Table 18. Percent Inhibition of R-138727 Formation in the Presence of Sulfaphenazole, Omeprazole, or Ketoconazole

Microsomal Sample	Sulfaphenazole (CYP2C9)	Omeprazole (CYP2C19)	Ketoconazole (CYP3A)
		2.0 µM R-95913	
HLC	-24 %	21 %[a]	29 %[a]
HLD[b]	5.6 %	40 %	50 %[a]
HLG	-30 %	31 %	55 %[a]
HLL[b]	-40 %	8.6 %[a]	8.6 %[a]
		20 µM R-95913	
HLC	13 %	8.3 %	33 %
HLD[b]	32 %	-37 %	47 %
HLG	12 %	11 %	83 %
HLL[b]	24 %	29 %	86 %[a]

[a] The LLOQ level was reached. Minimum inhibition percentages are indicated. Inhibition percentages are likely actually greater than the values indicated.
[b] HLD and HLL are deficient in CYP2C19.

Minor and more variable inhibition was observed with sulfaphenazole and omeprazole. To evaluate the effect of expression of CYP3A subfamily members CYP3A4 and CYP3A5 on R-138727 formation, enzyme kinetic parameters for the formation of R-138727 were determined in microsomes prepared from insect cells and engineered to express CYP3A4 + CYP reductase or CYP3A5 + CYP reductase.

Table 19. Enzyme Kinetic Profiles for the Formation of R-138727 by Expressed CYP3A4 and CYP3A5 Supersomes□

Expressed CYP	K_m (µM)	V_{max} (pmol/min/pmol CYP)	CL_{int} (V_{max}/K_m) (µL/min/pmol CYP)
CYP3A4	18 ± 2	0.50 ± 0.04	0.03
CYP3A5	48 ± 11	1.1 ± 0.2	0.02

In vitro, these two enzymes exhibited similar efficiency of converting R-95913 to R-138727, so the presence or absence of the polymorphically expressed CYP3A5 in patients would be expected to be of little consequence for this biotransformation.

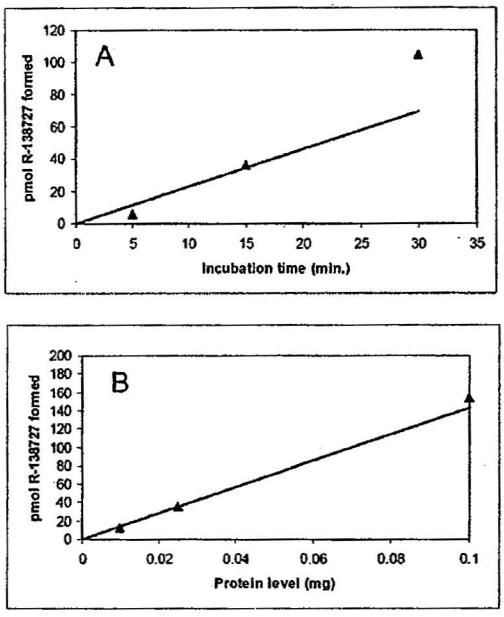


Figure 26. Formation of R-138727 from R-95913 (10 μM) with respect to time (A) and with respect to protein (B).

Reviewer's Comments

1. The sponsor' conclusion that CYP3A is the major enzyme responsible for the formation of R-138727 from R-95913 is reasonable.
2. The raw data of this study were not submitted for review.

Table 20. Enzyme Kinetic Profiles for the Formation of RS/RR and SR/SS R-138727 from R-95913 in Human Liver Microsomes

Microsomal Sample	R-138727 Isomer set	K_m (μM)	V_{max} (pmol/min/mg)	Cl_{int} (V_{max}/K_m) (μL/min/mg)
BHMQ	RS/RR	25.4 ± 1.0	176 ± 8	6.95
BHMQ	SR/SS	28.2 ± 0.7	119 ± 6	4.23

The enzymes capable of forming RS/RR of R-138727, in rank order, were CYP2B6 > CYP3A4 ~ CYP2C19 > CYP3A5 > CYP2C9 and SR/SS of R-138727, in rank order, were CYP2B6 > CYP2C19 > CYP3A4 > CYP3A5>CYP2C9 (Figure below). The ratios of the RS/RR to SR/SS isomer sets after incubation with 20 μM R-95913 for CYP2B6, CYP2C19, CYP2C9, CYP3A4, and CYP3A5 were 0.64, 0.85, 0.49, 1.6, and 1.6, respectively, indicating that only CYP3A4 and CYP3A5 generate more RS/RR over SR/SS isomers.

