RESULTS:

Study Population and Demographics:

Twenty-five healthy volunteers were recruited to the study. Subject 8 withdrew from the study at an early stage and was replaced by Subject 8A who began the study 2 days later than the rest of the volunteers. The results were determined from the 24 subjects who completed the protocol.

Demographic data can be found in Table 9. All subjects were white males ranging in age from 19-46 years. Many of the subjects had a history of smoking and almost all consumed alcohol.

Table 9. Summary of Demographic Data

Variable	Parameter	Group A (N=12)	Group B (N=12)
Age (years)	Mean (SD) Range	31 (9.4) 19-46	29 (5) 22-37
Height (cm)	Mean (SD) Range	173 (6.4) 158-183	178 (8.3) 164-189
Weight (kg)	Mean (SD) Range	71.8 (8.13) 55-82	76.5 (11.1) 52.2-88.2
Smoking Habit (cigarettes/day)	None (non-smoker) 2 6 8 10	8 0 1 2 1	3 1 1 1 6
Alcohol consumption (units/day)	Mean (SD) Range	0.9 (0.74) 0-2.9	1.1 (1.05) 0-2.9
Coffee consumption (cups/day)	None 1 2 3 4	6 1 3 0 2	4 0 5 2 1

Adverse Events:

There were no serious adverse events over the duration of this study. There was a statistically significant difference in the number of events in Group B, with more noted after BSZ than after MSZ (see Table 10). This trend was not observed in Group A.

Table 10.
Incidence of Adverse Events for each Sequence in each group

Group	Sequence	Event experienced?	BSZ	SSZ or MSZ	p value
A	BSZ-SSZ	No Yes	4 2	4 2	p>0.2*
	SSZ-BSZ	No Yes	6 0	2 4	p=0.061
	Both	No Yes	10 2	6 6	p=0.19
B	BSZ-MSZ	No Yes	2 4	3 3	p>0.2
	MSZ-BSZ	No Yes	1 5	6 0	**p=0.015**
	Both	No Yes	3 9	9 3	**p=0.039**

*as determined by Fishers exact test

Note: BSZ-SSZ = BSZ in Period 1 and SSZ in Period 2
SSZ-BSZ = SSZ in Period 1 and BSZ in Period 2
BSZ-MSZ = BSZ in Period 1 and MSZ in Period 2
MSZ-BSZ = MSZ in Period 1 and BSZ in Period 2

Analytical Methods:

Overall, the assays used for determination of BSZ and its metabolites in plasma, urine, and feces had some deficiencies. For example, there was often inadequate validation at assay LOQs. This is especially relevant for analytes for which the majority of the samples had concentrations near the LOQ, such as, BSZ and ABA. The majority of the fecal samples required dilution to bring them within range of the calibration curve for the compound of interest, however, the parallelism studies which were performed to ensure that these dilutions were accurate and precise were inadequate. (Parallelism studies include serial dilutions of QC samples that correspond to the dilutions needed for the study samples).

Some of the chromatograms were difficult to interpret due to interfering peaks, unstable baselines, or shifting retention times for the analytes. In addition, only a limited number of chromatograms from actual study samples were provided.

Many of the fecal samples collected during this study could not be identified due to "smudged labels" and separation of the labels from the sample bags. Those samples were discarded and a decision was made to analyze only those samples that could be fully identified and only those collected from BSZ-dosed individuals. Only ten of the 24 subjects in this study were identified as having complete sets of fecal samples.

Finally, there were no intraday precision or accuracy data reported for any of the compounds in the three biological matrices (except for the ABA/NABA fecal assay which was run as one batch only). Results of recovery determinations and stability testing, if undertaken, were also not reported.

Pharmacokinetic Results:

Mean plasma concentration vs time profiles for BSZ and each metabolite are located in the Appendix. The following tables display the results of the PK analyses for the study drugs and their metabolites. In all cases, values are reported as means±SD. Ranges are included in parentheses and are provided because of the high variability in the data. All concentrations that were below the LOQ of the relevant assay were reported as zero and included in calculations. Values for BSZ are now reported as the free acid (given as the disodium dihydrate for assay validation studies).

Table 11. Plasma PK Parameters* determined after a single 2.25 gm dose of BSZ.

	C_{max} (ng/ml)	T_{max} (hr)	AUC_{last} (ng*hr/ml)
BSZ	18.9±8.9 (0-39.4)	1.6±1.1 (0-6)	37.7±43.2 (0-166)
ASA	460±282 (98.7-1064)	9.3±1.8 (6-14)	3127±2150 (407-6709)
NASA	1148±401 (518-1843)	10.1±1.7 (8-14)	19613±5578 (10337-31962)
ABA	13.7±16.8 (0-50)	4.7±5.3 (0-12)	81±154 (0-584)
NABA	59.2±21.7 (21.9-114)	15.6±10.3 (8-48)	1422±742 (209-3208)

*N=24 in all cases.

BSZ appeared in the plasma of almost all of the subjects by 1 hour after dosing, and was no longer measurable after 4 hours. The majority of the plasma concentrations were near the assay LOQ. Furthermore, 18/24 subjects had only one or two plasma samples with detectable levels of BSZ or concentrations which were below the assay LOQ. In the subjects who had three or more plasma samples with measurable quantities of BSZ (6/24), the plasma concentration vs time profiles tended to be erratic. For this reason, the majority of the AUCs were inadequately characterized and determinations of half-lives could not be made with any degree of confidence.

All subjects had detectable quantities of ASA, which were not observed until 4 hours after a dose of BSZ. The majority of subjects had no quantifiable concentrations after 14 hours. As the majority of the plasma concentration vs time profiles for ASA were erratic, half-lives were not calculated. However, literature estimates of ASA half-life are 0.6 to 1.4 hours after treatment with oral ASA.[1] Conversely, 13/24 subjects had ABA concentrations which were below the assay LOQ for all samples obtained. Overall, there were only 31/336 samples which had detectable quantities of this metabolite and most of them were near the assay LOQ. As for BSZ, AUCs were difficult to characterize and half-life determinations could not be made. ABA did not appear in the blood until 6 hours after a BSZ dose and was not observed after 14 hours in the majority of subjects.

All subjects had readily quantifiable levels of NASA and NABA. Neither was detected until 4 hours after a BSZ dose. NASA was observed over 96 hours, while there were no measurable concentrations of NABA after 48 hours. Again, plasma concentration vs time profiles were too erratic too allow any reliable determinations of half-lives. Literature estimates of NASA half-life are from 6-9 hours after oral SSZ administration.[2]

Of interest is the appearance in plasma of the N-acetylated metabolites (NASA or NABA) prior to that of the "parent" metabolite (ASA and ABA, respectively) in some of the subjects. One possible explanation for this phenomenon is that ASA and ABA are either absorbed in quantities too low to be detected or are absorbed more slowly than their metabolites, NASA and NABA.

Tables 12 and 13 compare the PK parameters for the therapeutically active moiety, ASA, and for NASA following the administration of BSZ, SSZ, and MSZ in Groups A and B.

Table 12. Plasma ASA PK Parameters*

Drug received	C_{max} (ng/ml)	p value	T_{max} (hr)	p value	AUC_{last} (ng*hr/ml)	p value
Group A: BSZ	392±282 (98.7-959)		9.2±2.2 (6-14)		2593±2278 (407-6607)	
Group A: SSZ	286±221 (0-819)	0.118[a]	10.7±3.9 (0-14)	0.243	1175±1110 (0-4077)	0.040
Group B: BSZ	528±276 (137-1064)		9.3±1.3 (8-12)		3661±1962 (676-6709)	
Group B: MSZ	981±930 (93.9-2865)	0.118[b]	7.2±4.4 (4-14)	0.039	4797±3032 (705-10126)	0.358

*N=12 for each group.
[a]Results of ANOVA for BSZ vs SSZ in Group A.
[b]Results of ANOVA for BSZ vs MSZ in Group B.

1. Klotz, U, et al. Arzneimittel-Forschung 1985;35:636.
2. Meese, CO, et al. Brit J Clin Pharmac 1984;18:612.

All subjects had quantifiable concentrations of ASA in plasma with the exception of one subject treated with SSZ in Group A (below assay LOQ). There were differences between the AUC_{last} for Group A with the BSZ treatment having a significantly higher value than that observed for the SSZ treatment. There were no differences noted in this parameter in Group B (BSZ vs MSZ) nor in Cmax values for either group. The MSZ treatment resulted in a lower tmax for ASA as compared to the BSZ treatment in Group B. There were also some period and sequence effects noted in both Groups A and B; these are displayed in Table 14.

Table 13. Plasma NASA PK Parameters*

Drug received	C_{max} (ng/ml)	p value	T_{max} (hr)	p value	AUC_{last} (ng*hr/ml)	p value
Group A: BSZ	1116±419 (518-1790)		10±1.7 (8-14)		19786±6566 (11921-31962)	
Group A: SSZ	811±289 (293-1368)	0.018[a]	13±4.2 (8-24)	0.026	13946±3837 (8612-20385)	0.004
Group B: BSZ	1179±397 (611-1843)		10.2±1.8 (8-14)		19440±4679 (10337-24399)	
Group B: MSZ	1388±1085 (420-4194)	0.570[b]	16±18.4 (6-72)	0.252	20901±8783 (6508-37672)	0.565

*N=12 for each group.
[a]Results of ANOVA for BSZ vs SSZ in Group A.
[b]Results of ANOVA for BSZ vs MSZ in Group B.

All subjects in both groups had readily detectable levels of NASA in plasma. There were significantly greater Cmax and AUC_{last} values observed for the BSZ-treated individuals in Group A when compared to SSZ-treated subjects. In addition, Tmax for BSZ was shorter. There were no statistically significant differences noted for any of the parameters in Group B.

Table 14. p values for Dose, Period and Sequence Effects

	Dose			Period			Sequence		
	AUC_{last}	Cmax	tmax	AUC_{last}	Cmax	tmax	AUC_{last}	Cmax	tmax
ASA - Group A[a]	0.040	0.118	0.243	0.918	0.836	0.089	0.302	0.215	0.011
ASA - Group B[b]	0.358	0.118	0.039	0.083	0.035	0.006	0.397	0.003	0.010
NASA - Group A	0.004	0.018	0.026	0.721	0.486	0.702	0.856	0.831	0.834
NASA - Group B	0.565	0.570	0.252	0.012	0.067	0.076	0.853	0.012	0.143

*N=12 for each group
[a]BSZ vs SSZ
[b]BSZ vs MSZ

The effect of the treatment alone is difficult to interpret in some cases due to the significant effect of period and sequence. However, the significant differences observed in AUC_{last} for ASA and NASA and Cmax and tmax values for NASA in Group A, appear to be related to the treatment only (p values for sequence and period effects were well >0.05). Conversely, although there appear to be only minor differences in the results for the PK parameters from Group B (no treatment effects), there are period and sequence effects which could confound interpretation of the data.

Table 15 provides urinary and fecal excretion parameters. Specifically, the values represent the percent of a single dose of BSZ, SSZ, or MSZ recovered as the parent compound or as metabolite. In addition, Cl_R parameters were calculated for each of the compounds in the study, however, values were determined for individual urine collection intervals only, and total Cl_R data was not provided. Rough estimates of total Cl_R have been calculated by this reviewer according to the following: Au_{96hr}/AUC_{96hr} where Au_{96hr} is the mean value for amount of compound collected in the urine over 96 hours and AUC_{96hr} is the mean area under the plasma concentration vs time curve as determined over 96 hours. The values were calculated using mean data only; i.e., Cl_R parameters were not determined for each individual and then averaged. These data are included in Table 15 as well.

Table 15. Excretion data for BSZ, SSZ, MSZ and their metabolites.				
	% recovered in urine[a]	p value	% recovered in feces[b]	Cl_R (L/hr)
BSZ	0.13±0.08 (0-0.28)	-	0.04±0.14 (0-0.44)	63.7
ABA	0 -		36.55±26.63 (4.44-88.87)	0
NABA	2.97±1.74 (0-6.67)		2.62±1.6 (0.44-5.33)	26.9
Group A:				
ASA from BSZ	0 -	-	7.05±4.11[c] (1.35-13.81)	0[c]
ASA from SSZ	0 -	-		0
NASA from BSZ	20.5±8.19 (9-38)	-	17.24±11.13[c] (2.61-35.84)	11.2[c]
NASA from SSZ	14.1±8.05 (4.33-29)	0.016[d]		10.0
Group B:				
ASA from BSZ	0 -	-		-
ASA from MSZ	0.19±0.52 (0-1.76)	0.217		0.3
NASA from BSZ	29.2±7.32 (19.6-48.1)	-		-
NASA from MSZ	31.8±15.9 (12.7-58.4)	0.485		12.2
SSZ	4.88±3.31 (1.62-11.8)			0.7
SP	8.94±3.26 (4-14.39)			0.6
NASP	8.92±2.26 (5.81-14)			1.5

[a]N=24 for BSZ, ABA, and NABA, N=12 for ASA and NASA in both Groups A and B, and N=12 for SSZ, SP, and NASP.
[b]N=10 for all compounds
[c]Pooled amounts from both Groups A and B.
[d]Results from ANOVA for BSZ vs SSZ or MSZ.

