administered radioactivity or Day 10 was reached, whatever occurred first. The weight of each sample was documented.

For the purpose of deciding whether or not a subject met the release criteria "real time" determination of the radioactivity in urine and feces was performed using [redacted] spectrometry [redacted]. However, these measurements were not considered in the final mass balance analysis of the data.

Assays

Measurements of Total Radioactivity in Whole Blood, Plasma, Urine and Fecal Samples

Total radioactivity was measured in plasma and urine by direct [redacted]. Radioactivity in whole blood and homogenized fecal samples was by [redacted] after oxidation of the sample aliquots.

Measurement of Ambrisentan in Plasma, Urine, and Feces

Ambrisentan concentrations in plasma urine and fecal homogenates were determined using fully validated LC-MS/MS methodology. The LLOQ in plasma, urine and feces was [redacted] respectively.

Characterization of Ambrisentan Metabolites in Plasma, Urine and Feces

Pooled samples were used for the characterization of ambrisentan metabolites in plasma, urine and feces. The concentrated pooled plasma extracts, the centrifuged pooled urine samples and the concentrated pooled fecal homogenate extracts were analyzed by HPLC with radio-detection. These analyses should be considered to be only semi-quantitative.

Identification of Metabolites in Plasma, Urine and Feces

Pooled samples were used for the identification. The samples were injected in quadruplicate into the HPLC system. The eluates were collected into fractions of 0.5 min intervals. Elution-time matched fractions from the 4 injections were pooled for further analysis. After concentration to dryness under nitrogen gas and reconstitution in methanol/water the concentrated samples were submitted to LC-MS/MS.

PK Data Analysis

PK parameters for ambrisentan and total radioactivity in plasma, whole blood, urine and feces were obtained using non-compartment model dependent methods. WinNonlin™ Professional Network Edition Version 4.0, Pharsight Corp., Palo Alto, CA was used.

The total radioactivity parameters included: Cmax(blood,rd), tmax(blood,rd), AUCtlast(blood,rd),AUC0-00(blood,rd), λz(blood,rd), t1/2(blood,rd).

The parameters for ambrisentan and total radioactivity in plasma included: Cmax,tmax, tlag, AUC0-tlast, AUCo-00, λz, t1/2, CL/F, Cmax(rd),tmax(rd), tlag(rd), AUC0-tlast(rd), AUC0-00(rd), λz(rd), t1/2(rd), RCmax(blood/plasma,rd), RAUC(blood/plasma,rd)

The parameters for ambrisentan and total radioactivity derived from urine included:

Ae(urine,0-tlast), % Ae (urine,o-last,rd), t1/2, CLr, Ae (urine,0-tlast,rd), %Ae(urine,0-tlast,rd), λz(urine,rd), t1/2(rd)

The parameters for ambrisentan and total radioactivity in the feces included:

Ae(feces,0-tlast), %Ae(feces, 0-tlast), Ae(feces,0-tlast,rd), %Ae(feces,0-tlast,rd)

Ratios of ambrisentan and its metabolites to total radioactivity were calculated for Cmax and AUC0-tlast