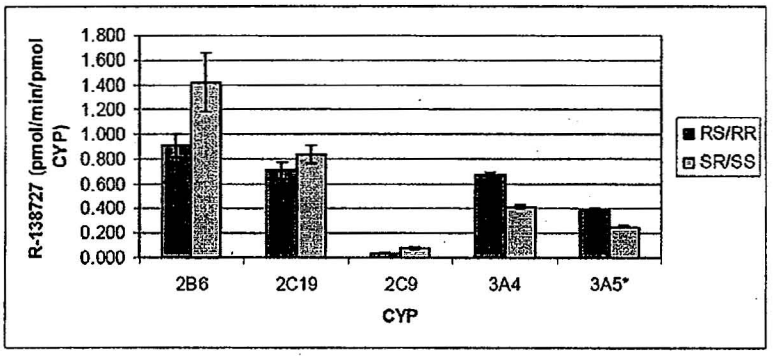


Figure 27 Formation of isomer sets of R-138727 by expressed CYPs following incubation with 20 μM R-95913.

Enzyme kinetic parameters for the formation of the two isomer sets of R-138727 by expressed CYP2B6, CYP2C19, CYP2C9, CYP3A4, and CYP3A5 are shown in the table below.

Table 21. Enzyme Kinetic Profiles for the Formation of RS/RR and SR/SS R-138727 from R-95913 in Expressed Supersomes™

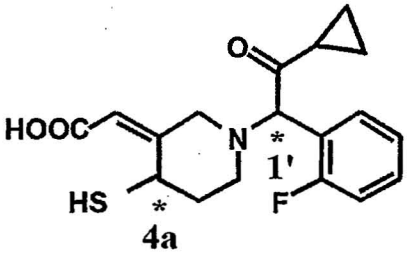
Microsomal Sample	R-138727 Isomer set	K_m (μM)	V_{max} (pmol/min/pmol)	Cl_{int} (V_{max}/K_m) (μL/min/pmol)
CYP2B6	RS/RR	4.52 ± 0.82	1.15 ± 0.04	0.255
CYP2B6	SR/SS	4.62 ± 0.67	1.75 ± 0.13	0.379
CYP2C19	RS/RR	4.98 ± 0.39	0.873 ± 0.096	0.175
CYP2C19	SR/SS	4.56 ± 0.33	0.978 ± 0.102	0.215
CYP2C9	RS/RR	26.8 ± 2.3	0.087 ± 0.007	0.003
CYP2C9	SR/SS	19.8 ± 1.0	0.147 ± 0.011	0.007
CYP3A4	RS/RR	15.2 ± 1.3	1.13 ± 0.07	0.074
CYP3A4	SR/SS	8.12 ± 0.62	0.569 ± 0.033	0.070
CYP3A5*	RS/RR	128 ± 8	2.94 ± 0.23	0.023
CYP3A5*	SR/SS	21.8 ± 1.2	0.563 ± 0.035	0.026