BSZ, NASA, and NABA were detected in the urine of almost every subject. Conversely, concentrations for ABA and ASA were all below the assay LOQ, with the exception of 2 subjects that had ASA detected after MSZ treatment. Only one subject had any detectable BSZ in feces, while all 10 subjects, from which fecal samples were available, had quantifiable levels of ASA, ABA, NASA, and NABA.

Approximately 88% of a 2.25 gm dose of BSZ was recovered in the urine and feces. Most of the dose was eliminated as NASA in the urine (21-29%) and ABA and NASA in the feces (37% and 17%, respectively). Very little unchanged BSZ was quantitated in either matrix.

Approximately 37% of a dose of SSZ was recovered in the urine as parent compound and metabolites. Of note is that approximately 9% of a SSZ dose appeared as the toxic "carrier" portion, SP, as compared to no quantifiable amounts for ABA from BSZ treatment. MSZ was excreted as ASA and NASA; about 32% of a single dose was present in the urine. No fecal analyses of either SSZ or MSZ were performed.

There was a statistically significant difference in the amount of NASA recovered in the urine in Group A, with BSZ-dosed individuals exhibiting greater quantities. There were no significant differences in urinary recovery of NASA in Group B.

Cl_R values for BSZ and NABA were high. This was probably due to incomplete characterization of the plasma profiles because of concentrations near or below the assay LOQs, coupled with borderline assay accuracy and precision. The renal clearance of NASA was similar after dosing with all three parent compounds (BSZ, SSZ, and MSZ) and exceeded normal creatinine clearance values (80-110 ml/min). There was virtually no ABA or ASA detected in urine.

CONCLUSIONS:

In general, the absorption and systemic exposure to BSZ in the individuals studied was very limited. Less than 1% of a single 2.25 gm dose was recovered in the urine or feces as intact drug, indicating reduction (presumably by colonic bacterial azoreductases) of this compound into its primary metabolites, ASA and ABA. ASA is considered to be the "active" therapeutic moiety, while ABA serves as a "carrier" which is intended to deliver ASA to its site of action in the colon, thus, minimizing systemic exposure to either compound. Although both of these compounds were detected in plasma, they appeared to be excreted only as their N-acetylated metabolites in urine in this study of normal, healthy, male volunteers. Furthermore, plasma concentration vs time profiles for BSZ, ASA, and ABA indicated erratic absorption of these three moieties. The majority of a dose of BSZ was detected in feces as both ABA and ASA and their N-acetylated metabolites (approximately 65%), thus, confirming that the active moiety is reaching the intended site of action.

The disposition of BSZ was also compared to established treatments for ulcerative colitis. SSZ is a compound in which ASA and SP are azo-bonded. This prodrug is cleaved in the colon by bacterial reductases, thus, releasing ASA at the active disease site. However, it is thought that

absorption of SP is responsible for many of the adverse side effects observed with administration of this drug. Therefore, it was postulated that BSZ would provide a safety advantage over SSZ, as its carrier (ABA) is nontoxic and poorly absorbed. MSZ is an enteric-coated preparation of ASA. Its main drawback is its erratic absorption profile resulting in variable exposure of the diseased colon to ASA.

Unfortunately, no fecal recoveries of either SSZ or MSZ were undertaken, therefore, comparisons with BSZ dispostion were limited to urinary recovery data. While the "carrier" portion of BSZ (ABA) did not appear in the urine, about 9% of a dose of SSZ was recovered as the toxic SP. Therefore, it would appear that BSZ offers a safety advantage over SSZ in that the body is not exposed to a toxic carrier compound. However, no clearcut PK advantages can be determined from the data provided as it appears that more ASA is absorbed into the systemic circulation in BSZ-treated subjects than in SSZ-treated individuals. This may imply that less ASA is available at the therapeutic site of action in BSZ-treated individuals, reducing the efficacy of this compound as compared to SSZ. On the other hand, the possibility that ASA has some efficacy in UC patients as a result of systemic exposure has not been completely ruled out. Firm conclusions are difficult to draw as relative fecal recoveries were not determined in subjects who received SSZ. Likewise, comparisons between BSZ and MSZ are not easy to make.

Overall, there were no serious adverse events associated with the administration of BSZ and it appeared to be generally well-tolerated. There was, however, a significantly greater incidence of events after treatment with BSZ as compared to treatment with MSZ.

Finally, the bulk of the PK data provided should be interpreted with caution in light of the high variability and the deficiencies noted in the assay validation. In addition, the plasma concentration vs time profiles for the vast majority of individuals were erratic for all of the analytes, therefore, half-lives could not be determined with any degree of certainty.

APPENDIX

APPEARS THIS WAY
ON ORIGINAL

Study Plan

	Screening	Pre-dose	Study Drug Administration & Test Period															Post-dose/ Post-study
		Day -1	Day 1										Day 2			Day 3	Day 4	
Hours		-12	0	1	2	4	6	8	10	12	14	16	24	30	36	48	72	96	
Demography & medical history	✓																		
Physical examination	✓	✓																	✓
Biochemistry, haematology & urinalysis	✓	✓																	✓
Virology	✓																		
Drug screen	✓	✓																	
Faecal occult blood	✓																		✓
Dosing			✓																
Pharmacokinetic blood samples			✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	
Urine Collection		•—	—•	——	——	—•	——	—•	——	—•	——	—•	—•	——	—•	—•	—•	—•	
Recent/concomitant medication	✓																		✓
Adverse event monitoring			✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	

Figure 1A Mean Plasma Concentrations of Balsalazide versus Time Groups A and B

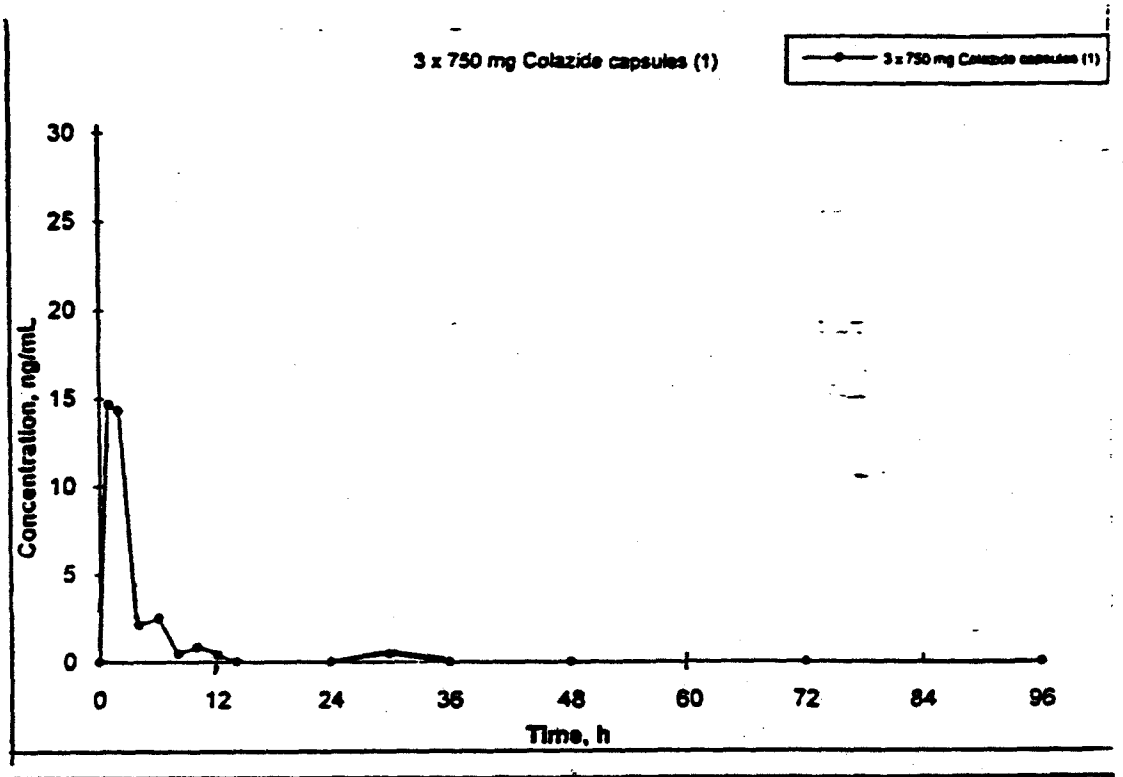

Figure 1B ln(Mean Plasma Concentrations) of Balsalazide versus Time Groups A and B

