Table 16 Multiple Dose Pharmacokinetic Metrics - 9.5 days - Protocol GPK:90:02

Parameter	Mean (90% CI)		
	Dose 1	Dose 19	Ratio Dose 19:Dose 1 p-Value
Dose Normalized Cmax (ng/ml)	6.775 (5.4, 8.525)	5.575 (4.45, 7.0)	0.82 (0.69 - 0.98) p = 0.031
Tmax (hours)	1 (0.5, 1.5)	1 (0.75, 2.0)	—
*Dose Normalized AUC** (ng*hr/ml)	17.675 (14.05, 22.25)	14.575 (11.925, 17.825)	0.82 0.70 - 0.97 p = 0.023
Half-Life (hours)	1.42 (1.34, 1.52)	1.48 (1.34, 1.65)	λz Difference Dose 19:Dose 1 p-Value
			-0.02 (-0.08 - 0.05) p = 0.568

* AUC - Dose 1 = AUC_{∞}; Dose 19 = AUC_{12}

Study S3BB1011

Study S3BB1011 was a randomized, open label study that administered alosetron 1mg po bid for 29½ days (57 doses) to 15 healthy male and 15 female volunteers, 18 to 50 years of age.

No difference in plasma alosetron concentrations was observed over this longer course of dosing, indicating no accumulation, auto-inhibition, or auto-induction (See Table 17).

Table 17 Alosetron 1 mg po Multiple Dose Pharmacokinetic Metrics in Males and Females over 29 days - Protocol S3BB1011

Dose	C_{max} (ng/mL)	t_{max} (h)	AUC_{12} (ng•h/mL)	$t_{½}$ (h)
1mg alosetron po BID x 29½ days				
Day 1:	5.03 (4.13-6.13)	1.00	14.66 (11.80-18.23)	1.41 (1.30-1.54)
Day 8:	5.28 (4.43-6.30)	1.00	15.50 (12.83-18.74)	1.48 (1.37-1.61)
Day 15:	5.33 (4.44-6.40)	1.00	16.49 (13.50-20.15)	1.53 (1.40-1.68)
Day 22:	5.64 (4.69-6.78)	1.00	17.08 (14.05-20.77)	1.53 (1.41-1.65)
Day 29:	5.54 (4.76-6.45)	1.00	17.08 (14.45-20.19)	1.51 (1.40-1.62)

All values of C_{max}, AUC_{12}, and $t_{½}$, are shown as geometric mean (95% confidence interval) or median (range) for tmax

Similar results are seen in the males alone, although the concentrations are somewhat lower (See Table 18). (Separate results were not reported for women.)

Table 18 Alosetron 1mg po Multiple Dose Pharmacokinetic Metrics in Males - 29 days

Dose (mg)	Cmax (ng/ml)		Tmax (hours)		AUC_{12} (ng*hr/ml)		Half-Life (hours)	
	Dose 1	Dose 57	Dose 1	Dose 57	Dose 1	Dose 57	Dose 1	Dose 57
1	4.06 ± 2.24	4.25 ± 2.28	1.10 ± 0.43	1.13 ± 0.35	12.17 ± 7.98	13.60 ± 7.8	1.43 ± 0.35	1.51 ± 0.29

All values of C_{max}, AUC_{12}, and $t_{1/2}$, are shown as geometric mean (95% confidence interval) or median (range) for tmax

C. Absorption and Bioavailability

Alosetron has a mean systemic availability after oral administration of approximately 50%, (F_{sys} = 0.5), with both an oral solution and two different tablet formulations. However, the systemic variability varies widely.

Most, if not all, of the drug is absorbed from the gastrointestinal track ($F_{abs} \cong 1.0$) with the lower systemic availability due to first pass elimination. The extent of the first pass effect is consistent with total absorption, alosetron clearance, and hepatic blood flow. The data indicates that there should not be a significant formulation effect with the to-be-marketed formulation.

Two studies were performed with two different doses (2 mg and 4 mg), and two different tablet formulations. The 4 mg dose study used tablet formulation H (4 mg alosetron with a total tablet weight of mg). The 2 mg dose study used formulation D (1 mg alosetron with a total tablet weight of mg). Formulation D differs from the to-be marketed formulation only with respect to the colorant of the film coating. Both formulations had similar absolute bioavailabilities. The bioavailability of 4 mg tablet relative to an oral solution was comparable, with a slightly greater extent of absorption and a slight delay in mean Tmax of 25 minutes compared to the solution. This is probably due to the time required for tablet dissolution.