Michaelis-Menten kinetic parameters for the formation of isomers of R-138727 were determined in pooled human liver microsomes from four different donors. The kinetic analyses were consistent with a single enzyme being responsible for the conversion of R-95913 to either isomer set, with the apparent Km values of 25.4 and 28.2 μM, and intrinsic clearances of 6.95 and 4.23 μl/min/mg, respectively, for the isomer sets RS/RR and SR/SS.
The specific CYPs were assessed for their abilities to produce both isomer sets of R-138727. Following incubations with 20 μM R-95913, all five CYPs formed the RS/RR isomer set of R-138727. The ratios of RS/RR to SR/SS isomer sets generated at this concentration were approximately 0.6:1, 0.8:1, 0.5:1, 1.6:1 and 1.6:1, respectively, for CYP2B6, 2C19, 2C9, 3A4 and 3A5, suggesting CYP3A4 and 3A5 preferentially form RS/RR while 2B6, 2C19 and 2C9 prefer SR/SS formation.
Considering the relative abundance of each CYP enzyme in human liver and proximal small intestine, the CYP3A subfamily would be suggested as the enzymes predominantly responsible for the formation of both isomer sets of R-138727. The role of CYP3A in R-138727 formation in vivo is further supported by the higher RS/RR intrinsic clearance value than that of SR/SS in human liver microsomes and the observation that only CYP3A is capable of forming more RS/RR than SR/SS among all CYPs examined. Relative to CYP3A5, CYP3A4 appears to be a relative high affinity but low capacity enzyme in formation of both isomer sets of R-138727.

Sponsor's Conclusions

1. The members of the CYP3A subfamily are primarily responsible for the conversion of R-95913 to both isomer sets of R-138727, with RS/RR being preferentially formed over SR/SS isomers at the ratio of 1.6.
2. CYP2B6, CYP2C9, and CYP2C19 are also capable of forming either isomer set of R-138727. These results imply that the effects of altered activity of any of the CYPs involved in R-138727 isomer formation in vivo would be compensated for by the abilities of multiple CYPs to form the active metabolites.
3. At the same time, factors other than the stereoselective formation of R-138727 enantiomers by CYPs may contribute to their stereoselective disposition.

From study TAAD:
The ratios of the distereomers found in the plasma samples are also listed in the bioanalytical data summary section of this report. The RS-and RR-diastereomers of R-138727 comprised about 84%, while the SR and SS diastereomers accounted for about 16%, of the R-138727 present in plasma. The RS- and RR-diastereomers of R-138727 (R-125690 and R-125689, respectively) are the most potent of the 4 isomers with IC50 values of 0.19 μM and 3.1 μM. The IC50 values of the other two diastereomers are 28 μM (R-125687) and 36 μM (R-125688). The ratios of the R-138727 stereoisomers were consistent among subjects, regardless of the dose, time of sample collection, or whether the blood was sampled after the first CS-747 dose or after a few weeks of therapy.



Compd No.	4a	1'
R-125690	R	S
R-125689	R	R
R-125687	S	S
R-125688	S	R

R-99224 (SR+RS)

R-138727 (SR+RS+RR+SS)

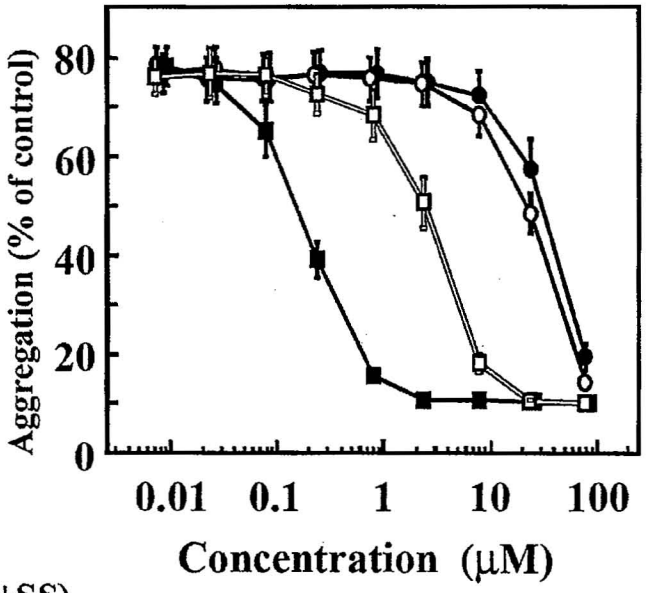


Figure 28. The structures of R-138727 stereoisomers and their relative activity towards inhibition of platelet aggregation.

4.2 Individual In Vivo Study Reviews

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

Investigator(s): M J. Goldberg, MD M. A. Turik, MD J. T. Callaghan, MD, PhD
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

b(4)

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.