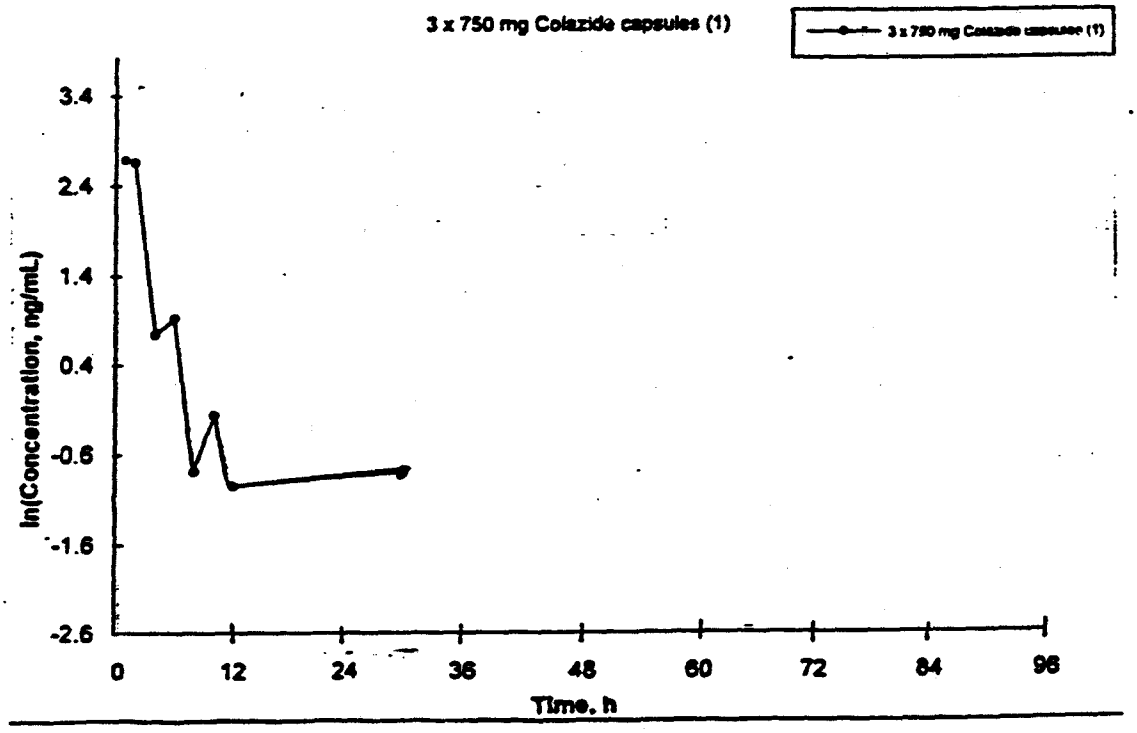

Figure 11A Mean Plasma Concentrations of 5-ASA versus Time Groups A and B

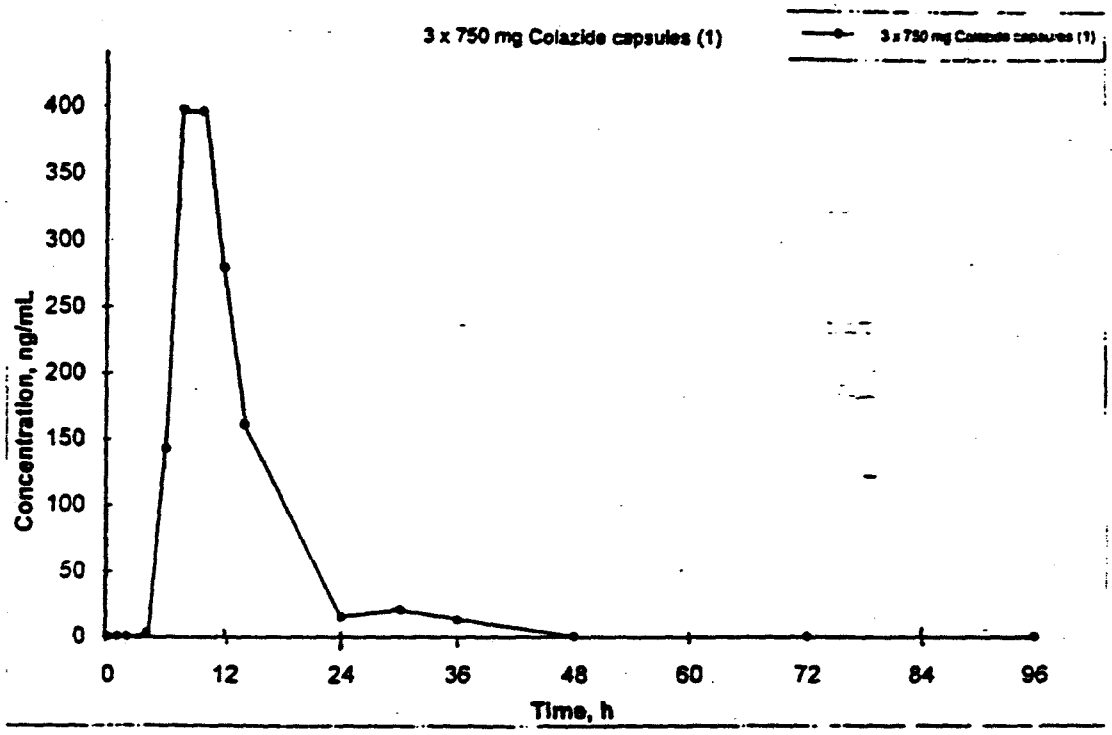

Figure 11B ln(Mean Plasma Concentrations) of 5-ASA versus Time Groups A and B

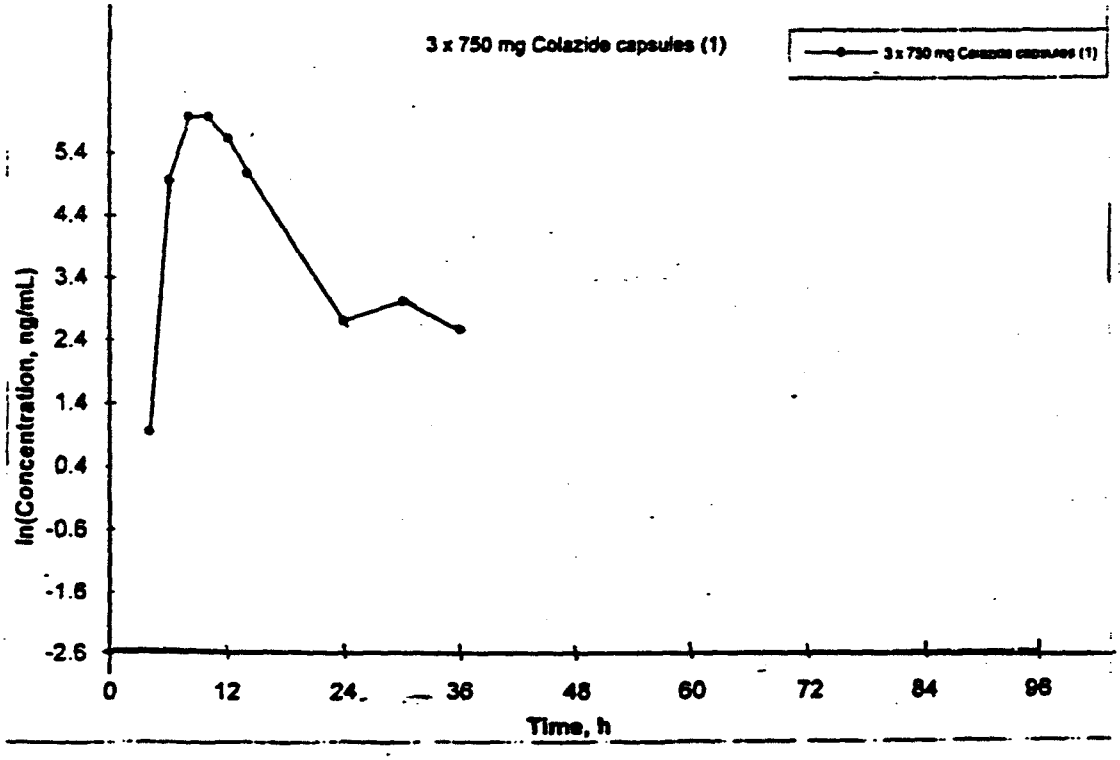

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Figure 12A Mean Plasma Concentrations of N-Ac-5-ASA versus Time Groups A and B

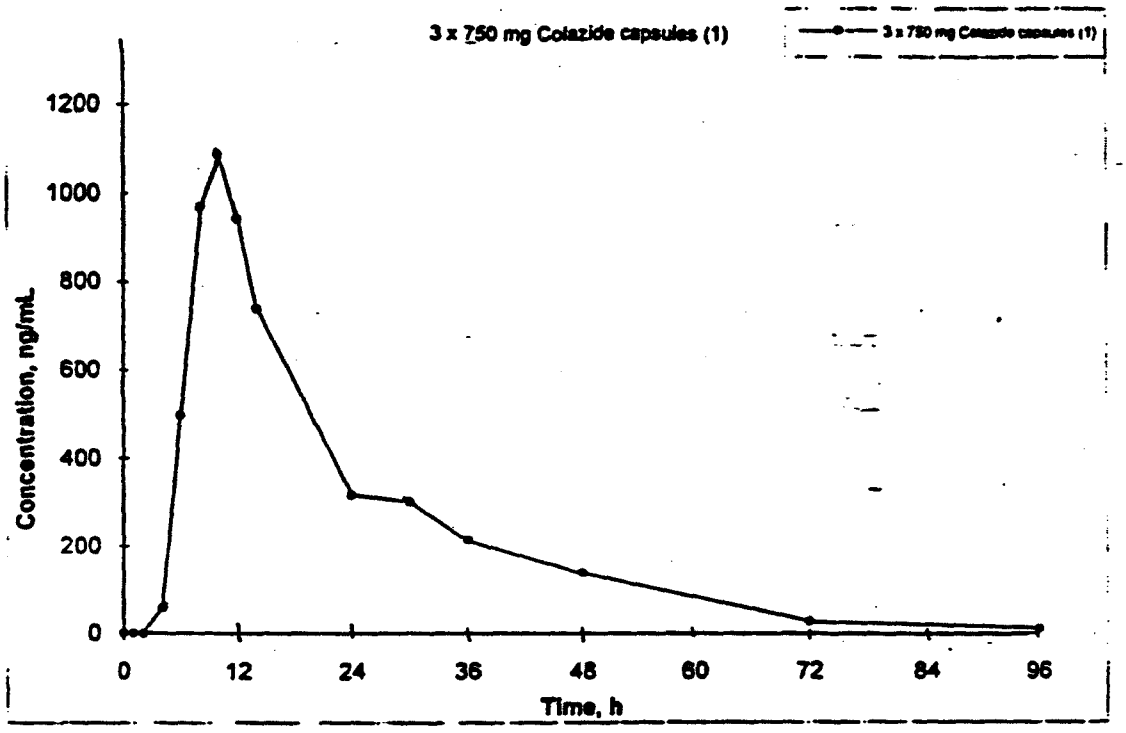

Figure 12B ln(Mean Plasma Concentrations) of N-Ac-5-ASA versus Time Groups A and B

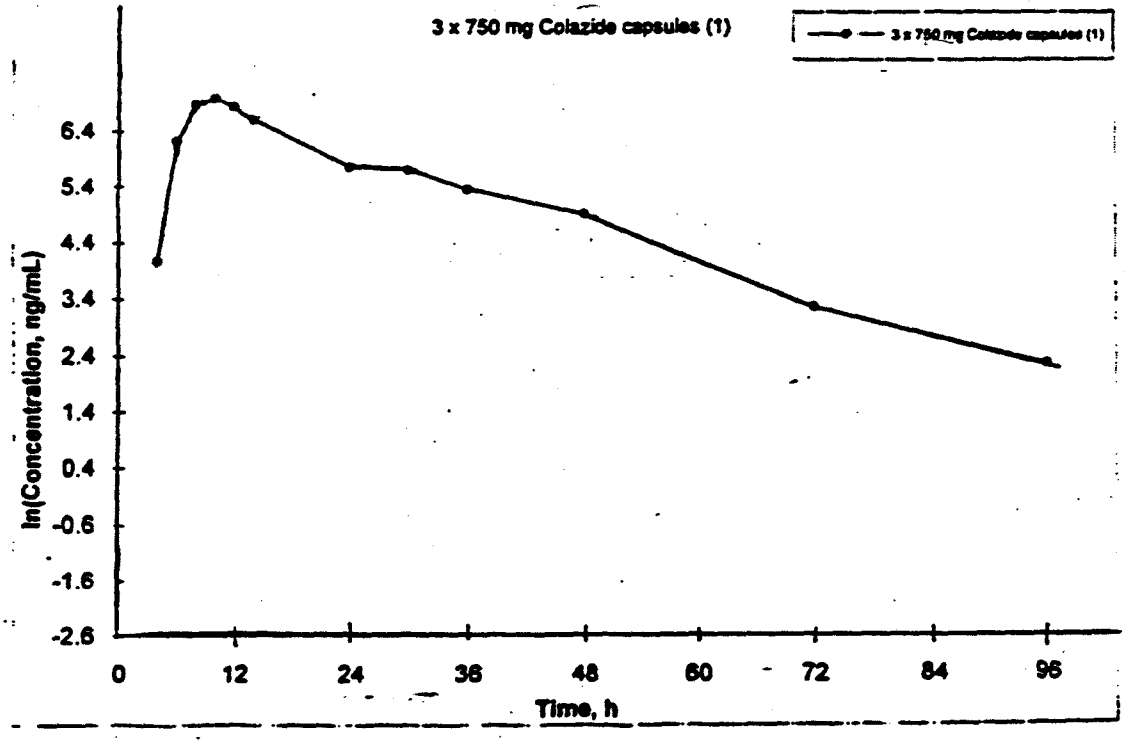

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Figure 2A Mean Plasma Concentrations of 4-ABA versus Time Groups A and B