With a pKa of 6.95 there is a possibility of an effect of drug substance on absorption in different parts of the GI tract. This has been seen with other imidizoles such as cimetidine and ketoconazole. It could be clinically significant in the presence of conditions or drugs that raise gastrointestinal pH. Alternatively, it may not be important due to the rapid release characteristics and low dose of the present formulation. It would become more important should a sustained release formulation be developed.

1. Bioavailability - Drug Substance

The absolute bioavailability of drug substance is 46%, with plasma concentrations peaking 1hour after oral dosing.

Study GHP:89:44

Study GHP:89:44 was a randomized, single dose, crossover, study in 8 healthy male volunteers. It was carried out to determine the absolute bioavailability of an alosetron solution.

The results indicated a mean bioavailability of 46%, with plasma concentrations peaking 1 hour after oral administration (See Table 19).

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Table 19 Absolute Bioavailability of Alosetron Drug Substance - Study GHP:89:44

Route of Admin. Dosage Form	Dose	C_{max} (ng/mL)	t_{max} (h)	AUC∞ (ng•h/mL)	$t_{½}$ (h)	CL_p (mL/min)	V_{ss} (L)	F (%)
PO solution	4mg	19.4 (15.2-24.8)	1.00	52.1 (43.6-62.4)	1.4 (1.3-1.6)	—	—	46 (37-56)
IV solution	4mg over 15 min	104.6 (81.8-133.9)	0.25	113.7 (95.0-136.0)	1.4 (1.3-1.6)	584 (455-757)	64 (49-83)	—

All values of C_{max}, AUC, $t_{½}$, CL_p, V_{ss}, and F are shown as geometric mean (95% confidence interval) or median (range) for t_{max}

The equation listed by the sponsor for Vdss appears to contain a typographical error. A check of the calculations for a single subject using the correct equation resulted in the same value as provided by the sponsor.

Studies GHP:89:38 and GHP:90:21

Other studies that administered alosetron oral solution to young healthy males include studies GHP:89:38 and GHP:90:21.

Study GHP:89:38 was a single rising oral dose study to assess the pharmacokinetic linearity of alosetron solution. Alosetron was administered in grapefruit juice in this study. Whereas as study GHP:90:21 was a mass balance study. The pharmacokinetic metrics seen in study GHP:89:38 are confirmatory for the absolute bioavailability study, GHP:89:44 (See Table 20). The 6% recovery of unchanged alosetron in the urine in the human mass balance study GHP:90:21, with no recovery of unchanged alosetron in feces supports 100% absorption.

Table 20 Alosetron Solution - Additional Oral Pharmacokinetic Metrics

Protocol (Report)	Alosetron Dose	C_{max} (ng/mL)	t_{max} (h)	$AUC_∞$ (ng•h/mL)	$t_{½}$ (h)	A_e (% of dose)
GHP:89:38 (GMH/90/004)	0.5 mg	—	—	—	—	—
	1 mg	4.8 (2.4-5.0)	1.00	15.0	1.7	—
	2 mg	4.8 (2.1-14.8)	1.00 (0.50-3.00)	25.8 (22.6-29.0)	1.4	—
	4 mg	17.6 (11.7-31.0)	2.00 (1.00-3.00)	65.6 (36.5-110.9)	1.7	—
	8 mg	55.7 (27.5-83.0)	1.50 (1.00-2.00)	198.2 (52.7-326.5)	1.6	—
	16 mg	118.0 (50.7-152.0)	0.98 (0.50-1.00)	533.6 (107.2-562.0)	1.7	—
GHP:90:21 (GMH/91/002)	4 mg	19.8	1.13	69	1.6	6

All values of C_{max}, t_{max}, AUC, and $t_{½}$ are shown as median (range)

2. Bioavailability - Drug Product

The data indicates that there should not be a formulation effect with the current rapidly dissolving oral formulations.

Study GHP:90:13

Study GHP:90:13 was a randomized, single dose, crossover study in 18 healthy male volunteers. It was conducted to examine the absolute bioavailability of 4mg doses of alosetron from both an oral solution and an oral tablet.

Initially the protocol was a 3 way cross-over design. After an incidence of arrhythmia with IV administration, the IV arm was stopped and the study continued as a 2 way cross-over bioequivalence trial between the tablet and solution. The arrhythmia was determined to be a pre-existing condition.

Similar absolute bioavailabilities were observed for the solution (51%) and the tablet (57%). Respective peak plasma concentrations were 22.2 and 19.6ng/mL, with respective Tmax's of 0.73 and 1.2 h.