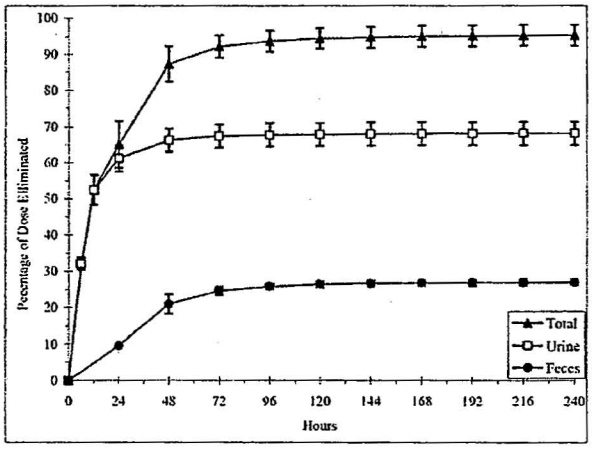


Figure 29. Mean (±SEM) of the percentages of ^{14}C dose eliminated in humans following a single oral 15-mg (100 µCi) dose of ^{14}C -CS-747.

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

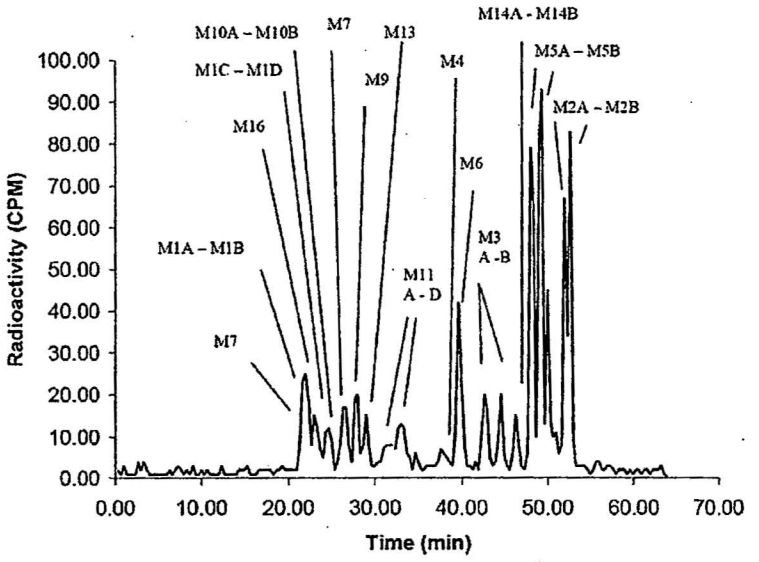
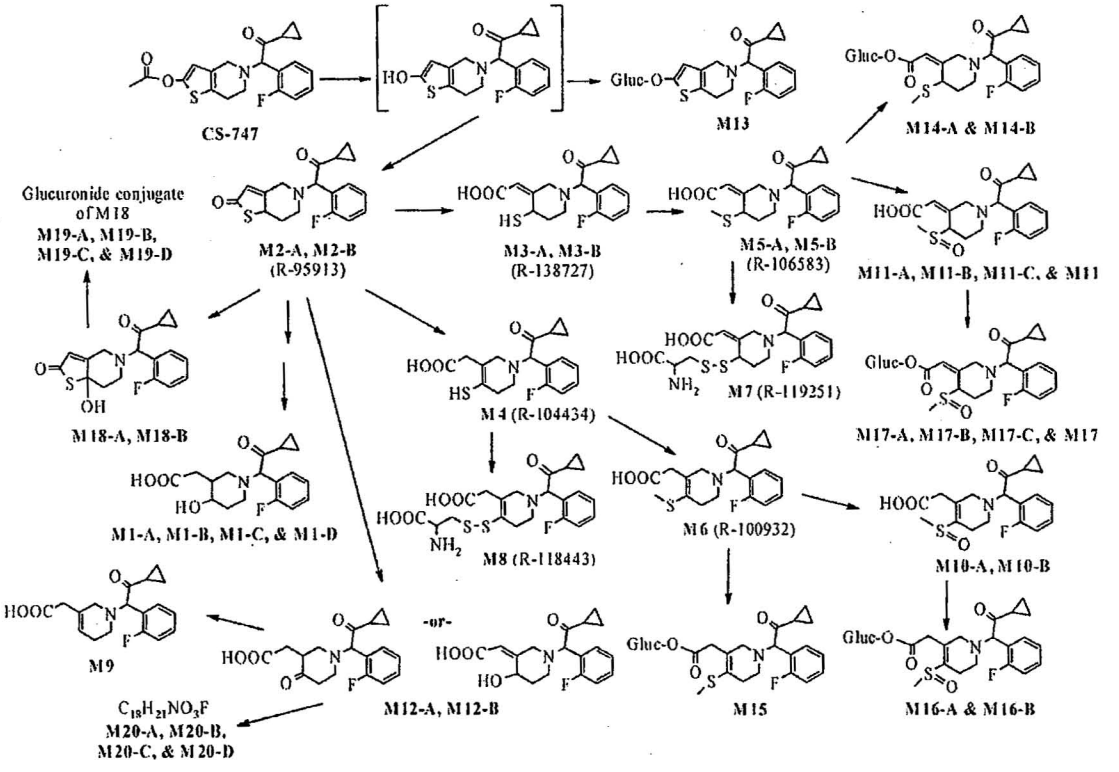