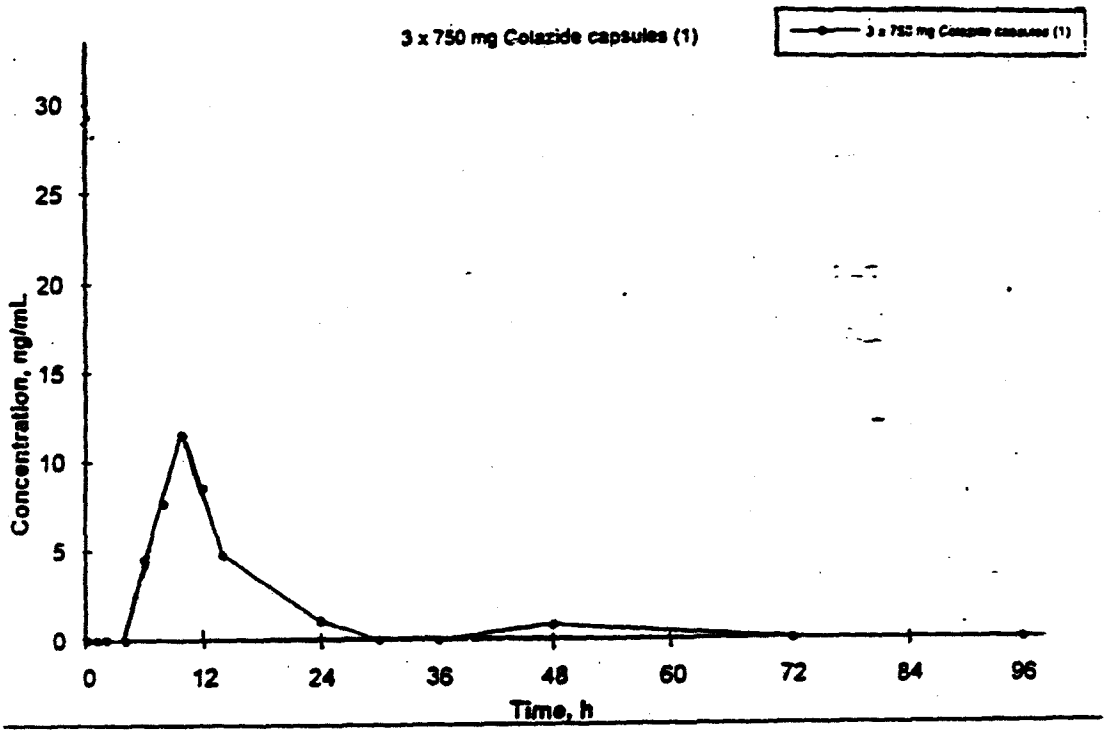


Figure 2B ln(Mean Plasma Concentrations) of 4-ABA versus Time Groups A and B

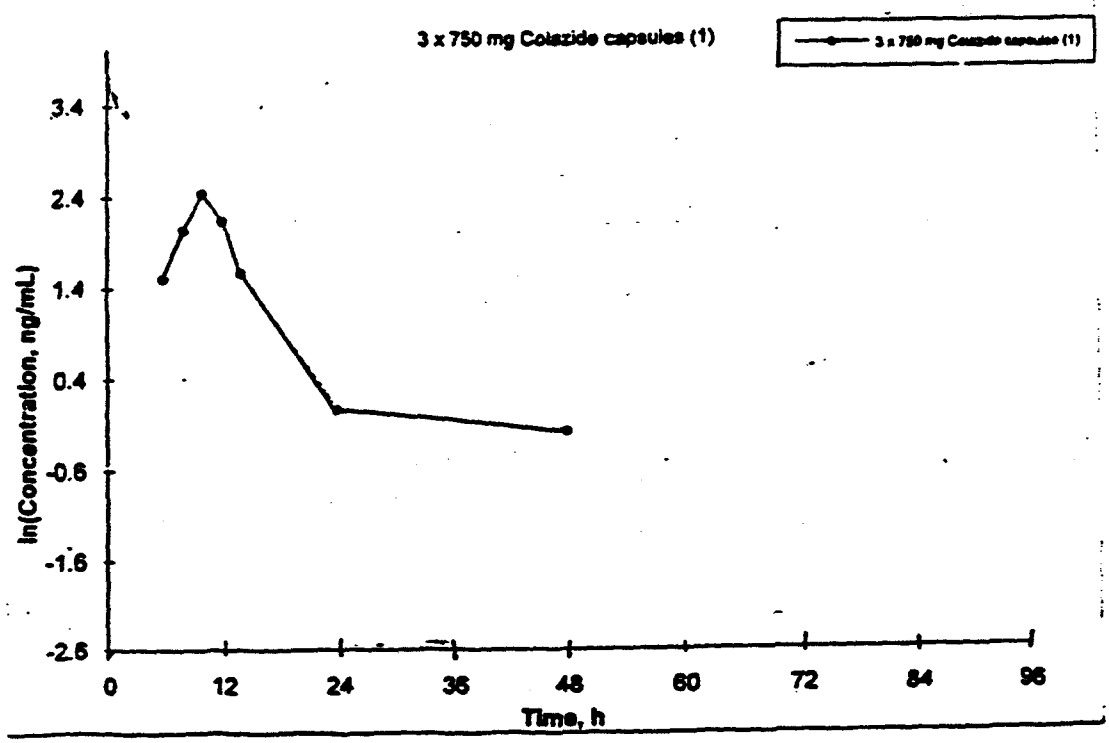


Figure 3A Mean Plasma Concentrations of N-Ac-4-ABA versus Time Groups A and B-

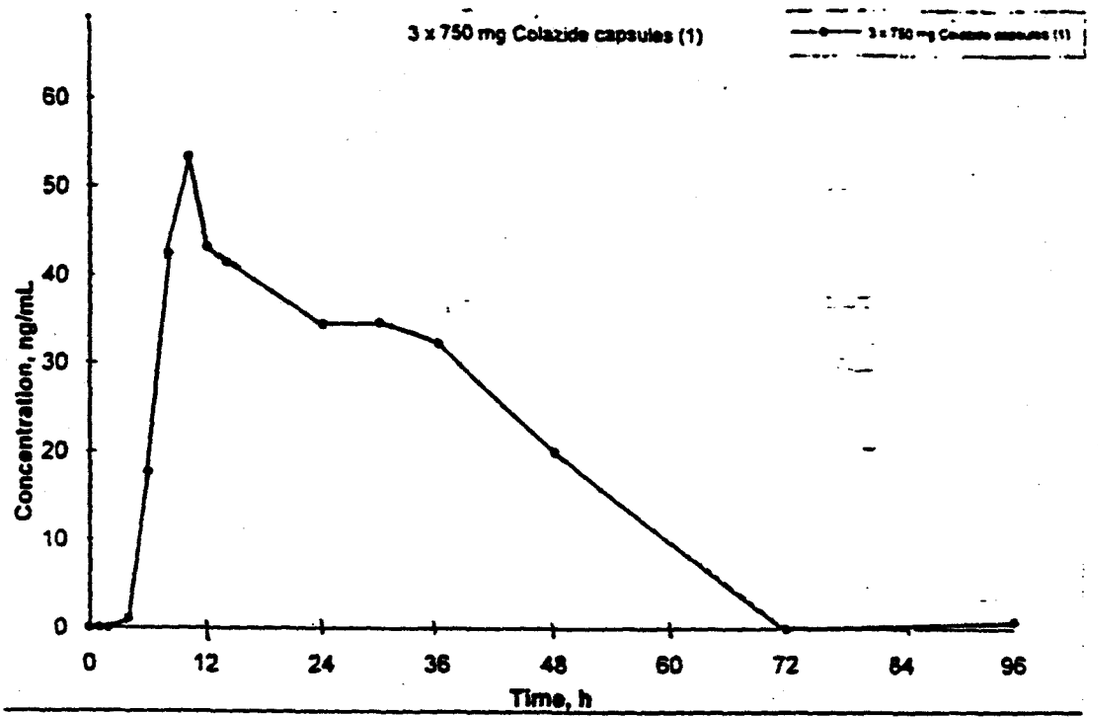
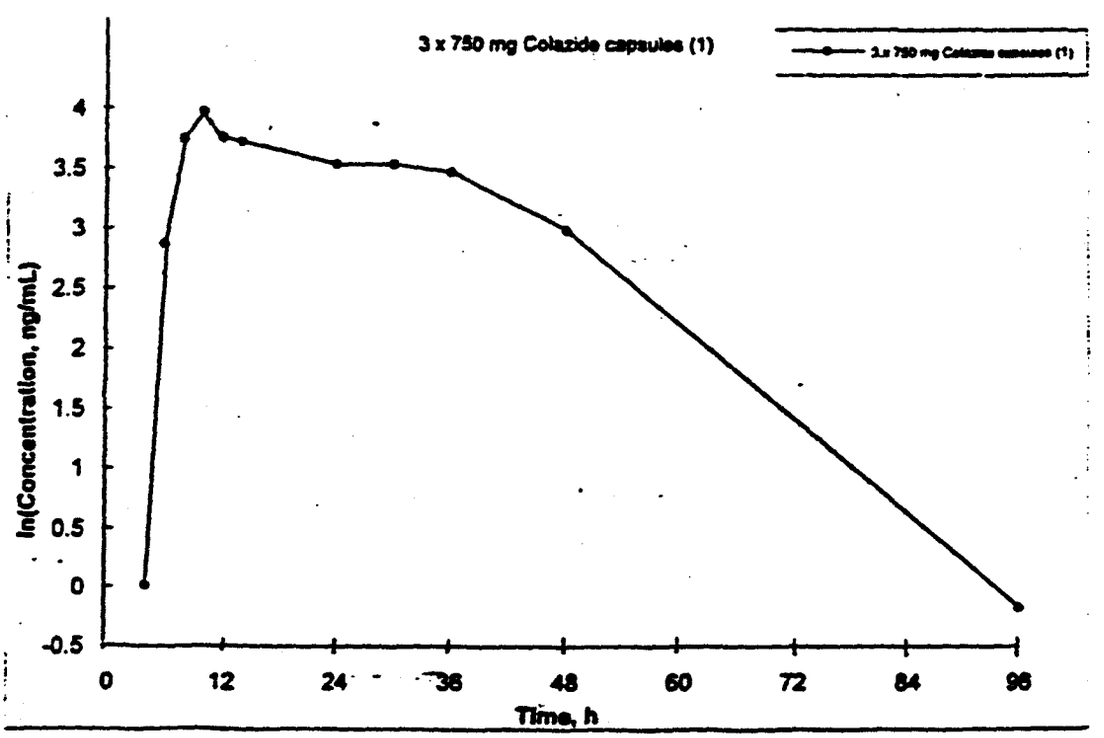


Figure 3B ln(Mean Plasma Concentrations) of N-Ac-4-ABA versus Time Groups A and B



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TITLE: The Tolerability and Pharmacokinetics of Single and Repeated Oral Doses of Balsalazide Disodium (Colazide®)

Study Number: 20061

Investigator and Site: ———

Study Date: August 27 - September 21, 1992.

Abbreviations used: BSZ = Balsalazide
ASA = 5-aminosalicylic acid
NASA = N-acetyl-5-aminosalicylic acid
ABA = 4-aminobenzoyl-β-alanine
NABA = N-acetyl-4-aminobenzoyl-β-alanine
AUC = area under the plasma concentration vs time curve
Cl_R = renal clearance
LOQ = limit of quantitation
PK = pharmacokinetic

OBJECTIVES:
1. To evaluate the absorption, disposition, and elimination of single and multiple doses of BSZ.
2. To evaluate the safety and tolerability of BSZ after repeated oral dosing.

METHODS:
Study Design:
This study was an open-label, noncomparative, single and multiple dose study.

Study Population:
The subjects were twelve healthy, adult, male volunteers.

Treatment and Administration:
Drug administration was comprised of three phases: a single dose of BSZ on Day 1, two daily doses on Days 4-13, and three daily doses on Days 18-19 as outlined in Table 1.

Table 1. BSZ administration.

Day	Time (hr)	Dose (gm)	Total Daily Dose BSZ (gm)
1	08:00	1.5	1.5
4-12	08:00 20:00	1.5 1.5	3.0
13	08:00	1.5	1.5
18	08:00 16:00 24:00	2.25 2.25 2.25	6.75
19	08:00	2.25	2.25

Each dose of study drug was taken with 250 ml of water. Subjects were confined to the clinic for 23 days. Standard drinks and meals were given from the eve of Day 1 until the morning of Day 23 (menus provided in NDA submission). Subjects were not permitted to consume any alcohol or caffeine-containing beverages in the 24 hours prior to the first dose and for the duration of their stay in the clinic. Meals and snacks were provided at the following times:

Breakfast - 07:30 hours
Lunch - 13:00 hours
Dinner - 18:00 hours
Snack - 22:00 hours

Blood, urine, and fecal samples were collected at predetermined times over the study period for PK analyses. A detailed Study Plan can be found in the Appendix.

Study Drug Supplies:

Generic name	Balsalazide disodium (BSZ)
Trade name	Colazide®
Dosage form	750 mg capsule
Lot number	2814
Expiry date	August 1995
Manufacturer	Biorex Lab., Limited

The BSZ used in this study was not used in the pivotal clinical trials nor is it the to-be-marketed formulation.

Adverse Events:
Adverse events were recorded with date and time of onset and cessation, severity, study drug relationship, and any action taken.

Biological Sampling:
1. Blood - samples were collected immediately before and at 1, 2, 4, 6, 8, 10, 12, 14, 24, 30, 36, 48, 72 after the first administration (Days 1-3). This served as a single-dose profile. The last sample doubled as the predose for the bid regimen, commencing on Day 4.

Pre-administration samples during the bid dosing phase were collected on the mornings of Days 5, 7, and 10. On the morning of Day 13, the last dose of the bid regimen was given. The 0-72 hour blood collection profile employed during the single dose phase (Days 1-3) was then repeated. Additional samples were collected on the mornings of Days 17 and 18, the second which also served as a pre-dose sample for the tid regimen.

Doses were administered tid throughout Day 18. During this phase, samples were collected at 1, 2, 4, and 8 hours after the first and second doses. Blood was collected at 8 hours after the third tid dose, which also served as the pre-dose sample for the final BSZ administration of this phase, which was given on the morning of Day 19. Immediately following this dose, the 72 hour blood collection profile employed during the single dose phase (Days 1-3) was again repeated. One additional sample was collected on the morning of Day 23 at 96 hours after the final dose.

2. Urine - samples were collected over the following intervals on Days 1-3: 0-4 hr, 4-8 hr, 8-12 hr, 12-16 hr, 16-24 hr, 24-36 hr, 36-48 hr, and 48-72 hr.

During the bid regimen (Days 4-13) urine was collected for one dosage interval (12 hours) immediately after the morning doses on Days 5, 7, and 10, and after the evening dose on Day 12. Following the final dose on Day 13, the single dose profile (Days 1-3) was repeated, with an additional 24 hour sample being collected to 96 hours post-dose.

During the tid regimen (Day 18) samples were collected as on Day 1. After the final administration on Day 19, the collection profile following the last bid dose was again employed.

3. Feces - all feces voided during the following periods were collected: up to 72 hours after the first dose, over 120 hours after the final dose of the bid regimen on Day 13, and for over 120 hours after the first dose of the tid regimen which commenced on Day 18.

Pharmacokinetic Analysis:
The following PK parameters were determined for BSZ and it metabolites (ASA, NASA, ABA, and NABA):
1. Cmax - maximum observed plasma concentration
2. tmax - time to Cmax
3. AUC_{last} - area under the plasma concentration time curve from time 0 to the time of the last quantifiable concentration
4. Time to establish steady state - comparison of Cmin (predose) values for the mornings of Days 5, 7, 10, and 13 during the bid dosing regimen. Comparison of predose values during the tid dosing regimen.

5. %Dose excreted in the urine - 100*amount excreted in the urine/dose corrected for the molecular weight of the compound
6. %Dose excreted in the feces - 100*amount excreted in the feces/dose corrected for the molecular weight of the compound
7. Cl_R - renal clearance computed as amount of drug excreted in the urine/plasma AUC over the collection interval.

Statistical Analysis:
Summary statistics were presented for demographic data. Adverse events were summarized by tabulating the number of subjects experiencing each event during the following periods: Days 1-3, Days 4-13, Days 14-17, and Days 18-22, corresponding to changes in dose.

Plasma, urine and fecal concentrations and computed parameters were listed and summarized by treatment (mean, SD, range). Mean and individual plasma concentrations versus time curves were plotted for each dose.

Analytical Methods:

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RESULTS:

Study Population and Demographics:

Fourteen healthy volunteers were recruited to the study. Subjects 1 and 8 withdrew from the study and were replaced by Subjects 1A and 8A. The results, therefore, were determined from the 12 subjects who completed the protocol with one exception. Subject 8A was withdrawn from the study on Day 19 due to diarrhea and stomach cramps. He completed the single and bid dosing phases to Day 13 but did not receive any BSZ thereafter.