Following intravenous dosing, plasma clearance was 556 mL/min, volume of distribution was 70L, and elimination half-life was 1.42 hours (See Table 21). The less than complete systemic bioavailability (50-60%) is consistent with the clearances reported (after IV dosing) relative to hepatic blood flow. These results suggest that absorption is near complete (almost 100%) and that the less than complete systemic availability is due primarily to first-pass effect. In conclusion, it appears that neither the rate nor extent of absorption should be significantly effected by the to-be-marketed tablet formulation (See Table 22).

Table 21 Absolute Bioavailability of Alosetron Drug Substance and Drug Product - Protocol GHP:90:13

Route of Admin. Dosage Form	Dose	C_{max} (ng/mL)	t_{max} (h)	AUC_∞ (ng•h/mL)	$t_{½}$ (h)	CL_p (mL/min)	V_{ss} (L)	A_e (% of dose)	F (%)
PO solution	4mg	22.2 (19.4-25.7)	0.73 (0.5-1.0)	61.9 (53.6-71.6)	1.50 (1.40-1.55)	—	—	—	51 (30-87)
PO tablet	4mg	19.6 (17.0-22.6)	1.2 (1.0-2.0)	57.3 (49.5-66.3)	1.44 (1.38-1.52)	—	—	—	57 (33-97)
IV solution	4mg	78.2 (52.8-115.8)	0.25 (0.25-0.25)	119.9 (76.0-189.1)	1.42 (1.21-1.72)	556 (398-802)	70 (52-98)	—	—

All values of C_{max}, t_{max}, AUC_∞, $t_{½}$, CL_p, and V_{ss}, are shown as geometric mean (95% confidence interval). F is shown as geometric LS mean (90% confidence interval)

Table 22 Relative Bioavailability of Alosetron Tablets and Solution - Protocol GHP:90:13

	Geometric Mean Ratio (90% CI) [a]		
	Tab / IV	Soln / IV	Tab / Soln
Cmax (ng/ml)	0.29 (0.18, 0.45)	0.27 (0.17, 0.43)	0.88 (0.75, 1.03)
Tmax (hours)	—	—	—
$AUC_{0-\infty}$ (ng*hr/ml)	0.57 (33-97)	0.51 (30-87)	0.92 (0.78, 1.09)

a - period 1 data only n = 6

The 4 mg tablet used (formulation H) was comparable to the proposed commercial 1 mg tablet (formulation E). In addition to the difference in the amount of active ingredient and lactose the composition used had a mg total tablet weight whereas the to be marketed formulation has a mg total tablet weight. The difference is due to the amount of lactose added Since, absorption is nearly 100%, these results should reflect what would be expected with the commercial 1 mg tablet (See Table 2 and Table 3).

In-process quality control samples indicate that the clinical sample analysis was mostly acceptable for this study. However, Cmax's after IV administration (≥52.8) were above the upper limit of the assay (50 ng/ml). There is no indication that these samples were diluted, nor is there any mention of validation of sample dilution for the assay method used for this protocol. The inter-day quality control precision was within 12% and the inter-day quality control accuracy ranged from 95.3% to 105% of nominal.

Study C92-058

Similar absolute bioavailabilities were seen in study C92-058 that compared the pharmacokinetic metrics of alosetron in young and elderly males and females. Elderly females tended to have a higher mean bioavailability (26% higher) and this was consistent with the 34% lower mean clearance in this group (See Table 23).

The tablet formulation used in this study (F) had 2 mg of alosetron and a total tablet weight of mg. The similar results seen in this study support the conclusion that the extent of absorption is not significantly different with the different formulations used in the clinical development program.

Table 23 Absolute Bioavailabilities in Young and Elderly Males and Females with a 2 mg Tablet (Formulation F) - Study C92-058

Dose Route of Admin. Dosage Form	C_{max} (ng/mL)	t_{max} (h)	AUC_∞ (ng•h/mL)	$t_{½}$ (h)	CL_p (mL/min)	V_{ss} (L)	F (%)
2mg alosetron							
Young Males: PO tablet	9.4	1.00	24.8	1.4	—	—	0.50
Young Males: IV solution	40.2	0.25	49.4	1.5	675	82	—
Elderly Males: PO tablet	9.8	0.75	26.5	1.6	—	—	0.51
Elderly Males: IV solution	49.8	0.25	52.2	1.7	639	83	—
Young Females: PO tablet	12.0	1.00	30.5	1.4	—	—	0.49
Young Females: IV solution	44.6	0.25	61.3	1.6	544	65	—
Elderly Females: PO tablet	17.2	0.75	47.1	1.7	—	—	0.63
Elderly Females: IV solution	62.9	0.25	74.1	1.8	450	62	—

All values of C_{max}, AUC, $t_{½}$, CL_p, V_{ss}, and F are shown as geometric LS mean (range) with t_{max} shown as median (range)

3. Food Effect

Administration of alosetron with food slows and decreases the extent of absorption to a moderate extent. The slowed absorption appears to be at least partially due to an increase in lag time. Alosetron was administered with food in the dose ranging study.