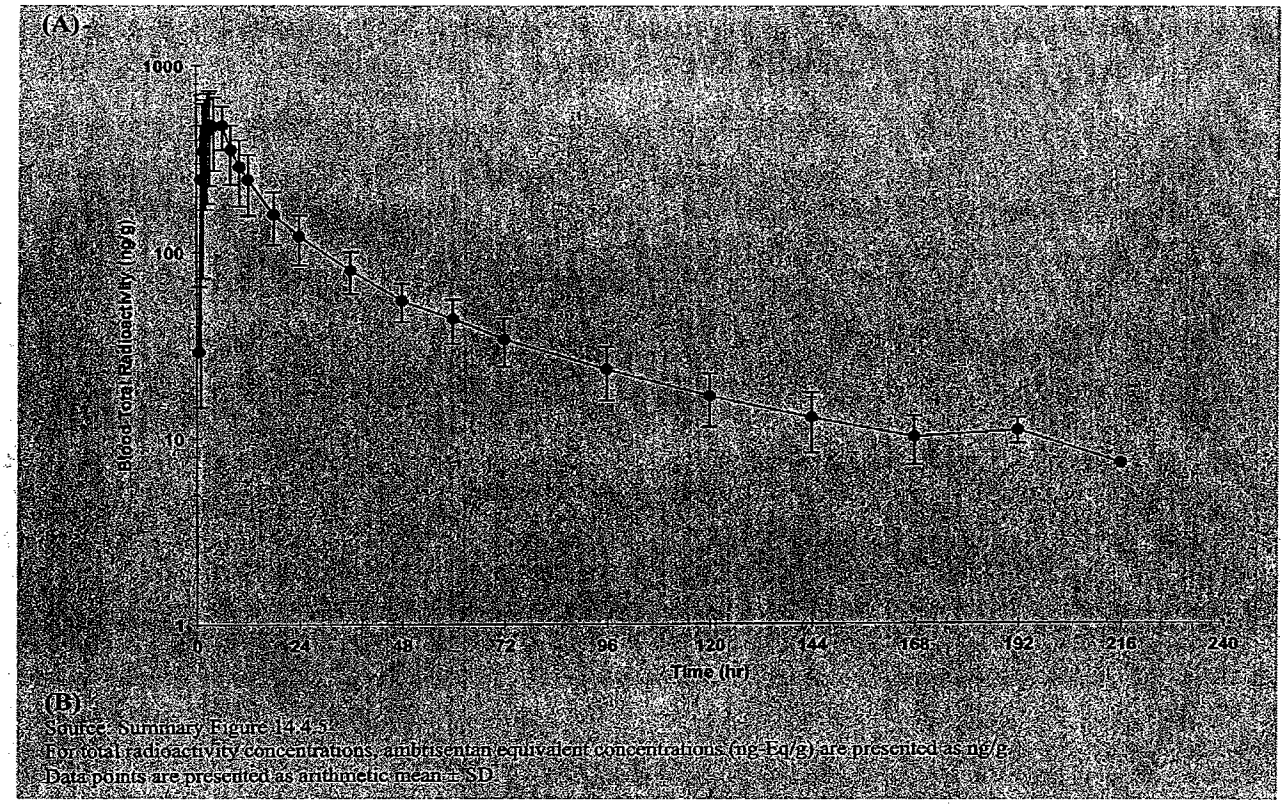
Results

Eight healthy male subjects of mean age 32 y and body weight 77.4 kg were enrolled and completed the study.

A retrospective determination of the total radioactivity in the excreta using definitive [redacted] showed that the recovery in 4 of the 8 subjects was lower than 90 % of the administered dose. Thus the "real time" analysis overestimated the recovery of total radioactivity in the excreta. The true recoveries in these 4 subjects were [redacted] and are acceptable.

Plasma and Whole Blood Data

A semi-logarithmic plot against time of the mean concentrations of total radioactivity in whole blood is shown in the below figure:



Source: Summary Figure 14.4-52

For total radioactivity concentrations, ambrisentan equivalent concentrations (ng-Eq/g) are presented as ng/g.

Data points are presented as arithmetic mean ± SD.

Table 11.2 lists the values of the derived parameters:

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Table 11.2 Summary of Total Radioactivity Pharmacokinetic Parameters in Whole Blood (Pharmacokinetic Full Analysis Set)

PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
$C_{max,d}$ (ng/g)	320.16 (83.364) n = 8	311.40	26.04
$t_{max,d}$ (hr)	4.00 (1.50, 6.00) n = 8		
$AUC_{t1,last,d}$ (ng·hr/g)	5910.9 (1223.78) n = 8	5797.7	20.70
$AUC_{t1,\infty,d}$ (ng·hr/g)	6353.6 (1317.58) n = 8	6229.3	20.74
$\lambda_{z,d}$ (1/hr)	0.01124 (0.001620) n = 8	0.01114	14.42
$t_{1/2,d}$ (hr)	62.797 (8.8322) n = 8	62.246	14.06

Source: Summary Table 14.2.5
Values for $C_{max,d}$ are reported by median (minimum, maximum).
n = number of subjects included in the calculation of mean values
d = total radioactivity
d parameters based on antimicrobial equivalent concentrations (ng Eq/g)

Mean maximum whole blood concentrations of total radioactivity are attained after about 4 h post-dose. The estimated mean apparent t1/2 of total radioactivity in whole blood is about 63 h. The report does not state the criteria used in determining the slope of the terminal log linear phase.

A semi-logarithmic plot of the mean total radioactivity in plasma against time and derived parameters are shown in Figure 11.2 and Table 11.3, respectively:

(B)

Source: Summary Figure 14.4.3

For total radioactivity concentrations, zimbrescinan equivalent concentrations (ng Eq/mL) are presented as ng/mL.
Data points are presented as arithmetic mean ± SD.