Figure 30. Radiochromatogram of the 0.5-hour underivatized plasma (Subject 4503)

The identified metabolites are shown below:



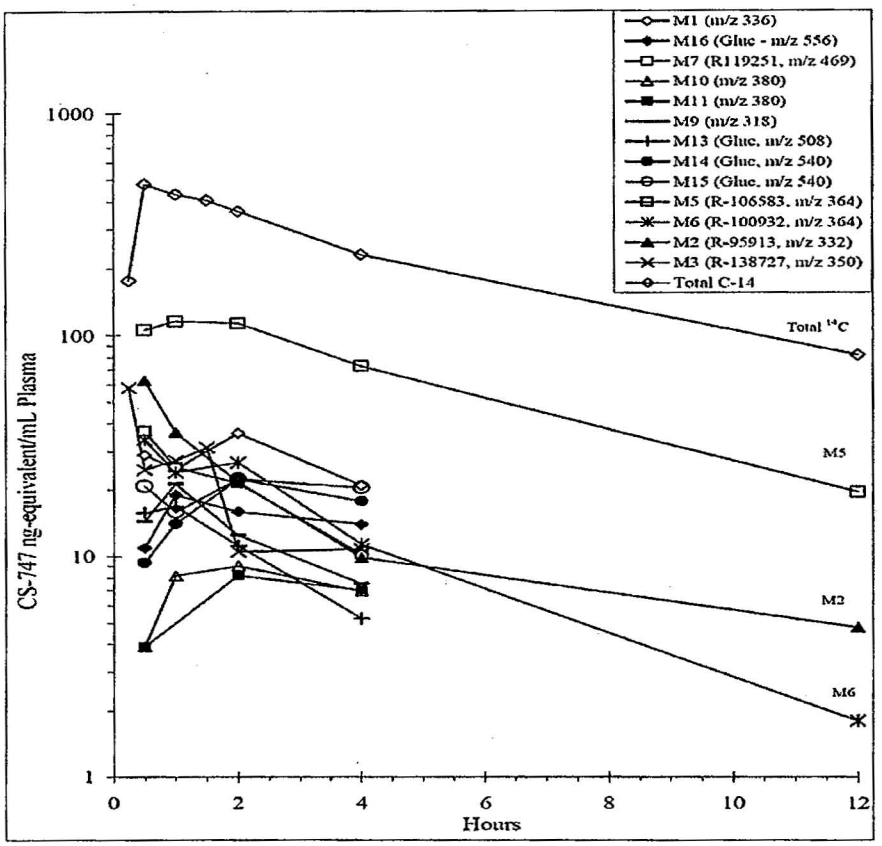


Figure 31. Mean plasma concentrations of LY640315 metabolites in plasma following a 15-mg oral dose of [^{14}C]LY640315, (N = 5).

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.