In the presence of food mean Cmax decreases by 16 - 25%, and mean AUC decreases by 17 - 20%. If the difference in efficacy between men and women is due to lower plasma concentrations and shorter duration of receptor blockade, the food effect could be clinically significant.

Study S3BB1004

Study S3BB1004 was a well designed trial in 10 male and 10 female subjects administered a single 4 mg tablet of alosetron. The total tablet weight of this formulation was mg and it only differed from the to-be-marketed formulation in the amount of active ingredient.

There was a 25% decrease in peak concentration with a slight delay in Tmax and a 20% decrease in the extent of absorption (See Table 24). This might be clinically significant an will be discussed later.

A lag time was not determined, however in the fasting state only 50% of subjects had detectable concentrations at 0.5 hr, with all subjects having detectable concentrations at 1 hour. In contrast, under fed conditions only 1 of 10 subjects had detectable concentrations at 0.5 hours with 8 of 10 subjects having detectable concentrations at 1 hour.

Table 24 Effect of Food on Alosetron Absorption - Protocol S3BB1004

Metric	Fast	Fed	Fed/Fasted
C_{max} (ng/mL)			
Geometric LS Mean	26.7	19.8	
95% CI	(20.5-34.9)	(15.0-26.3)	
Geometric LS Mean Ratio			0.74
90% CI			0.67, 0.82
p-value			<0.001
Range			
Tmax (hours) mean ± SD	1.05 ± 0.26	1.30 ± 0.51	
Median	1.00[a]	1.00[a]	
Range			
Difference			0.25
90% CI			0.00, 0.50
p-value			0.043
t_{lag} (hours) mean ± SD	0.02 ± 0.07	0.07 ± 0.14	
Median	0.00	0.00	
Range			
Difference	n = 1	n = 4	0.00
90% CI		first time point 20	0.00, 0.17
p-value		minutes	0.067
AUC_∞ (ng•h/mL)			
Geometric LS Mean	75	60	
95% CI	(53-106)	(43-85)	
Geometric LS Mean Ratio			0.8
90% CI			0.75, 0.86
p-value			<0.001
Range			
t½ (hours)			
mean ± SD	1.439 ± 0.248	1.459 ± 0.201	
Mean Ratio			101[b]
90% CI			(95, 107)

a - not transformed
b - log transformed

Study AS-03 (Japan)

Similar results were seen with a Japanese study that used a single 1 mg dose in 8 males (See Table 25). This study used formulation D, which only differs from the to-be-marketed formulation in the colorant of the film coating.

Table 25 Effect of Food on Alosetron Absorption - Protocol AS-03 - Japan

Metric (mean ± SD)	Fast	Fed	p Value	Ratio of Arithmetic Means
Cmax (ng/ml)	3.89 ± 2.33	3.25 ± 1.39	0.199	0.836
Tmax (hours)	1.63 ± 0.37	2.01 ± 0.57	0.003	—
AUC0-∞ (ng*hr/ml)	13.34 ± 8.26	11.04 ± 3.65	0.254	0.826
t½ (hours)	1.32 ± 0.17	1.27 ± 0.14	0.591	—

The mean amount of drug eliminated as N-desmethyl-alosetron doubled in the presence of food in this study from 6.69 ± 4.41% to 9.15 ± 9.07% of the dose, and in one subject it accounted 30.54% of the dose when dosed with food.

D. Bioequivalence - with the To-Be-Marketed Formulation

A number of pharmacokinetic studies were conducted using formulations that differ from the to be marketed formulation only in the colorant of the film coating or in the amount of active and lactose The data generated indicate that all conclusions should be applicable to the to-be-marketed formulation.

E. Drug Metabolism

The metabolite pharmacokinetics of alosetron is poorly presented and poorly analyzed by the sponsor.