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Table 11.3 Summary of Total Radioactivity Pharmacokinetic Parameters in Plasma (Pharmacokinetic Full Analysis Set)

PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
$C_{max,rd}$ (ng/mL)	615.96 (176.581) n = 8	595.19	28.67
$AUC_{0\text{-}last,rd}$ (ng·hr/mL)	11174.6 (2515.20) n = 8	10927.4	22.51
$AUC_{0\text{-}\infty,rd}$ (ng·hr/mL)	11890.9 (2614.70) n = 8	11636.5	21.99
$t_{max,rd}$ (hr)[1]	3.50 (1.00, 6.00) n = 8		
$t_{lag,rd}$ (hr)[1,2]	0.50 (0.50, 0.50) n = 8		
$\lambda_{z,rd}$ (1/hr)	0.0113 (0.00175) n = 8	0.0112	15.43
$t_{z,rd}$ (hr)	62.71 (10.680) n = 8	61.97	17.03
$R_{Cmax(blood/plasma),rd}$	0.52359 (0.021873) n = 8	0.52319	4.18
$R_{AUC(blood/plasma),rd}$	0.53098 (0.022809) n = 8	0.53056	4.30

Source: Summary Table 14.2.6
[1]Values for $t_{max,rd}$ and $t_{lag,rd}$ are reported by median (minimum, maximum)
[2]Values for t_{lag} may also represent the first sampling time, and therefore should be interpreted with caution.
n = number of subjects included in the calculation of mean values
rd = total radioactivity
rd parameters based on ambrisentan equivalent concentrations (ng-Eq/mL); as 1 g plasma was assumed to be equivalent to 1 mL plasma, ambrisentan equivalent concentrations are presented as ng/mL.
$R_{Cmax(blood/plasma),rd} = C_{max,rd(blood)}/C_{max,rd(plasma)}$
$R_{AUC(blood/plasma),rd} = AUC_{0\text{-}last,rd(blood)}/AUC_{0\text{-}last,rd(plasma)}$

Mean tmax and t1/2 derived from the plasma concentrations of total radioactivity in plasma are similar to those in whole blood. The ratios of the concentrations in whole blood to plasma are slightly greater than 0.5 indicating that the moieties measured exhibit substantial plasma protein binding resulting in small unbound fractions in plasma available for penetration into red cells.

A semilogarithmic plot of the mean plasma concentrations of ambrisentan against time and derived PK parameters are shown in Figure 11.3 and Table 11.4, respectively:

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Table 11.4 Summary of Pharmacokinetic Parameters for Ambrisentan in Plasma (Pharmacokinetic Full Analysis Set)

PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
C_{max} (ng/mL)	380.88 (165.500) n = 8	351.60	43.45
AUC_{0-t} (ng·h/mL)	6743.9 ([illegible]) n = 8	[illegible]	[illegible]
$AUC_{0-\infty}$ (ng·h/mL)	[illegible] ([illegible]) n = [illegible]	[illegible]	[illegible]
t_{max} (h)	4.00 (1.00, 6.00) n = 8		
t_{lag} (h)	0.75 (0.50, 1.50) n = 8		
λ_z (1/h)	0.05015 (0.011784) n = 6	0.04900	[illegible]
$t_{1/2}$ (h)	14.61 (4.4242) n = 6	[illegible]	30.28
CL/F (L/h)	2.54 (0.545) n = 6	2.49	21.45

Source: Summary Table 14.2-6
For 2 subjects λ_z could not be determined, therefore the [illegible] $AUC_{0-\infty}$ and CL/F [illegible]
Values for t_{max} and t_{lag} are reported by median (minimum, maximum)
Values for t_{lag} may also represent the first sampling time and therefore should be interpreted with caution
n = number of subjects included in the calculation of mean values

On average ambrisentan attained maximum plasma concentrations about 4 h after administration. An apparent mean t1/2 of about 15 h was estimated for ambrisentan. Relative to ambrisentan the total radioactivity declined much slower indicating that ambrisentan metabolites are formed whose residence time in plasma is longer than that of the parent drug.

Data in Urine

A linear plot of the mean amounts of total radioactivity cumulatively excreted in urine and expressed in % of the dose as well as derived parameters are shown in Figure 11.4 and Table 11.5:

Figure 11.4: Mean Cumulative Percent of Dose Excreted in Urine Over Time Following a 10 mg Dose of [^{14}C] Ambrisentan Measured as (A) Total Radioactivity and as (B) Ambrisentan