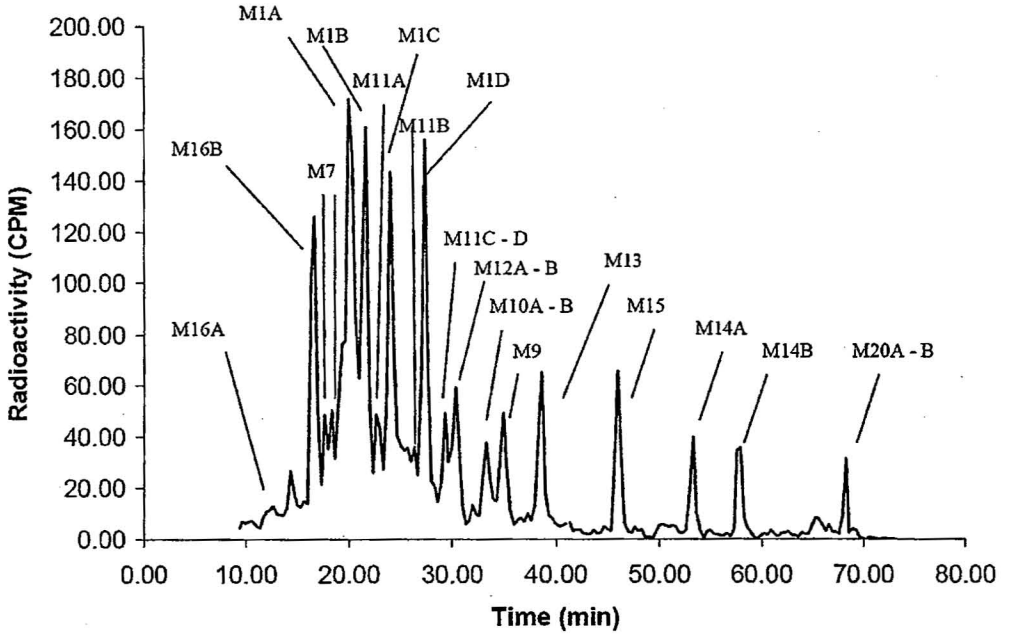


Figure 32 Radiochromatogram of the pooled 0-24 hour urine of Subject 4503

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) [e]	9.9 ± 1.1	6.0 ± 1.0
M7 (R-119251)	3.3 ± 0.7	2.0 ± 0.5
M10 (A - B) [e]	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) [e]	4.6 ± 1.9	2.7 ± 1.0
M13	5.5 ± 1.4	3.3± 0.8
M14 (A - B) [e]	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.

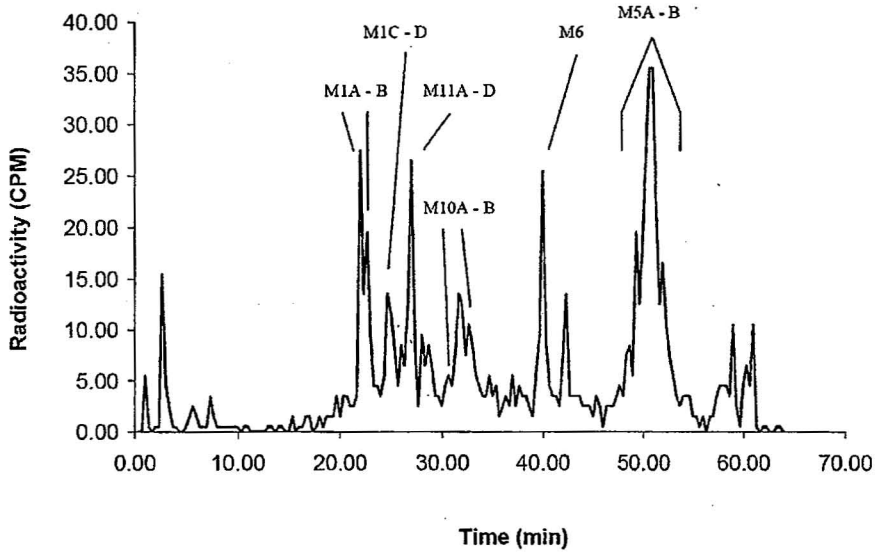


Figure 33 Radiochromatogram of a 0-24 hour fecal extract of Subject 2949

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.