According to the sponsor: *'Alosetron is a substrate (in vitro) for a variety of cytochrome P450 (CYP) enzymes: 2C9 (30%), 3A4 (18%), 1A2 (10%), and some non-CYP-mediated Phase I metabolism (11%), and does not undergo glucuronidation or sulfation in vivo. Apart from two primary urinary metabolites each accounting for 15% of the dose, no other urinary metabolite accounts for more than 4% of the dose. This diversity of metabolic pathways renders alosetron less vulnerable to alterations in any one pathway. Inhibition of one pathway would likely be compensated for by other pathways. And induction of one pathway would be diluted by the contributions of other pathways to elimination of alosetron. Therefore, it is unlikely that drug-drug interactions will occur in which another drug could alter the metabolism of alosetron to a clinically meaningful extent.'*

The sponsor has failed to account for secondary metabolism. Consequently, the sponsor's statements implying that no single pathway contributes more than 15% of the clearance is clearly erroneous. Consequently, alterations in single metabolic pathways for alosetron due to drugs, environmental causes, illness, or inter-individual variation with clinically meaningful consequences are possible. Consequently, this issue needs to be examined further. The effect of alosetron on the metabolism of other compounds has not been adequately examined.

This reviewer has tried to interpret the available information on alosetron metabolism. However, due to the conflicting and confusing presentation, this reviewer's analysis of the relative contribution of specific metabolic pathways should be approached cautiously.

1. *In Vitro* Oxidative Metabolism of Alosetron

CYP1A2, CYP2C9 and CYP3A4 are likely to be major contributors to alosetron metabolism. There is also a significant contribution by non-P450 oxidative enzymes. Since alosetron is an indole, with certain

structural similarities to 5-hydroxytryptamine, non-P450 oxidative metabolism might be expected to occur via flavin monooxygenase (FMO) and/or monoamine oxidase (MAO). No *in vitro* studies were performed to evaluate the P450 oxidative metabolism of alosetron metabolites.

Alosetron 5 μM was incubated with specific P450 isozyme inhibitors. Except for incubations with 1-aminobenzotriazole, a suicide substrate inhibitor, all incubations used microsomes prepared from a single donor liver. It's claimed that the metabolite formation profile was followed. However, no metabolite data was presented. Results are presented in Table 26.

Table 26 *In Vitro* P450 Isozyme Metabolism of Alosetron

Isozyme[a]	Inhibitor	Concentration (μM/L)	Mean % Inhibition of Alosetron Metabolism (n=2)
CYP1A2	Furafylline	25	9.7
CYP2C8	Quercetin	10	0.0
CYP2C9	Sulphaphenazole	50	29.5
CYP2C19[b]	Omeprazole	5	1.6
CYP2D6[b]	Quinidine	25	0.0
CYP2E1	diethyldithiocarbamate	100	0.0
CYP3A4	Ketoconazole	1	18.2
Non-P450 Phase I Oxidation	1-Aminobenzotriazole[c]	1000	11.0

a Except where noted microsomes were from a single donor liver (Human 26)
b Polymorphisms
c Suicide Substrate Inhibitor - incubated with pooled hepatic microsomes (n=6)

There are several potential issues with this study.

- Microsomes were prepared from a single donor liver in almost all cases. Since, there is a large amount of inter-individual variability in microsomal expression, the relative contribution in any particular individual *in vivo* could be quite different.

- The concentration of alosetron used, 5 μM, is equal to 1654 ng/ml. This is well in excess of the 5-10 ng/ml peak concentrations expected with a 1 mg dose. Consequently, the relative contribution from various isozymes could be different than observed with clinically achieved concentrations. Especially, if there is autoinhibition at these higher concentrations or if there's inhibition by metabolites.

- Only a single isozyme specific inhibitor was used per isozyme. Since, metabolism by an isozyme could potentially involve multiple sites and/or result in multiple products, use of a single inhibitor does not preclude additional metabolism by that isozyme.

- There was no *in vitro* assessment of P450 metabolism of alosetron metabolites to form secondary metabolites

2. *In Vivo* Metabolism and Mass Balance of Alosetron

The mass balance of ^{14}C labeled alosetron was examined in study GHP:90:21, a single dose study in two healthy male volunteers, mean age 53 yo. Subjects were administered a nominal dose of 4 mg alosetron base using a 1 mg/ml solution. ^{14}C labeled alosetron accounted for 25% of the dose (1 mg).

The mean total recovery in the urine and feces was 97.4% of the dose, with only 1% of the dose recovered in feces as unchanged drug. Thus, absorption is nearly complete (i.e. 100%). The amount of unchanged alosetron recovered in the urine was 6% of the dose.

