hours). Although the half-life of plasma radioactivity should be interpreted cautiously, these data suggest that unmeasured and/or covalently protein-bound metabolites are persisting in the circulation.
5. The plasma-to-whole blood ratio was generally greater than one, suggesting that radioactivity in the plasma was greater than that in an equivalent volume of blood cells.

Reviewer Comments

1. This study adequately evaluated the mass-balance of CS-747 in 5 healthy males.
2. The gender differences were not evaluated in this study.
3. CS-747 was rapidly absorbed and extensively metabolized and renal excretion (up to 68% over 10 days) is the major route for elimination of CS-747 metabolites in humans.
4. R-106583 is the major metabolite in human plasma, followed by R-95913 and R-138727, these metabolites represent a mixture of interconverting diastereomers.
5. Although the measured total radioactivity plasma-to-whole blood ratio was generally greater than one, this does not prove that the penetration into red blood cell was limited to a specific molecular entity.