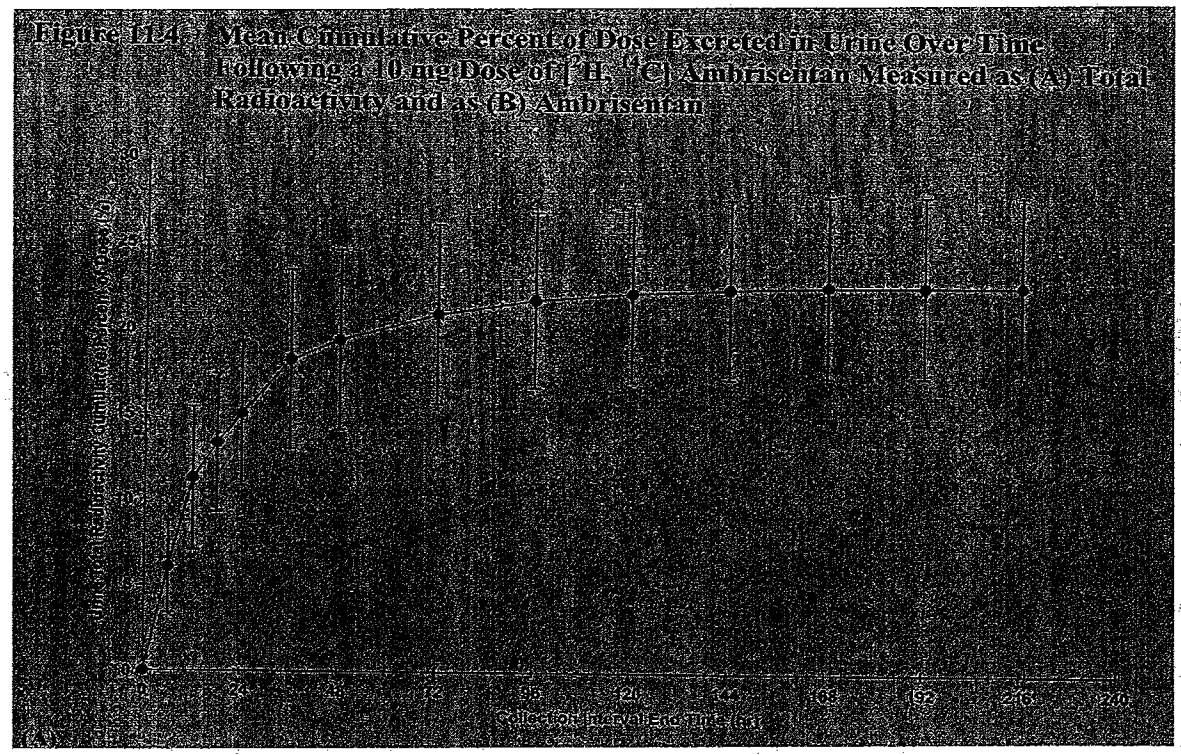

Table 11.5: Summary of Total Radioactivity Pharmacokinetic Parameters in Urine (Pharmacokinetic Full Analysis Set)

PK Parameter (Unit)	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
A_e (mg)	2.26 (0.527)	2.21	23.35
f_e (%)	22.58 (5.274)	22.10	23.35
k_e (1/h)	0.0216 (0.00750)	0.0213	0.35
$t_{1/2}$ (h)	33.12 (5.945)	32.1	17.95

Source: Summary Table 12.2.7
n = number of subjects with the specified type of parameter value
A_e = total radioactivity
Parameters based on ambrisentan equivalent concentrations (ug Eq/g)
A_e ambrisentan equivalent amounts (mg Eq) excreted in urine

On average about 22 % of the dose is excreted as total radioactivity in urine over a period of 8-10 days after administration. The sponsor estimated from the data in urine that the radioactivity is eliminated from the body with an apparent mean t1/2 of about 33 h.

Table 11.6 provides a summary of the parameters for ambrisentan derived from the data in urine:

Table 11.6 Summary of Pharmacokinetic Parameters for Ambrisentan in Urine (Pharmacokinetic Full Analysis Set)

PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
Ae[illegible] (mg)	0.33 (0.071) n = 8	0.33	21.34
%Ae[illegible]	3.35 (0.071) n = 8	3.28	21.34
$t_{1/2}$ (h)	11.19 (2.538) n = 6	10.983	22.67
CL_R (L/h)	0.00913 (0.00192) n = 8	0.0090	21.05

Source: Summary Table 14.2.7
n = number of subjects included in the calculation of mean values.

The data indicate that mean recovery of unchanged ambrisentan (about 3% of the administered dose) and estimated mean renal clearance (< 1ml/min) are small.

Data in Feces

The mean amounts of radioactivity excreted cumulatively in the feces and expressed in % of the dose as well as related parameters are shown in Figure 11.5 and Table 11.7, respectively.

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Figure 11.5 Mean Cumulative Percent of Dose Excreted in Feces Over Time Following a 10 mg Dose of [^{3}H, ^{14}C] Ambrisentan Measured as (A) Total Radioactivity and as (B) Ambrisentan

(A)

Table 11.7 Summary of Total Radioactivity Pharmacokinetic Parameters in Feces (Pharmacokinetic Full Analysis Set)

PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
$Ae_{feces\,0\text{-}last\,ni}$ (mg)	6.57 (0.665) n = 8	6.54	10.12
$\%\,Ae_{feces\,0\text{-}last\,ni}$ (%)	65.73 (6.65) n = 8	65.43	10.12

Source: Summary Table 14.2.8
n = number of subjects included in the calculation of mean values
ni = total radioactivity
pi = parameters based on ambrisentan equivalent concentrations (ng-Eq/g)
ni ambrisentan equivalent amounts (mg-Eq) are presented as mg

On average about 66% of the administered radioactivity is recovered in the feces over 8-10 days after administration. It appears that the accumulation of total radioactivity in the feces is slower than in urine, but it must be considered that fecal recovery depends on intestinal mobility in addition to elimination of systemically available ambrisentan and metabolites.