Approximately 71% of the dose is recovered in the urine by 24 hours, with only an additional 2-3% of the dose recovered in the urine by day 6. The total amount recovered in urine in these two subjects were 71.7% and 75% of the dose, with a mean of 73.4%

Radioactivity in feces was not detected until approximately 55 hours post dose with the majority of radioactivity recovered between 80 - 102 hours post dose. The amount of drug recovered in feces from these two subjects was, 26.1% and 21.9% of the dose, with a mean of 24% (See APPENDIX 2).

There were 13 radioactive polar chromatographic peaks detected in urine in addition to parent drug; indicating the presence of 13 urinary metabolites.

In total there were at least 9 peaks in feces from man, dog, and rat (although this reviewer counted 10 peaks in human feces). Almost all the peaks in human feces have similar retention times to the peaks found in the urine.

The sponsor implies that since no single metabolite contributes more than 15% of the recovery in urine no single elimination pathway comprises more than 15% of total elimination. Since, there is secondary metabolism and fecal elimination the sponsor's statements are clearly erroneous. It should be possible for the sponsor to figure out the relative contribution of various pathways.

Based upon the presented data the relative contribution from various pathways to the systemic clearance of alosetron can be estimated, and an analysis follows below.

None of the urinary metabolites were cleaved by beta-glucuronidases or sulfatases to yield parent drug, indicating that parent drug is neither sulfated nor glucuronidated to a readily cleaved sulfate or glucuronide conjugate to a significant extent.

The metabolic pathways for alosetron are shown in APPENDIX 2.

In the following discussion the following conventions have been used:

UX - Urinary metabolite chromatographic peak number X as labeled by the sponsor
UXY - Urinary metabolite Y resolved from urinary metabolite chromatographic peak X
FX - Feces metabolite chromatographic peak number X as labeled by the sponsor
FUX - Feces metabolite with a similar chromatographic retention time to urinary metabolite UX

Urinary metabolite U3 represented the largest fraction of the dose in urine representing approximately 18% of the dose respectively. The sponsor indicates that a urinary metabolite peak comprising 18% of the dose was composed of 6-OH-alosetron and 7-OH-alosetron, each accounting for 15% and 3 % of the dose respectively.

Metabolite U1 accounted for 14% of the dose. Metabolite U2 was the 4th largest urinary metabolite peak and accounted for slightly less than 4% of the dose. Metabolites U1 and U2 are hydrolyzable by β-glucuronidase to a compound having a similar retention time to metabolite peak U3. It was stated in the pharmacology/toxicology section of the submission that urinary 6-O-alosetron glucuronide represented 14% of the dose and the amount present was 5 times larger than the amount of urinary 7-O-alosetron glucuronide.

There was a major chromatographic peak in feces (F2), representing approximately 7% of the dose. This peak has a similar retention time to urinary metabolite peak U3, the primary aglycone metabolite(s) found in urine. This peak is renamed by this reviewer FU3.

F_{U1} is a chromatographic peak in feces that corresponds to urinary peak U1, and probably represents the glucuronidated metabolite of either 6-O-alosetron glucuronide or 7-O-alosetron glucuronide. F_{U1} represents much less than 1% of the dose. This low amount found in feces may be due to hydrolysis by GI flora and fauna.

Taken together U3 (or F_{U3}) and their daughter metabolites found in urine and feces represent approximately 44% of the dose (See Table 27).

Table 27 Recovery for Metabolite U3 and Daughter Metabolites

Metabolite Peak	Equivalent to	Equivalent to	% of Dose		
			Total 6 & 7-OH alosetron	6-OH	7-OH
U3			18		
U3a	6-OH alosetron (GR96105)			15	
U3b	7-OH alosetron (GR163860)				3
F_{U3}	6&7-OH alosetron	U3	7		
F_{U3a} - estimated	6-OH alosetron (GR96105)			5.5	
F_{U3b} - estimated	7-OH alosetron (GR163860)				1.5
U1	6-O-alosetron glucuronide	U3-gluc-1	14	14	
F_{U1}	U1	U3-gluc-1	<1	<1	
U2	7-OH alosetron	U3-gluc-2	~4		~4
F_{U2}	U2		<<1		<<1
Total	—	—	**44**	**35**	**8.5**

U - Urinary Metabolites
F - Feces Metabolites

Hydroxylation of indole rings is frequently mediated by CYPIA2, which *in vitro* has high activity towards alosetron. Indole compounds typically exhibit 'methylcholanthrene' type effects on P450 CYPIA2. In addition, CYPIA2 classically shows initial inhibition followed by induction with indole compounds. This could contribute to some of the conflicting results and the variability seen with alosetron pharmacokinetics with different doses and in the various multiple dose studies. This is opposite the pattern seen with alosetron and may indicate inhibition due to a cumulative factor. Indoles may also be metabolized by CYPIIC9, consequently 6-OH and/or 7-OH alosetron may be formed by CYPIA2 and/or CYPIIC9.