Demographic data can be found in Table 7. All subjects were white males ranging in age from 19-40 years. Most of the subjects had a history of smoking and alcohol and caffeine consumption.

Table 7. Summary of Demographic Data

Variable	Parameter	Subjects (N=12)
Age (years)	Mean (SD) Range	27 (6.4) 19-40
Height (cm)	Mean (SD) Range	176 (5.5) 166-183
Weight (kg)	Mean (SD) Range	70.4 (8.3) 57.6-84.6
Smoking Habit (cigarettes/day)	None (non-smoker) 7 8 10	3 3 5 1
Alcohol consumption (units/week)	Mean (SD) Range	7 (5.1) 0-16
Coffee consumption (cups/day)	None 1 2 3 4	3 1 4 3 1

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Adverse Events:
As stated previously, one subject was withdrawn from the study. The following Table lists the sequence of adverse events for Subject 8A:

Table 8. Adverse events experienced by Subject 8A.

Day	Date	Adverse Events
8	9/10/92	Diarrhea
		Sore stomach
8	9/11/92	Stomach cramps
		Diarrhea
		Sore stomach
11	9/13/92	Stomach pains
		Diarrhea
12	9/14/92	Diarrhea
		Nausea
13	9/15/92	Vomiting
		Headache
		Dyspepsia
15	9/17/92	Rash on left foot
18	9/20/92	Stomach cramps
		Headache

Most of the events resolved within 24 hours of onset. The exception was the stomach pains, which arose on Day 11 and lasted one week. These were described as moderately severe while all other symptoms were mild. The investigator judged the relationship between the study drug and all of the adverse events to be remote. The only exception was skin rash, which was judged to be possibly related to BSZ. However, the timing of events in relation to BSZ dosing make a drug relationship possible.

The following table displays the adverse events for all of the subjects. All of these were considered to be mild and they resolved without any action required (Subject 8A excluded).

Table 9.
Number of Subjects experiencing Adverse Events during different study days.

Disorders classified by WHOART system	WHOART preferred term	Days 1-3 (N=12)	Days 4-13 (N=12)	Days 15-17 (N=11)	Days 18-23 (N=12)
Body as a whole	Fatigue	1	0	0	0
Central and peripheral nervous system	Dizziness	0	1	0	0
	Headache	0	5	0	4
	Hypoasthesia	0	0	0	2
Gastrointestinal	Abdominal pain	0	1	1	2
	Constipation	1	0	0	0
	Diarrhea	0	2	0	0
	Epigastric pain	0	0	0	1
	Nausea	0	3	1	1
	Vomiting	0	2	0	0
Hearing and Vestibular	Earache	0	0	1	0
Skin and Appendages	Furunculosis	0	1	0	2
	Pruritus	0	4	1	0
	Pustular rash	0	0	0	1
	Rash	0	0	1	0
	Urticaria	0	1	1	0

Note: WHOART = World Health Organization Adverse Reaction Tables

Analytical Methods:

Overall, the assays used for determination of BSZ and its metabolites in plasma, urine, and feces had some major deficiencies. For example, most of the fecal samples required dilution to bring them within range of the calibration curve for the compound of interest, however, the measures taken to ensure that these dilutions were accurate and precise (parallelism studies) were inadequate.

Assay validation for plasma, urine, and feces at the lower limits of the respective calibration curves was inadequate or inaccurate. For example, none of the urine or fecal assays analyzed QC samples at the respective LOQs. This is especially relevant for analytes for which the majority of the samples had concentrations near the LOQ, such as, BSZ and ABA. In addition, all of the plasma assays had numerous individual samples with unacceptable accuracy (greater than ±20%). No intraday precision or accuracy data were reported for any of the compounds in the three biological matrices. Results of recovery determinations and stability testing, if undertaken, were also not reported.

Many of the fecal samples collected during this study could not be identified due to "smudged labels" on the sample containers. Those samples were discarded and a decision was made to analyze only those that could be fully identified. Only 19 samples were assayed altogether.

Pharmacokinetic Results:

Mean plasma concentration vs time profiles for BSZ and each metabolite as determined after the single 1.5 gm dose can be located in the Appendix.

Tables 10-14 list the PK parameters as determined from the plasma analysis of BSZ and its metabolites. In all cases, values are reported as means±SD. Ranges are included in parentheses and are provided because of the high variability in the data. **All concentrations that were below assay LOQs were reported as zero and included in calculations.** BSZ values are now reported as the free acid (given as the disodium dihydrate for assay validation studies). N=12 on Days 1 and 13 and N=11 on Day 19. AUC_{last}= AUC_{0-12} for Day 13 and AUC_{0-8} for Day 19.

Table 10. BSZ Plasma PK Parameters

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{last} (ng*hr/ml)
Day 1 (1.5 gm BSZ)	-	7.9±4.9 (0-12.9)	1.1±0.8 (0-2)	5.7±5.7 (0-17.4)
Day 13 (1.5 gm BSZ bid)	0±0	7.2±5.5 (0-13.5)	1.0±0.9 (0-2)	7.5±9.6 (0-32.4)
Day 19 (2.25 gm BSZ tid)	0±0	11.9±7.6 (0-19.6)	3.9±10.1 (0-36)	13.9±10.4 (0-28.3)

Very few of the plasma samples obtained over the course of the entire study had detectable levels of BSZ. For example, only 11/156, 11/180, and 17/154 individual blood samples drawn on Days 1, 13, and 19, respectively, contained quantifiable levels of BSZ (all others were below the assay LOQ). Furthermore, 3, 4, and 2 subjects had no detectable quantities of BSZ in their plasma on Days 1, 13, and 19, respectively. In the plasma samples which did contain BSZ, all quantities were near the assay LOQ. In general, BSZ appeared in the plasma by 1 hour after a dose, with no measurable quantities observed after 2 hours. The BSZ plasma concentration vs time profiles

were incomplete, with virtually every subject exhibiting only 1 or 2 quantifiable levels of BSZ over the respective AUC time intervals. For this reason, the half-life of BSZ could not be determined nor could mean AUC values for the different treatments be compared with any degree of confidence.

Mean tmax values indicate some absorption of BSZ in the upper gastrointestinal tract, as drug would not be expected to reach the colon and absorbed within 1 hour. Although the mean tmax for Day 19 was around 4 hours, 8/9 subjects with measurable BSZ concentrations had tmax values of 1 or 2 hours.

Table 11. ASA Plasma PK Parameters

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{last} (ng*hr/ml)
Day 1 (1.5 gm BSZ)	-	292±156	7.2±2.6	1581±1182
Day 13 (1.5 gm BSZ bid)	182±107	377±178	9±1.8	2590±1329
Day 19 (2.25 gm BSZ tid)	205±137	413±220	12.4±7.9	1408±813

ASA was detected in the plasma of all subjects on all three days (with the exception of one subject on Day 1). It was not observed until at least 2 hours after the single BSZ dose was administered on Day 1. No ASA was detected after 14, 36, and 48 hours on Days 1, 13, and 19, respectively. Of note is that all of the subjects with BSZ plasma concentrations that were below the assay LOQ had detectable quantities of ASA. Again, the majority of the ASA plasma concentration vs time profiles were erratic, making it difficult to estimate half-life. Interestingly, the mean AUC_{last} was lowest on Day 19 when the largest dose was administered. Furthermore, 11/12 subjects all had individual AUC values which were lower on Day 19 compared to Day 13. Although the mean tmax appeared to increase with dose, the value for Day 19 is misleading in that 10/11 subjects in that group had tmax values of 8-12 hours.

Table 12. NASA Plasma PK Parameters

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{last} (ng*hr/ml)
Day 1 (1.5 gm BSZ)	-	900±278	8.7±1.0	10708±1977
Day 13 (1.5 gm BSZ bid)	551±245	852±301	8.3±2.6	7429±3230
Day 19 (2.25 gm BSZ tid)	618±196	951±287	12.5±7.9	4751±1628

All subjects had detectable levels of NASA on all three study days. NASA appeared by 4 hours after a single BSZ dose on Day 1 and was detectable for up to 72 hours on all three days. Of note is the appearance of NASA in one subject on Day 1 for which no ASA was detected. As for ASA, most of the NASA plasma concentration vs time profiles were erratic. Mean AUC_{last} values for NASA appeared to decrease with increased dosing. Once again, all subjects on Day 19 had individual AUC values which were less than those observed on Day 13. Although the mean tmax value on Day 19 is greater than that observed for the other two days, 10/11 subjects had tmax values from 8-12 hours.

Table 13. ABA Plasma PK Parameters

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{last} (ng*hr/ml)
Day 1 (1.5 gm BSZ)	-	11.9±14.2	3.8±4.4	29.7±45.6
Day 13 (1.5 gm BSZ bid)	8.1±12.6	24.7±14.2	8.5±7.9	130±153
Day 19 (2.25 gm BSZ tid)	14.7±13.2	31.7±18.2	11±8.1	103±115

Only 9/143 of the blood samples drawn had quantifiable levels of ABA on Day 1. The first observation of ABA in any subject was not until 6 hours after the single BSZ dose. 6/11, 2/11 and 1/10 subjects had ABA levels which were below the assay LOQ on Days 1, 13 and 19, respectively. In the subjects who did have measurable concentrations of ABA, all values observed were near the assay LOQ. ABA was detected in plasma for up to 36 hours after multiple BSZ dosing. The majority of the ABA plasma concentration vs time profiles were incomplete or erratic, therefore, comparisons between AUC values were difficult to make and no half-life could be determined. All of the subjects with measurable quantities of ABA on Day 1 had tmax values of 8 or 10 hours as did the majority of patients on Days 13 and 19.

Table 14. NABA Plasma PK Parameters

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{last} (ng*hr/ml)
Day 1 (1.5 gm BSZ)	-	49.4±14.4	13.5±11.7	892±562
Day 13 (1.5 gm BSZ bid)	54.9±21.7	72.6±23.5	9.4±6.2	643±261
Day 19 (2.25 gm BSZ tid)	59.5±32.1	85.3±37	13.6±6.7	451±235

All of the subjects had quantifiable plasma levels of NABA during all three of the study days. This metabolite was first detected at 4 hours after the single dose of BSZ and was observed for

up to 96 hours with multiple dosing. Like the other compounds, NABA plasma concentration vs time profiles were erratic. Mean AUC_{last} values decreased with increased dosing; >90% of the subjects had lower AUC values on Day 19 compared to Day 13. Tmax values were 8-10 hours for the majority of individuals on all three study days.

Of interest is the appearance in about 50% of the subjects of the N-acetylated metabolites (NASA or NABA) prior to that of the "parent" metabolite (ASA and ABA, respectively) in the systemic circulation. Furthermore, one subject had readily quantifiable levels of NASA without any plasma ASA detected, while 4 subjects had detectable levels of NABA, but no ABA. All of these occurred on Day 1 after a single dose of BSZ. One possible explanation for this phenomenon is that ASA and ABA are either absorbed in quantities too low to be detected or are absorbed more slowly than their acetylated metabolites. N-acetylation of ASA has been shown to occur in the feces and colonic mucosa of various species, including man,[1] however, no information is available as to the location of ABA acetylation.

Time to reach Steady-State

The trough levels for each of the five compounds at the predetermined times during the bid and tid dosing regimen are provided in Tables 15 and 16.

Table 15. Trough levels (ng/ml)[a] for BSZ and its metabolites with bid dosing.

	BSZ	ASA	NASA	ABA	NABA
Day 5	0	12.2±25.9[b]	251±83.5	0±0	28.3±15.2
Day 7	0	148±63.5	582±137	14.1±13.7	69.5±13.7
Day 10	0	123±72	527±139	11.2±13.2	71.3±24.1
Day 13	0	100±75.5	416±193	4.6±10.5	59.1±17

[a]N=10-12

[b]Values are reported as means±SD. Ranges are in (). All concentrations <LOQ were reported as zero.

It was assumed that BSZ had reached steady-state, as all samples collected were below the assay LOQ. There was no apparent accumulation of BSZ with multiple dosing. Based on the mean data, all of the metabolites appeared to reach peak levels by Day 7, and declined thereafter. Examination of the individual data, however, revealed that the time required to achieve steady state varied considerably within subjects for all of the metabolites, and ranged from 7 to 13 days.

1. Biochem Pharmac 36:3372, 1987.

Table 16. Mean trough levels (ng/ml)[a] of BSZ and its metabolites with tid dosing.

	BSZ	ASA	NASA	ABA	NABA
$Cmin_1$ [b]	0	206±189[c]	534±346	13.5±19.5	26.9±25.4
$Cmin_2$	0	88.6±108	376±155	4.1±8.6	25.5±20.8
$Cmin_3$	0	72.8±72.6	403±176	5.4±11.5	50.5±27.9

[a]N=10-12

[b]$Cmin_1$, $Cmin_2$, and $Cmin_3$ indicate the trough plasma concentrations after the first, second, and third doses, respectively.

[c]Values are reported as means±SD. Ranges are in (). All concentrations <LOQ were reported as zero.