The data on the mean recovery of ambrisentan in feces are summarized in Table 11.8:

Table 11.8 Summary of Pharmacokinetic Parameters for Ambrisentan in Feces (Pharmacokinetic Full Analysis Set)

PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
$Ae_{feces\ 0-last}$ (mg)	3.65 (0.645) n = 8	3.60	17.67
$\%\ Ae_{feces\ 0-last}$ (%)	36.49 (6.448) n = 8	35.99	17.67

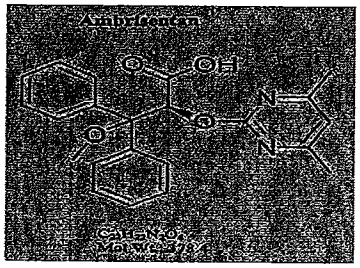
Source: Summary Table 14.2.8
n = number of subjects included in the calculation of mean values

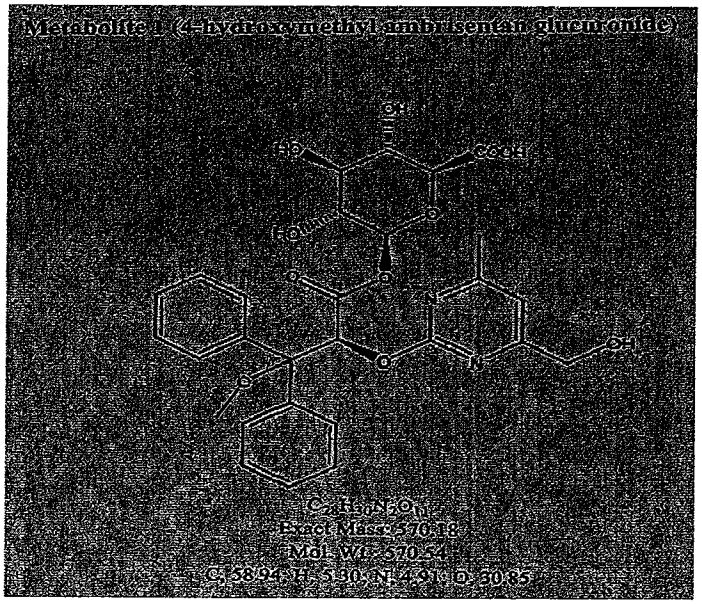
The mean fecal recovery of ambrisentan is about 36% of the dose and hence substantially smaller than the 66% fecal recovery of total radioactivity. This is indicates that ambrisentan metabolites are substantially excreted in the feces.

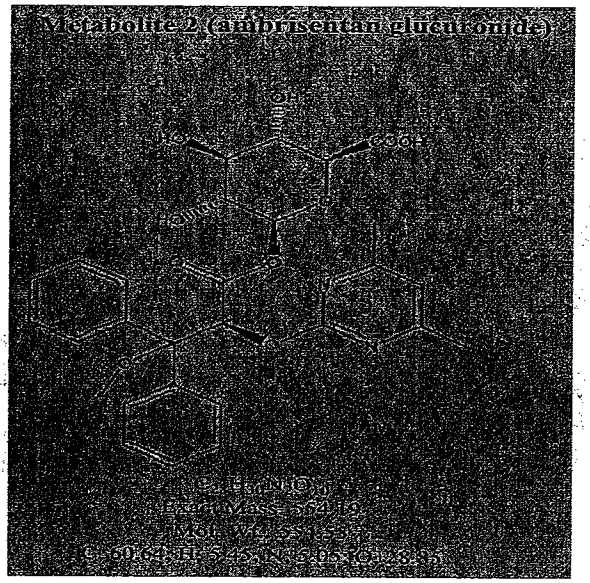
Characterization and Identification of Ambrisentan Metabolites in Plasma, Urine and Feces

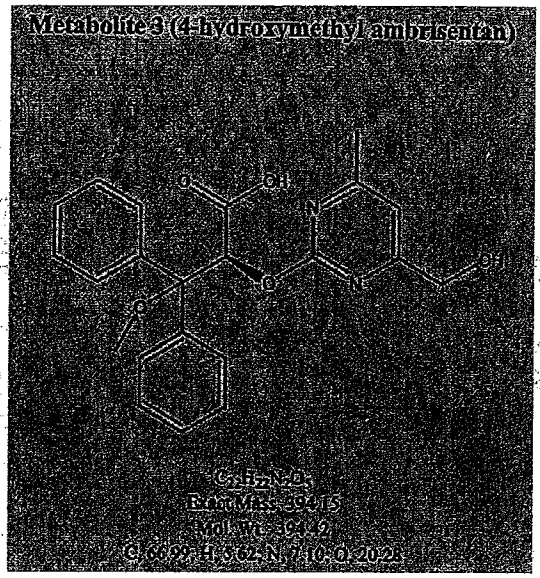
In addition to ambrisentan which was found in plasma, urine and feces, the following 3 metabolites were identified in the tested biological fluids:

4-hydroxymethyl ambrisentan glucuronide (Metabolite 1) was present in plasma and urine, ambrisentan glucuronide (Metabolite 2) was found in plasma and urine and 4-hydroxymethyl ambrisentan (Metabolite 3) was identified in plasma and feces. The structures of ambrisentan and its 3 metabolites are shown below:









Metabolite 1 is the acyl glucuronide of hydroxylated ambrisentan. Metabolite 2 is the acyl glucuronide of ambrisentan and Metabolite 3 is hydroxylated ambrisentan.

Metabolic Profiling of Ambrisentan in Plasma

Using a semi-quantitative, non-validated method based on radio-HPLC analysis approximate plasma concentration profiles of ambrisentan and identified metabolites were constructed. Profiles and derived parameters are shown in Figure 11.6 and Tables 11.9 and 11.10:

Figure 1146 Mean Radioactivity Plasma Concentration - Time Profiles of Ambrisentan and its Metabolites Following a 10 mg Dose of [$^{2}H_{5}$, ^{14}C] Ambrisentan Based on %HPLC Peak Following Radio-HPLC Analysis: [(A) Linear Scale and (B) Semi-Logarithmic Scale]

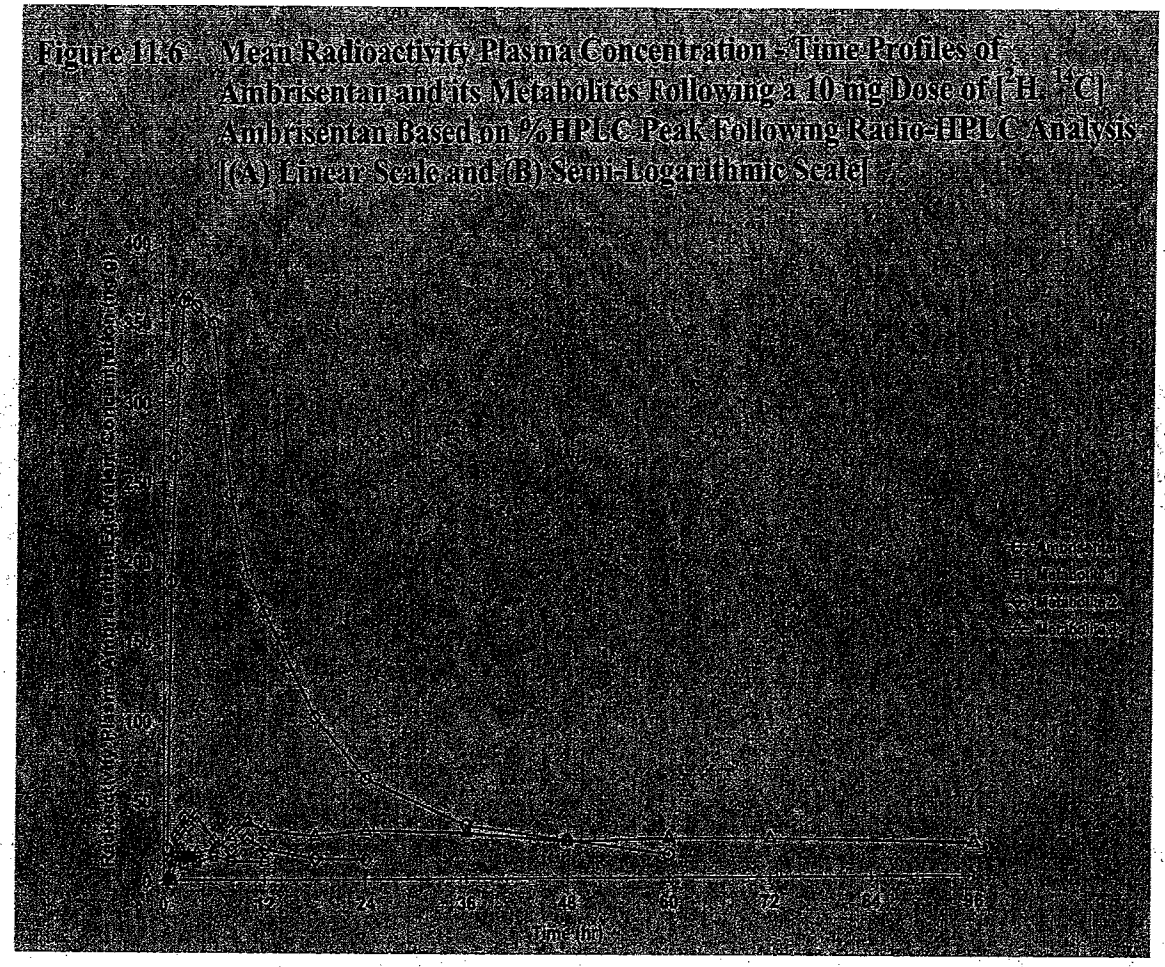


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Table 11.9 Summary of Relative Pharmacokinetic Parameters for Ambrisentan and Metabolites in Plasma, based on %HPLC Peak Following Radio-HPLC Analysis[1] (Pharmacokinetic Full Analysis Set)

Compound	PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
Ambrisentan	C_{max} (ng/g)	465.45 (137.545) n = 8	448.03	29.55
	t_{max} (hr)[2]	3.50 (2.50, 6.00) n = 8		
	AUC_{inf} (ng·hr/g)	5366.61 (1494.287) n = 8	5202.40	27.84
Metabolite 1	C_{max} (ng/g)[3]	14.27 (5.157) n = 6	13.64	36.13
	t_{max} (hr)[2,3]	3.50 (1.50, 8.00) n = 6		
	AUC_{inf} (ng·hr/g)[3]	59.79 (57.65) n = 6	[illegible]	96.26
Metabolite 2	C_{max} (ng/g)	41.26 (34.077) n = 8	34.63	82.59
	t_{max} (hr)[2]	2.75 (2.00, 10.00) n = 8		
	AUC_{inf} (ng·hr/g)	306.11 (323.290) n = 8	228.61	105.61
Metabolite 3	C_{max} (ng/g)	38.46 (15.619) n = 8	35.59	40.61
	t_{max} (hr)[2]	15.00 (8.00, 60.00) n = 8		
	AUC_{inf} (ng·hr/g)	1666.80 (757.088) n = 8	1515.49	45.42

Source: Summary Table 14.2.10

n = number of subjects included in the calculation of mean values.

[1] %HPLC Peak Radio-HPLC analysis is a non-validated, semi-quantitative analytical method; therefore, the radioactivity concentration values are considered estimates. Furthermore, the radioactivity PK parameters derived using these concentration values are considered as estimated and therefore, may not be as accurate as the PK values obtained using the LC-MS/MS method described in Section 9.5.