The amount of bis-oxidized metabolite, the dicarbonyl (GR153732), in urine is reported to account for 9% of the dose in the mass balance study, and 14% of the dose in another study that examined pooled urine (WBP/91/108).

No other urinary metabolite accounted for more than 4% of the dose. The metabolite contributing 4% was identified as the monocarbonyl (GR168355).

A fecal metabolite, F1, has a similar retention time to the monocarbonyl found in urine. This fecal metabolite (F1) accounts for 6% of the dose in a sample from volunteer 1 (See Table 28).

Table 28 Recovery for Metabolite F1/ Monocarbonyl

Metabolite	Equivalent to	% of Dose Recovered
Urinary Monocarbonyl		4
F1	Monocarbonyl	6
Total		10

The monocarbonyl is the parent compound of the dicarbonyl (GR153732). Consequently, at least 19-24% of the dose is eliminated through the monocarbonyl prior to some of it being further metabolized to the dicarbonyl. Since, these carbonyl are formed by metabolism of the imidizole ring their formation may be mediated by CYPIIIA4.

The dicarbonyl is formed via an NIH shift. Consequently, a potentially reactive expoxide intermediate is formed.

The sponsor did not state if recovery of radioactivity from feces was taken into account. Recovery of radioactivity from spiked and feces was >95% for urine, and 64% for feces. Neither did the sponsor state if the total amount recovered in feces was taken into account. (The feces sample assayed only accounted for ~75% of the total radioactivity found in feces, thus the amounts recovered in feces could be 33% higher). Consequently, if these factors were not taken into account by the sponsor, the elimination via these primary pathways could be even higher than calculated.

Other primary metabolites that are formed include hydroxymethyl-alosetron and N-desmethyl-alosetron.

Urinary elimination of Hydroxymethyl-alosetron (GR169307) accounts for 3% of the dose.

N-desmethyl-alosetron (GR87620) was not detected in the mass balance study. However, there were measurable plasma concentrations in all three Japanese studies with mean concentrations in Japanese males after a 1 mg dose in the range of 2 ng/ml. In the Japanese food effect study it accounted for 6.69 ± 4.41% of a 1 mg dose under fasted conditions and 9.15 ± 9.07% of the dose when administered with food. In one subject it accounted for 30.54% of the dose, when alosetron was administered with food.

Indoles may also be metabolized by flavin monooxygenase (FMO), and monoamine oxidase (MAO).

3. Metabolite Kinetics

Plasma total radioactivity peaked 2-fold higher and declined with a half-life 2-fold longer than that of alosetron, indicating the presence of metabolites in plasma (APPENDIX 2). Thus, on average alosetron metabolite kinetics are elimination rate limited.

The sponsor claims that metabolite exposure is 3-4 times alosetron exposure. This appears to be based upon the relative AUC of total plasma radioactivity compared with plasma alosetron AUC determined by a specific radioimmunoassay. However, the sponsor does not appear to have corrected for administration of non-radiolabeled alosetron (i.e. specific activity). Consequently, metabolite exposure is actually 13 fold larger than the central compartment's exposure to alosetron. This higher exposure is partially due to the slower elimination of the metabolites and partially due to a smaller volume of distribution. This reviewer's estimate of the mean volume of distribution of the metabolites is consistent with distribution that is limited primarily to the extracellular fluid.

Pharmacokinetic metrics for alosetron and total radioactivity (^{14}C) are shown in Table 29.

Table 29 Pharmacokinetic metrics for alosetron and total radioactivity

Analyte	Dose (mg)	C_{max} (ng/mL)	t_{max} (h)	AUC_{∞} (ng•h/mL)	$t_{½}$ (h)
Total Alosetron*	4	19.8	1.13	69	1.6
^{14}C-Alosetron†	1	4.95	—	17.25	—
Total ^{14}C Radioactivity	—	44.0	1.5	239	2.9

All values of C_{max}, t_{max}, AUC, $t_{½}$, CL_p, V_{ss}, A_e, and F shown as geometric LS mean and arithmetic mean
* Assayed by
† estimated as 1/4 of the values for alosetron reported by the sponsor

The following shows the correct ratio.