There was no BSZ detected in any of the predose plasma samples, therefore, it was assumed that steady-state had been achieved. Again, there was no accumulation with the multiple tid dosing regimen used in this study. ASA levels appeared to stabilize by the third BSZ dose, as did those for ABA. The mean ABA concentrations reflect the results of only a few individual subjects, as most were below the assay LOQ. Although mean NASA Cmin levels were higher after the third dose as compared to the second, 50% of the subjects actually had lower $Cmin_3$ values compared to $Cmin_2$.

Trough concentrations for NABA appeared to increase with multiple dosing. Three and four of the subjects had undetectable concentrations of NABA before the second and third dose, respectively, while only one subject was below the assay LOQ prior to the fourth dose. Furthermore, 9/11 of the subjects with measurable NABA concentrations exhibited the highest trough levels after the third dose of BSZ.

Extent of Accumulation with Multiple Dosing

Table 17 displays the BSZ and metabolite AUC results for 3 of the 4 dosing intervals during the tid dosing regimen which commenced on Day 18; i.e., $AUC_{0\text{-}8hr}$, $AUC_{8\text{-}16hr}$, and $AUC_{24\text{-}32hr}$. (There was no blood sampling during the 16-24 hour interval). Corresponding Cmax values are also displayed in Table 18.

Table 17. AUC values for tid dosing intervals at Day 18 and 19.

	AUC_{0-8hr} (ng*hr/ml)	AUC_{8-16hr} (ng*hr/ml)	$AUC_{24-32hr}$[a] (ng*hr/ml)
BSZ	9±13.3[b]	3.6±7.5	13.9±10.4
ASA	290±297	853±673	1408±813
NASA	1048±671	3422±1420	4751±1628
ABA	26.1±41.1	26.6±40.3	103±115
NABA	35.5±36.4	209±161	451±235

[a]$AUC_{24-32hr}$ is equivalent to AUC_{0-8hr} on Day 19.
[b]All values are means±SD. Ranges are provided in parentheses. N=11 for all values except ABA where N=9.

Table 18. Cmax values for tid dosing intervals at Day 18 and 19.

	$Cmax_{0-8hr}$ (ng/ml)	$Cmax_{8-16hr}$ (ng/ml)	$Cmax_{24-32hr}$[a] (ng/ml)
BSZ	6.4±7.5[b]	4.3±7.6	11.9±7.6
ASA	206±189	223±179	413±220
NASA	534±346	583±314	951±287
ABA	15±20.1	19.7±18.2	31.7±18.2
NABA	26.9±25.4	38.9±22	85.3±37

[a]$AUC_{24-32hr}$ is equivalent to AUC_{0-8hr} on Day 19.
[b]All values are means±SD. Ranges are provided in parentheses. N=11 for all values except ABA where N=9.

Although no formal statistical analysis was performed, there was no apparent accumulation of BSZ with the multiple tid dosing regimen. Mean exposure appeared to be higher after the fourth dose (AUC_{24-32}), however, examination of the individual data revealed that <50% of the subjects had greater AUC values after the fourth dose compared with the first dose. Cmax values revealed similar trends in individual data. In addition, all of the quantifiable BZS levels were very close to the assay LOQ.

The data for ABA were more variable, probably because there were very few measurable

concentrations, and these were all near the assay LOQ. Comparison of AUC values for this metabolite is nearly impossible as almost all of the ABA plasma concentration vs time profiles were incompletely defined.

The mean data for ASA, NASA, and NABA does indicate accumulation throughout the tid dosing regimen, especially by the fourth dose. Furthermore, examination of the individual data revealed an increase in AUC and Cmax values with each successive dose for ASA, NASA, and NABA in almost every subject. No PK determinations were made beyond the fourth dose, therefore, there may be further accumulation that is not apparent with the data provided. It should be noted that the proposed clinical dosage regimen is 2.25 gm BSZ tid for 8 weeks.

Dose Proportionality

The dose-normalized Cmax and AUC values for the final dose of the bid and tid regimens are provided in Table 19.

Table 19. BSZ dose-normalized Cmax and AUC values.[a]

	Cmax (ng/ml)	$AUC_{0-\tau}$ (ng*hr/ml)[b]
Day 13 (1.5 gm BSZ bid)	7.2±5.5	7.5±9.6
Day 19 (2.25 gm BSZ tid)	7.9±5.1	9.3±6.9

[a]Adjusted to a 1.5 gm dose.

[b]$AUC_{0-\tau}$=AUC_{0-12} for the bid dosing regimen and AUC_{0-8} for the tid dosing regimen.

Dose-adjusted mean values for AUC appear to indicate that BSZ is dose-proportional, however, there is large variability in the data. As discussed earlier, the majority of the subjects had very few detectable levels of BSZ throughout any of the dosing regimens. Therefore, the BSZ plasma concentration vs time profiles were not completely defined so that comparison of the dose-normalized AUC values is virtually meaningless.

Percent of dose excreted in urine and feces

The percent of a dose of BSZ excreted as parent compound or metabolite in urine and feces after each dosing regimen are provided in Tables 20-24. Renal clearance values are provided as well. The urinary and fecal collection intervals are described in the **Methods Section** under "Biological Sampling".

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Table 20. BSZ excretion in urine and feces.

	Percent of BSZ recovered in feces	Percent of BSZ recovered in urine	Renal Clearance[a] (L/hr)
Day 1 (1.5 gm BSZ)	0 - [N=1]	0.1±0.1 (0-0.2) [N=12]	150±85 (47-302) [N=9]
Day 13 (1.5 gm BSZ bid)	9.2±11.5 (0-29.6) [N=8]	0.1±0.1 (0-0.3) [N=12]	143±73 (42-254) [N=8]
Day 19 (2.25 gm BSZ tid)	7.9±7.5 (0.2-26.2) [N=9]	0.2±0.1 (0.1-0.3) [N=11]	182±212 (0-604) [N=9]

[a]Renal clearance was determined over the 0-4 hr collection interval.

BSZ was recovered in the urine of all the subjects, although quantities were very limited. The very large values obtained for the renal clearance of BSZ is most likely a function of the incomplete characterization of the BSZ plasma concentration vs time curves used in the Cl_R calculations.

Table 21. ASA excretion in urine and feces.

	Percent of BSZ dose recovered as ASA in feces	Percent of BSZ dose recovered as ASA in urine	Renal Clearance (L/hr)
Day 1 (1.5 gm BSZ)	3.7 - [N=1]	0 - [N=12]	0[a] - [N=2]
Day 13 (1.5 gm BSZ bid)	52.5±14.8 (30.4-75.9) [N=8]	0.6±1.6 (0-5.6) [N=12]	1.3±2.6[a] (0-8) [N=10]
Day 19 (2.25 gm BSZ tid)	20.5±12.6 (6.5-40.6) [N=9]	0.8±2.2 (0-7.3) [N=11]	1.1±1.3[b] (0-3.0) [N=11]

[a]Renal clearance was determined over the 0-4 hr collection interval.

[b]Renal clearance determined over the 8-12 hr collection interval.

Low concentrations of ASA were detected in the urine of only 4 subjects on Day 13 and 5 subjects on Day 19. Therefore, Cl_R values are representative of a very small number of subjects.

Table 22. NASA excretion in urine and feces.

	Percent of BSZ dose recovered as NASA in feces	Percent of BSZ dose recovered as NASA in urine	Renal Clearance[a] (L/hr)
Day 1 (1.5 gm BSZ)	6.0 - [N=1]	17.4±8.9 (5.1-34.3) [N=12]	16.1±6.1 (7.7-26) [N=12]
Day 13 (1.5 gm BSZ bid)	39.6±17.3 (21.4-67.8) [N=8]	12.8±6.6 (4.3-25) [N=12]	0.01±0.01 (0-0.04) [N=12]
Day 19 (2.25 gm BSZ tid)	8.4±4.7 (2.8-18.6) [N=9]	4.4±1.8 (2.2-7.7) [N=11]	12.5±6.3 (4.3-22.4) [N=11]

[a]Renal clearance was determined over the 8-12 hr collection interval.

NASA was readily detected in the urine of all subjects on all three of the study days. Renal clearance values on Days 1 and 19 exceeded normal creatinine clearance values (approximately 7 L/hr), which probably indicates active tubular secretion of NASA. Indeed, Rasmussen[2] and coworkers observed renal clearances for NASA that were 2 times higher than the corresponding creatinine clearances following oral ingestion of 1.5 gm ASA/day in healthy subjects. There is no readily apparent explanation for the low Cl_R obtained on Day 13.

Table 23. ABA excretion in urine and feces.

	Percent of BSZ dose recovered as ABA in feces	Percent of BSZ dose recovered as ABA in urine	Renal Clearance (L/hr)
Day 1 (1.5 gm BSZ)	2.1 - [N=1]	0 - [N=12]	0 - [N=4]
Day 13 (1.5 gm BSZ bid)	118.5±100.6 (35.5-358.4) [N=8]	0 - [N=12]	0 - [N=3]
Day 19 (2.25 gm BSZ tid)	43.4±28.8 (14-90.9) [N=9]	0 - [N=12]	0 - [N=8]

There was virtually no ABA recovered in the urine on any of the three study days.

2. Gastroenterology 1983;85:1350.

Table 24. NABA excretion in urine and feces.

	Percent of BSZ dose recovered as NABA in feces	Percent of BSZ dose recovered as NABA in urine	Renal Clearance* (L/hr)
Day 1 (1.5 gm BSZ)	1.6 - [N=1]	2.2±1.5 (0-4.5) [N=12]	52±33.5 (0-95.9) [N=12]
Day 13 (1.5 gm BSZ bid)	19.7±11.8 (9.7-48.1) [N=8]	2.2±2.1 (0.1-5.7) [N=12]	31.4±43.7 (0-122) [N=12]
Day 19 (2.25 gm BSZ tid)	7.3±4.9 (2.1-14.7) [N=9]	0.8±0.7 (0-2.4) [N=11]	34.4±20.4 (0-58.8) [N=11]

*Renal clearance was determined over the 8-12 hr collection interval.

All subjects had readily quantifiable concentrations of NABA in the urine. As for NASA, renal clearance values exceeded normal creatinine clearance values, and may indicate active secretion of this metabolite.

Values provided for the percent of a single dose of BSZ recovered in the feces as parent compound or metabolite on Day 1 are meaningless since N=1. Furthermore, the observations for fecal recovery on Day 13 are highly variable and appear to contain a large degree of error, as the mean sum total of BSZ and metabolites appearing in feces amounts to >200% (ABA alone contributes to>100% of the dose recovered). Finally, it is difficult to interpret the values obtained for Day 19, as fecal recovery determinations were made over a 120-hour period after the first dose of a 4-dose tid regimen. This makes it nearly impossible to determine the contributions of each single dose to the overall fecal recovery of each compound.

The data for urinary recovery and, therefore, renal clearance must be viewed with equal speculation as the sponsors admit that "The total amount in urine (Au) and the estimated renal clearance values (both Clr ml/min and Clr ml/min/kg) were also analysed but these should only be considered supporting data since there is no guarantee that urine collections were complete and accurately timed."

The following table summarizes the percent of a dose of BSZ recovered as parent drug and/or metabolite in both the urine and feces for each treatment regimen.

Table 25. BSZ and metabolite recovery in urine and feces.*

	Feces	Urine	Total
Day 1	13.4% (N=1)	19.7% (N=12)	33.1%
Day 13	239.5% (N=8)	15.7% (n=12)	255.2%
Day 19	87.5% (N=9)	6.2% (n=11)	93.7%

*Fecal and urine collection times and intervals are described in the **Methods Section**, under "Biological Sampling".

CONCLUSIONS:

The safety and tolerability of single and multiple doses of BSZ were studied in healthy males. Overall, the drug appears to be safe, however, adverse events with multiple dosing did necessitate the withdrawal of one subject from the study.

In general, BSZ was quickly absorbed into the systemic circulation, but only in very limited quantities. Furthermore, only small amounts were recovered in either the urine or feces. Likewise, ABA appeared almost exclusively in plasma as the N-acetylated metabolite with virtually none detected in the urine. It was not possible to determine the half-lives of these two compounds due to the incomplete definition of their plasma concentration vs time profiles.

ASA, NASA, and NABA were readily detected in the plasma of all subjects, although limited quantities of these compounds were recovered in the urine. Most of the plasma concentration vs time curves were erratic for all three of these compounds, most likely indicating inconsistent absorption from the colon. Due to the suboptimal characterization of the plasma profiles, estimations of half-lives were not attempted. This also made comparison of AUCs difficult, although there was a clear trend towards a decrease in AUC values with increasing BSZ doses. Relatively small quantities of ASA, NASA, and NABA were recovered in the urine.

The bulk of a 2.25 gm dose of BSZ was recovered in the feces as the parent compound and its four metabolites. ASA and ABA were detected in the largest quantities. It should be kept in mind that all of the PK data should be viewed in light of the small number of fecal samples, potentially inadequate collection of urine samples, and the assay validation limitations.