[2] Values for t_{max} are reported by median (minimum, maximum).

[3] Two subjects did not have any quantifiable peaks for Metabolite 1 over the time course of the plasma sampling. Therefore, n is equal to 6 for all Metabolite 1 parameters.

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Table 11.10 Summary of the Relative Pharmacokinetic Parameters Ratios for Ambrisentan and Metabolites in Plasma, based on %HPLC Peak Radio-HPLC Analysis[1] (Pharmacokinetic Full Analysis Set)

Ratio	PK Parameter	Arithmetic Mean (SD)	Geometric Mean	Coefficient of Variation (%)
Ratio of ambrisentan/total[2]	C_{max}	0.84 (0.0359) n = 8	0.84	4.27
	$AUC_{0\text{-last}}$	0.73 (0.0488) n = 8	0.73	6.65
Ratio of Metabolite 1/total[2]	C_{max}[3]	0.027 (0.0078) n = 6	0.026	29.30
	$AUC_{0\text{-last}}$[3]	0.0082 (0.0056) n = 6	0.0066	68.36
Ratio of Metabolite 2/total[2]	C_{max}	0.070 (0.0315) n = 8	0.065	45.00
	$AUC_{0\text{-last}}$	0.039 (0.0311) n = 8	0.032	78.89
Ratio of Metabolite 3/total[2]	C_{max}	0.069 (0.0185) n = 8	0.067	26.91
	$AUC_{0\text{-last}}$	0.22 (0.059) n = 8	0.21	26.68

Source: Summary Table 14.2.10

[1] %HPLC peak radio-HPLC analysis is a non-validated, semi-quantitative analytical method; therefore, the radioactivity concentration values are considered as estimates. Furthermore, the radioactivity PK parameters derived using these concentration values are considered as relative.

[2] Total is the sum of ambrisentan, Metabolite 1, Metabolite 2, and Metabolite 3 PK parameter values for the indicated PK variable. Ratios were calculated for individual subject data.

[3] Two subjects did not have any quantifiable peaks for Metabolite 1 over the time course of the plasma sampling. Therefore, n is equal to 6 for all Metabolite 1 parameters.

The mean AUC data suggest that ambrisentan is the major circulating moiety (73% of total identified compounds in plasma), whereas metabolites 1, 2 and 3 represent only about 1%, 4% and 22%, respectively, of the total compounds identified in plasma. The respective Cmax values indicate about the same relationships. The respective mean tmax values of for ambrisentan and metabolites 1 and 2 occur between 3 and 4 h. In contrast metabolite 3 peaks much later, about 15 h after administration. More important are the ratios of the AUC's of ambrisentan and metabolites to the AUC of total radioradioactity in plasma.