Equation 1

$$\frac{AUCm}{AUCp} = \frac{239 - 17.25}{17.25} = \frac{221.75}{17.25} = 12.85$$

Since

$$\frac{AUCm}{AUCp} = fm * \frac{Clp}{Clm}$$

and since the fraction metabolized (*fm*) is > 0.9, alosetron clearance (*Clp*) must be more than 13 fold greater than the weighted mean metabolite clearance (*Clm*). This is consistent with the longer half-life of total radioactivity and the longer Tmax indicating elimination rate limited kinetics. It should be remembered that the values reported for half-life and Tmax are based on total radioactivity. They are thus composite values based on the half-lives and Tmax's of alosetron and multiple metabolites (See Table 29). Consequently, the half-lives and Tmax's of one or more metabolites must be greater than the Tmax of 1.5 hours and the half-life of 2.9 hours that are reported.

Assuming nonreversible kinetics, the Tmax of any metabolite would have to be less than approximately 4x's the half-life of alosetron added to alosetron's Tmax. Thus, the Tmax of any specific metabolite is likely less than 7.5 hours.

Although, alosetron clearance is 13 fold greater than mean metabolite clearance, the mean metabolite half-life is only twice as long as alosetron's half-life. This is because half-life is directly related to the ratio of volume of distribution to clearance.

Since

$$\frac{t_{1/2}m}{t_{1/2}p} = \frac{kp}{km} = \left(\frac{Clp/Vp}{Clm/Vm} \right) = \left(\frac{Clp}{Clm} * \frac{Vm}{Vp} \right)$$

and since $\frac{t_{1/2}m}{t_{1/2}p}$ is > 2, $\frac{kp}{km}$ must be > 2.

Consequently, since $\left(\frac{Clp}{Clm} \right)$ is approximately 13, $\frac{Vm}{Vp}$ must be < 1/6)

Since Vp is approximately 0.9 - 1.4 liter/kg, alosetron likely distributes into slightly more than total body water. There are no estimates of Vm, but, since Vm must be much less than Vp, most of the metabolites likely don't distribute into tissues very well. A mean metabolite volume of 1/6 Vp, where Vp is 1.4 L/kg, is approximately 0.23 L/kg. This is approximately the volume of the extracellular fluid. Since we don't know the amount of metabolite in the body, the relative exposure to various metabolites cannot be calculated. However, it appears that most tissue exposure is to parent drug and not to metabolites (based on the above calculations). The volume of distribution of parent drug is in a range where changes in body composition might effect volume. With respect to metabolites, the volume of distribution is in the range where hydration status would be expected to effect the volume of distribution.

In conclusion, the higher total metabolite concentrations in plasma are likely due to smaller volumes of distribution. As a corollary, the exposure of tissues to metabolites is likely much less than tissue exposure to parent drug. Half-lives of most metabolites are thus unlikely to be influenced by tissue sequestration to any great extent.

F. Renal Elimination

The renal clearance of alosetron can be determined from data in 3 male subjects in study C92-087. The sponsor estimates renal clearance to be 83 ml/min by combining data from all 3 subjects. When the renal clearance of each individual is calculated, mean renal clearance is 93.6 ± 41.5 ml/min (mean ± SD). Since, demographic data is not provided it's not possible to tell if there is net reabsorption or not. The mean percent of the dose recovered in urine over 24 hours was 7.42% ± 1.03%. Indicating that this is a minor pathway. This value for alosetron recovery in urine is consistent with data reported for the mass balance study (6%).

G. Protein Binding

Alosetron is moderately protein bound to human plasma proteins and should not cause any protein binding interactions with other drugs. Binding to human plasma proteins was determined by equilibrium dialysis of ^{14}C-alosetron utilizing The percent bound was independent of concentration (mean ± SD) 81.7% ± 0.9% with a range of 80.5% - 82.7% over the concentration range of alosetron base/ml.

H. RBC Partitioning (WBP/91/047)

The RBC concentration to plasma concentration ratio in humans (Cb/Cp) is 0.94.

I. Alosetron Pharmacokinetics - Pharmacodynamics

1. Pharmacokinetic - Pharmacodynamic Correlations

a) 5-HT$_3$ Receptor Blockade

(1) Flare Response - IV Alosetron (Study GHP:90:16)

Study GHP:90:16 was a randomized, double-blind, placebo controlled, four period cross over study in 12 healthy male volunteers. Each subject received three dose levels of alosetron (0.1 mg, 1 mg, 4 mg) and placebo infused intravenously over 10 minutes. Venous blood was sampled at 10 and 35 minutes post infusion for determination of alosetron concentrations. Twenty minutes following treatment, subjects received intradermal injections in duplicate of 6 dose levels of 5-HT into 12 sites on the back using an