As there were no detectable concentrations of BSZ in the predose blood samples prior to either the bid or tid dosing regimens, it was assumed that steady-state had been achieved for the parent compound. Dose-proportionality could not be established because of the sparse data, which resulted in incomplete definition of the plasma concentration vs time profiles. Although it is not likely that either BSZ or ABA accumulated during multiple dosing, accumulation of ASA, NASA, and NABA was observed during the administration of 2.25 gm BSZ tid for four doses. As the proposed dose for this drug is 2.25 gm tid for 8 weeks, further accumulation could result in safety issues if any of these metabolites were toxic. However, this is unlikely as there were no serious adverse events suffered by patients on long-term BSZ therapy (Study #GLY01/93 in this

NDA submission) who had much higher plasma concentrations of these metabolites in most cases.

APPENDIX

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Study Plan : Dosing, Pharmacokinetic Blood Samples, Urine & Faecal Collection

Hours	-12	0	1	2	4	6	8	9	10	12	14	16	24/0
Day 1 Dosing Pharmacokinetic blood samples Urine collection		✓ ✓	✓	✓	✓	✓	✓		✓	✓	✓		✓
Day 2 P'K blood samples Urine collection						✓				✓			✓
Day 3 P'K blood samples Urine collection													✓
Day 4-12 Dosing P'K blood samples* Urine collection*		✓ ✓								✓			
Day 13 Dosing P'K blood samples Urine collection		✓ ✓	✓	✓	✓	✓	✓		✓	✓	✓		✓
Day 14 P'K blood samples Urine collection						✓				✓			✓
Days 15-16 P'K blood samples Urine collection													✓
Day 18 Dosing P'K blood samples Urine collection		✓ ✓	✓	✓	✓	✓	✓ ✓	✓	✓	✓	✓	✓ ✓	✓
Day 19 Dosing P'K blood samples Urine collection		✓	✓	✓	✓	✓	✓		✓	✓	✓		✓
Day 20 P'K blood samples Urine collection						✓				✓			✓
Days 21-22 P'K blood samples Urine collection													✓

* P'K blood and urine collection Days 5, 7 and 10 only
Faeces were collected on defecation during certain periods throughout the study

Figure 1A Mean Plasma Concentrations of Balsalazide versus Time Day 1

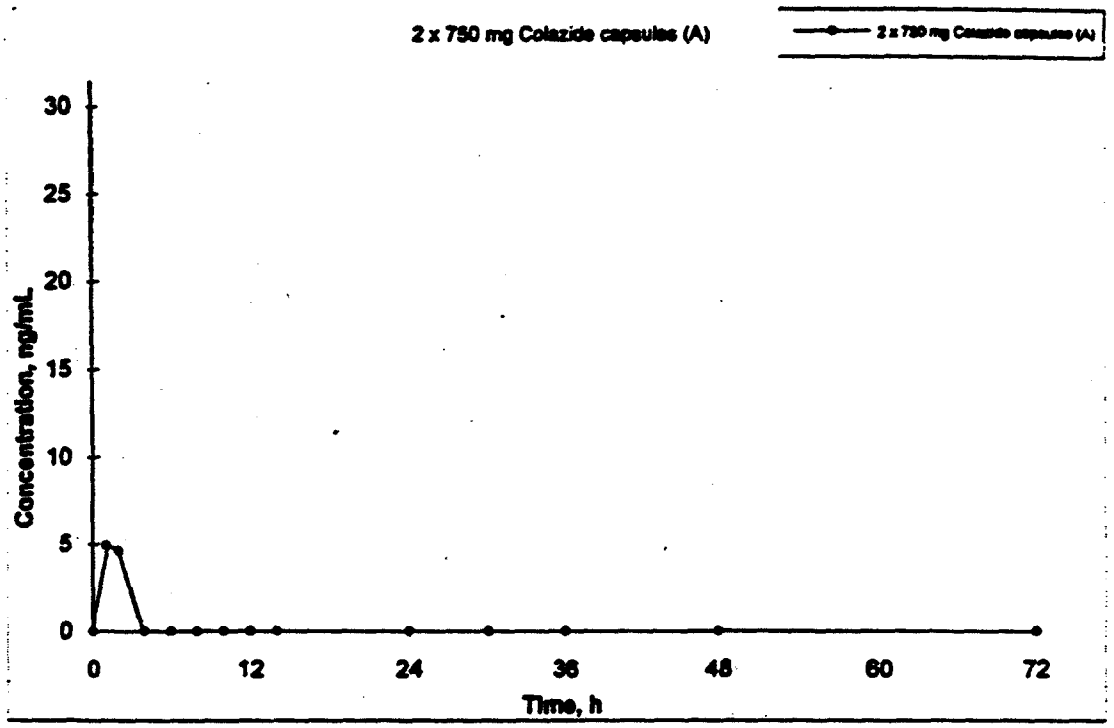

Figure 1B ln(Mean Plasma Concentrations) of Balsalazide versus Time Day 1

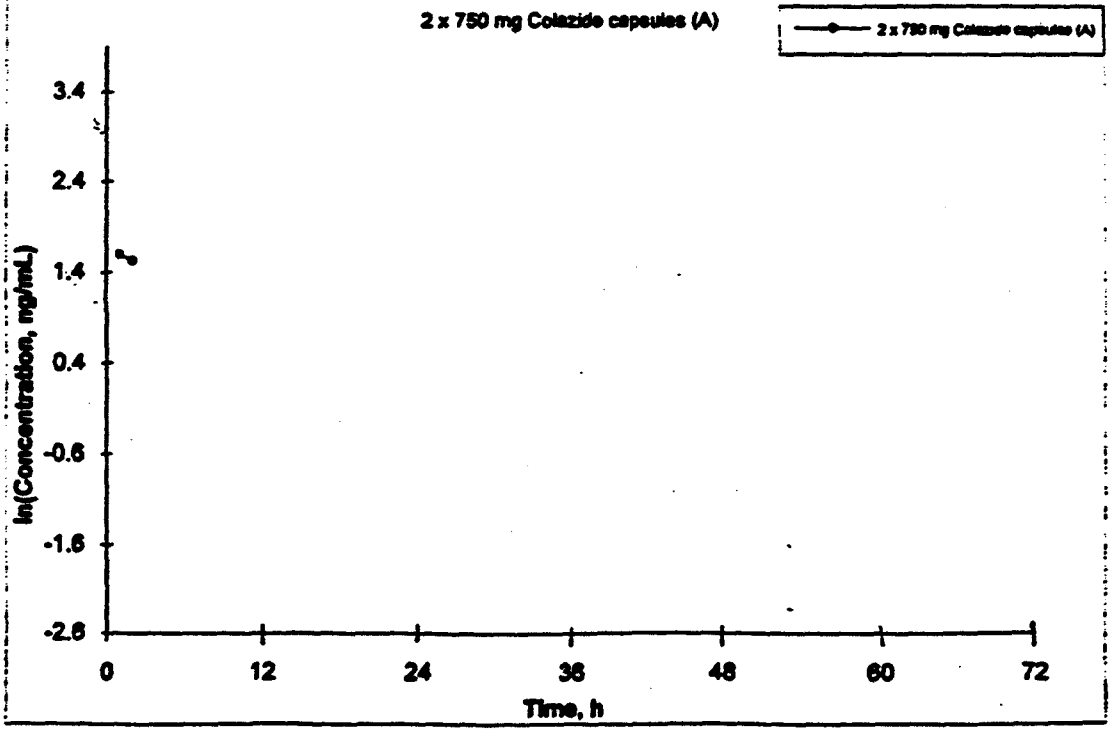

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Figure 4A Mean Plasma Concentrations of 5-ASA versus Time Day 1

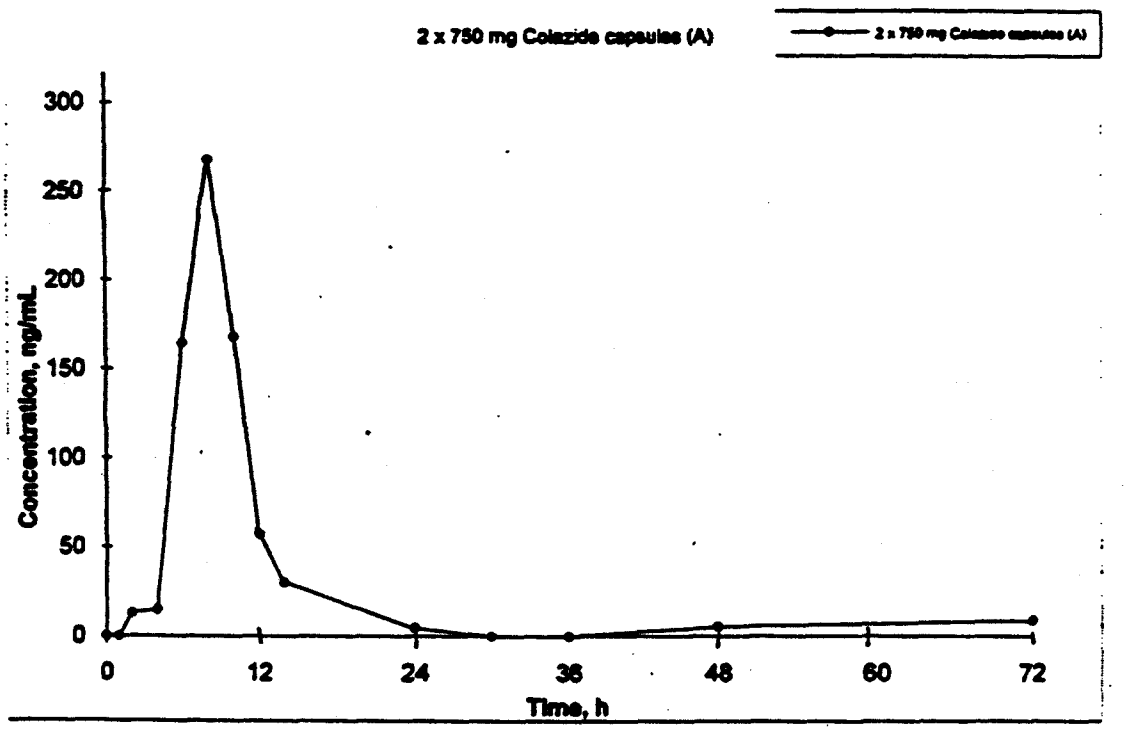

Figure 4B ln(Mean Plasma Concentrations) of 5-ASA versus Time Day 1

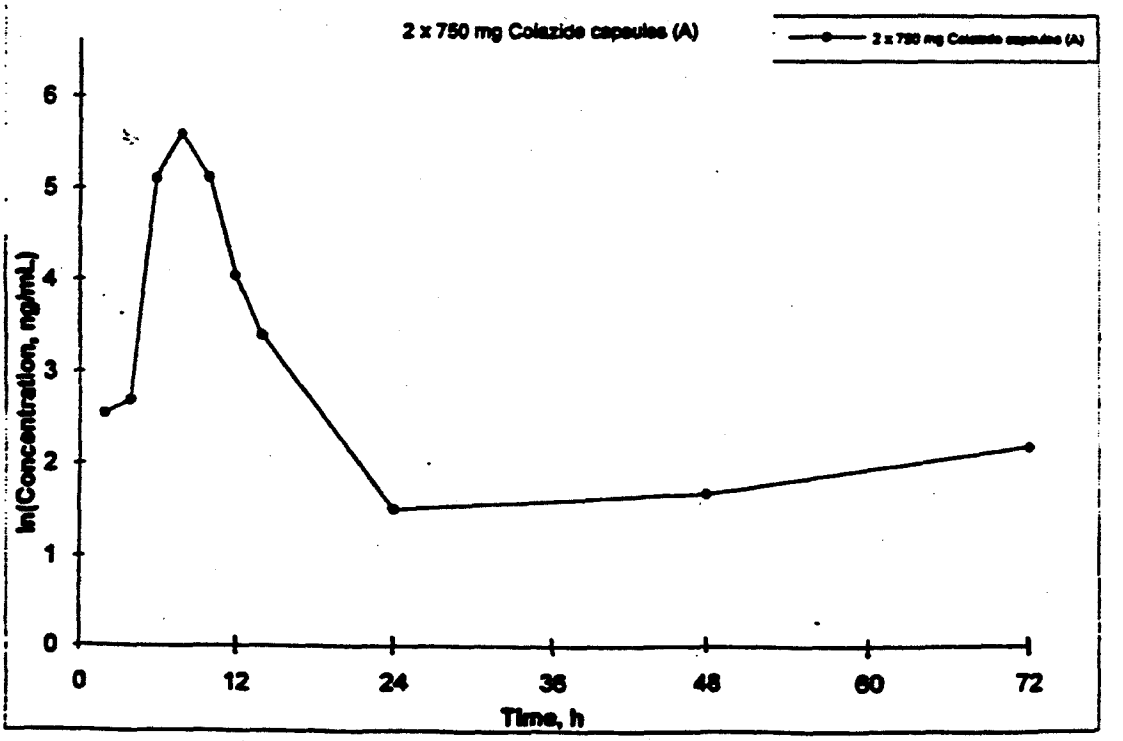

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Figure 7A Mean Plasma Concentrations of N-Ac-5-ASA versus Time Day 1