Conclusion

The mean total recovery in urine (22.58%) and feces (65.73%) of the administered ^{14}C ambrisentan is 88.31% and acceptable. On average 11.69% of total radioactivity is not accounted for. About 80% of radioactivity recovered in urine is excreted within 48 h after administration. Total radioactivity and ambrisentan in plasma decline with approximate t1/2's of about 63 h and 15 h, respectively, indicating the residence time of the metabolites in the body is greater than that of ambrisentan. The recovery of ambrisentan in urine is much smaller (3.35%) than that of total radioactivity (22.58%). Thus, the metabolites are excreted in urine to a larger extent than ambrisentan. The fecal recovery of ambrisentan is substantially smaller (36.49 %) than that of

total radioactivity (65.73%) suggesting that systemically available metabolites are eliminated into the bile and/or are formed in the intestine. Three metabolites M1, the acyl glucuronide of hydroxylated ambrisentan, M2, the acyl glucuronide of ambrisentan, and M3, hydroxylated ambrisentan, were identified. Among the circulating compounds in plasma ambrisentan is by far the most important moiety.

Additional calculations were performed by the reviewer and summarized in the below Table:

Mean Recoveries of Radioactivities, Total Radioactivity, Radioactivity Associated with Ambrisentan and Radioactivity not Associated with Ambrisentan

	Mean Recovery in % of Dose		
Matrix	Total Radioactivity	Ambrisentan	Non-Ambrisentan
Feces	65.73	36.49	29.24
Urine	22.58	3.35	19.23
Total	88.31	39.84	48.47

The respective recovery of radioactivity in urine and feces not associated with ambrisentan is 19.23% and 29.24% of the dose. It is not known whether the radioactivity not associated with ambrisentan in the feces represents metabolites excreted into the bile or metabolites generated in the lumen of the intestine or a combination of both. It is also not known whether the radioactivity non-assignable to ambrisentan represents the 3 identified metabolites or whether there are unidentified metabolites. The fraction of the dose of ambrisentan absorbed is unknown. It can be estimated that minimally 22.58% and maximally 63.51% of a dose of ambrisentan is absorbed. If 22.58% of ambrisentan is absorbed then metabolism is the pre-dominant route of elimination for ambrisentan (85.2% of the absorbed dose) and the impact of enzyme inhibitors and liver impairment on the exposure to ambrisentan could be substantial. If 63.51% of the dose of ambrisentan is absorbed then the contribution of metabolism to the elimination of ambrisentan is smaller, and biliary excretion could be a major route of elimination. However, in any case metabolic inhibitors and hepatic impairment cannot be excluded as important covariates for the exposure to ambrisentan.

The respective ratios of AUC0-tlast of radioactivity assignable to ambrisentan, M3, M2 or M1 to the AUC0-tlast of total radioactivity are 0.35, 0.15, 0.03 and 0.005 suggesting the presence of not yet defined metabolites in plasma.

Comments

1. A validated quantitative method for the measurement of the identified ambrisentan in the biological fluid of interest should have been developed and applied.

2. The radioactivity recovered in urine and feces not assignable to ambrisentan should have been analyzed to determine identity and quantity of the metabolites in the excreta. The

possible fecal generation of metabolites from ambrisentan in vitro should also have been examined. This information could have been useful in estimating the dose of ambrisentan absorbed after oral administration and could have helped to establish the relative contributions of the metabolic and biliary routes of elimination for ambrisentan.

3. The criteria used in estimating λz for the different moieties measured should have been defined in the protocol.

4. Values for AUC0-tlast instead of AUC were determined. The former values may underestimate the AUC of ambrisentan and the metabolites relative to that of total radioactivity.

Study Report: AMB-103" A Phase I, Open-Label, Randomized, Crossover Study to Compare Ambrisentan Formulations for Bioequivalence at Multiple Drug Doses in Healthy Adults" Volunteers

Study Investigator and Site: [redacted]

Objectives

Primary

To compare the clinical trial and commercial formulations of ambisentan [redacted] tablets for bioequivalence
To compare the clinical trial and commercial formulations of ambrisentan 5 mg tablets for bioequivalence
To compare the clinical trial and commercial formulations of ambrisentan 10 mg tablets for bioequivalence
To compare the clinical and commercial formulations of ambrisentan 10 mg tablets manufactured with drug substance having [redacted] to the commercial formulation of ambrisentan 10 mg tablets manufactured with drug substance having a [redacted] for bioequivalence

Secondary

To examine the safety and tolerability of ambrisentan in healthy adult subjects

Formulations