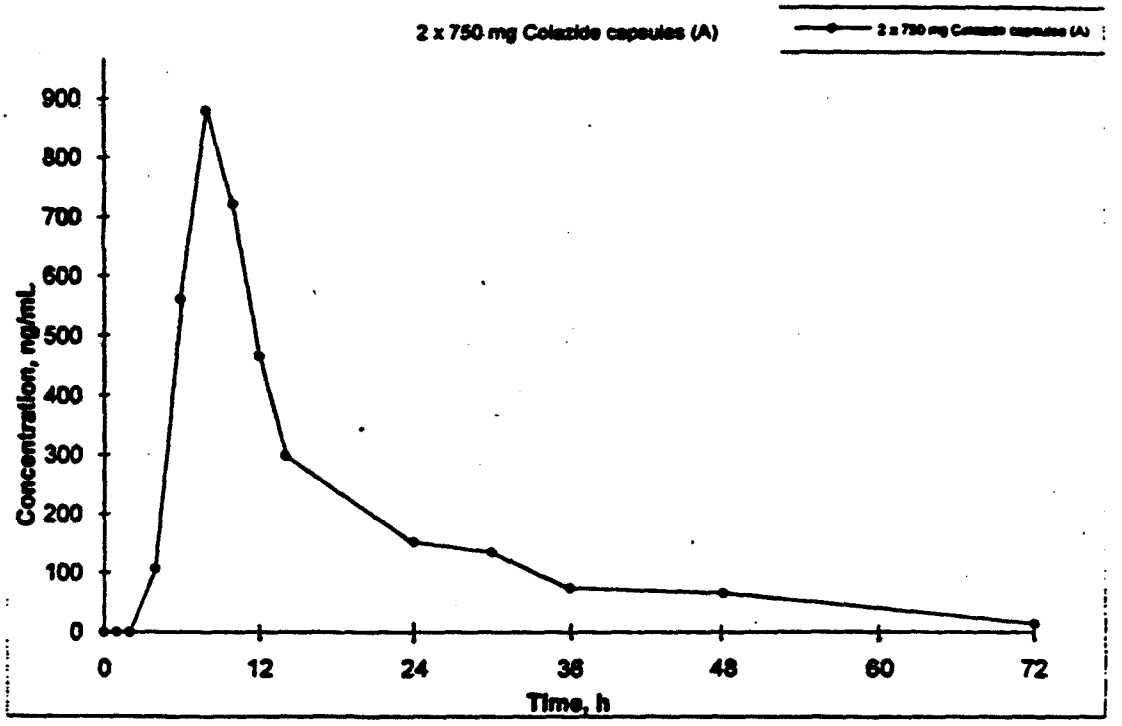

Figure 7B ln(Mean Plasma Concentrations) of N-Ac-5-ASA versus Time Day 1

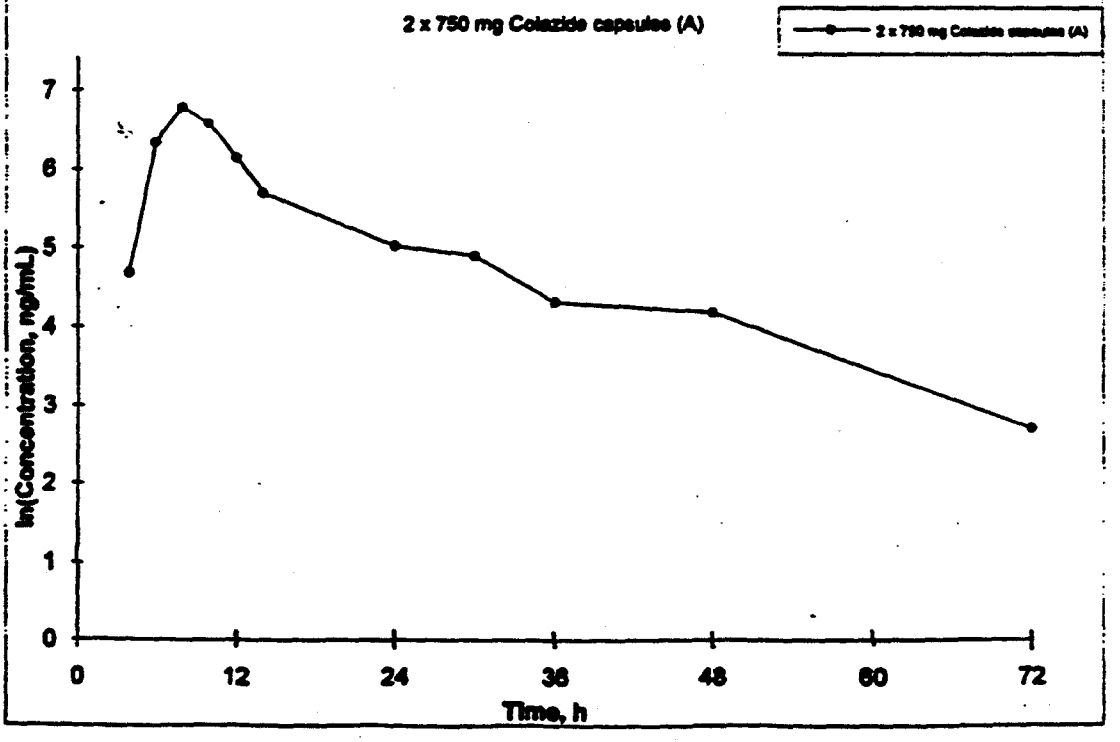

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Figure 10A Mean Plasma Concentrations of 4-ABA versus Time Day 1

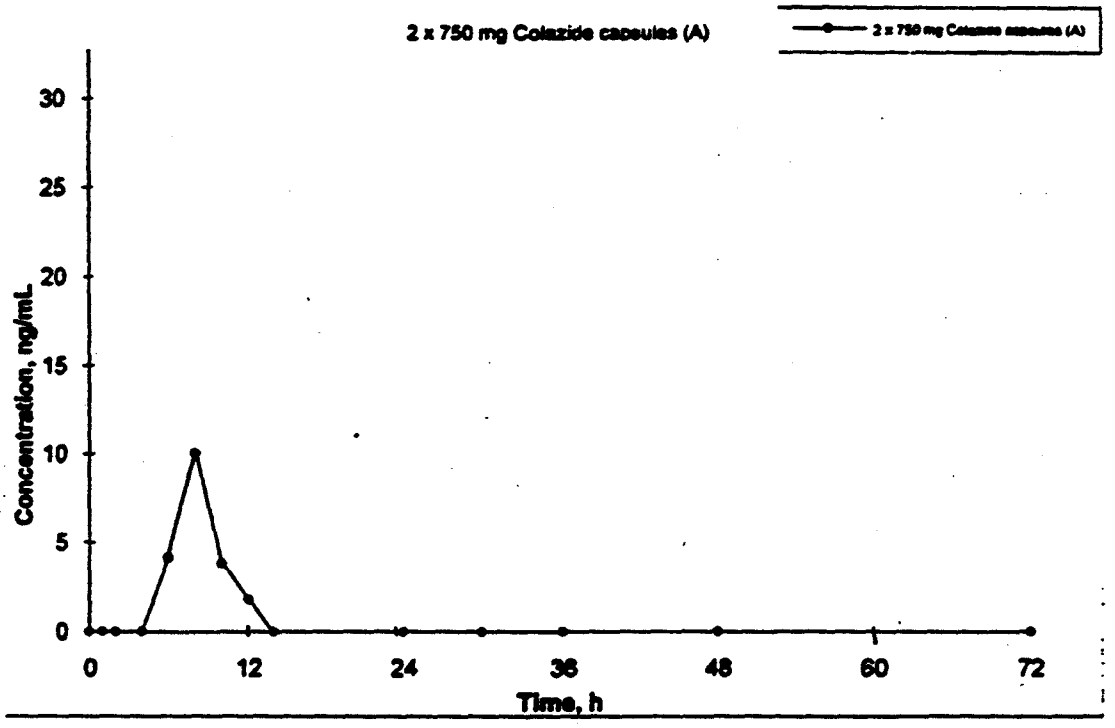

Figure 10B ln(Mean Plasma Concentrations) of 4-ABA versus Time Day 1

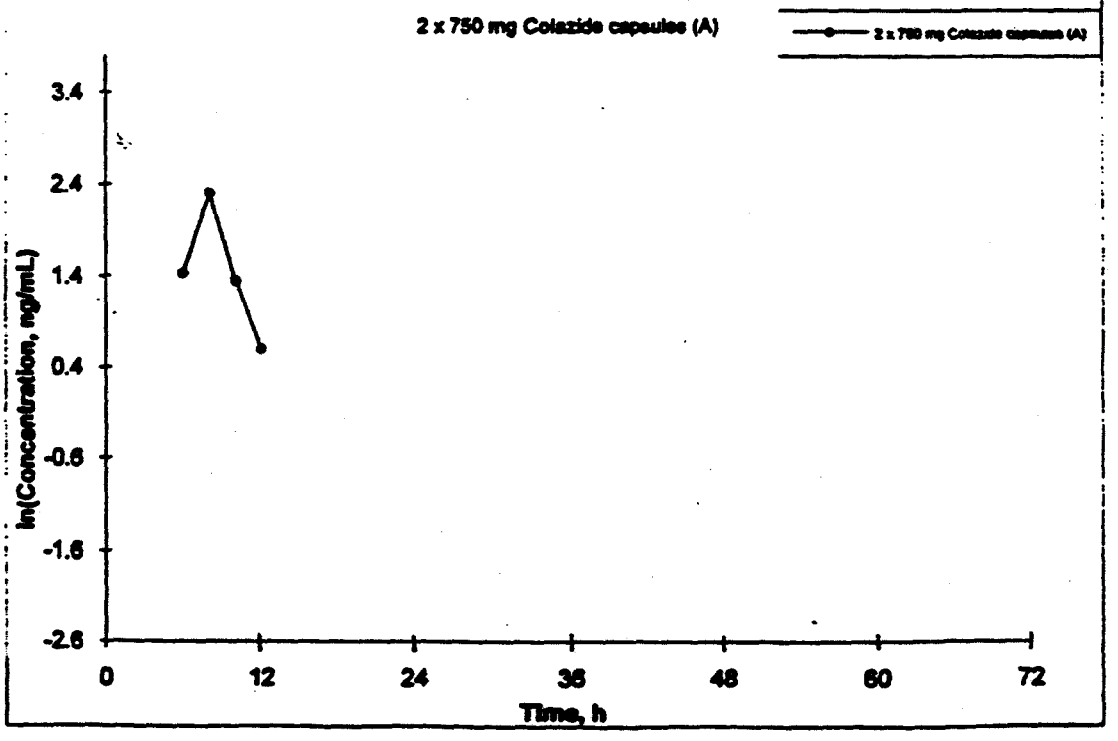

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Figure 13A Mean Plasma Concentrations of N-Ac-4-ABA versus Time Day 1

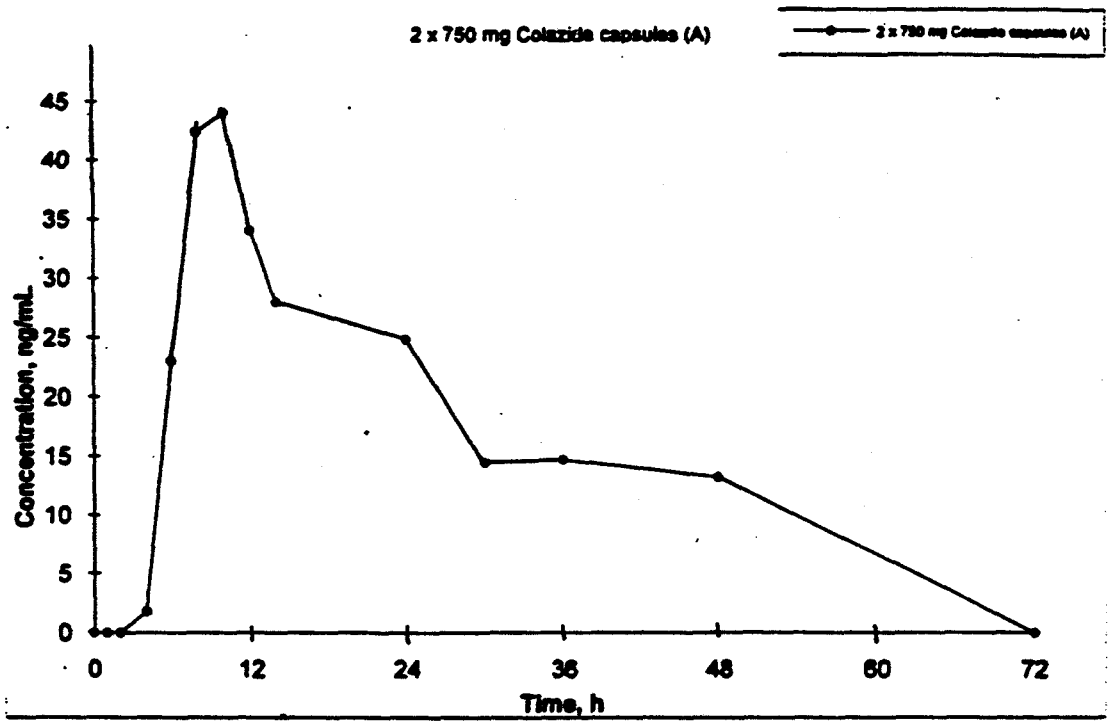
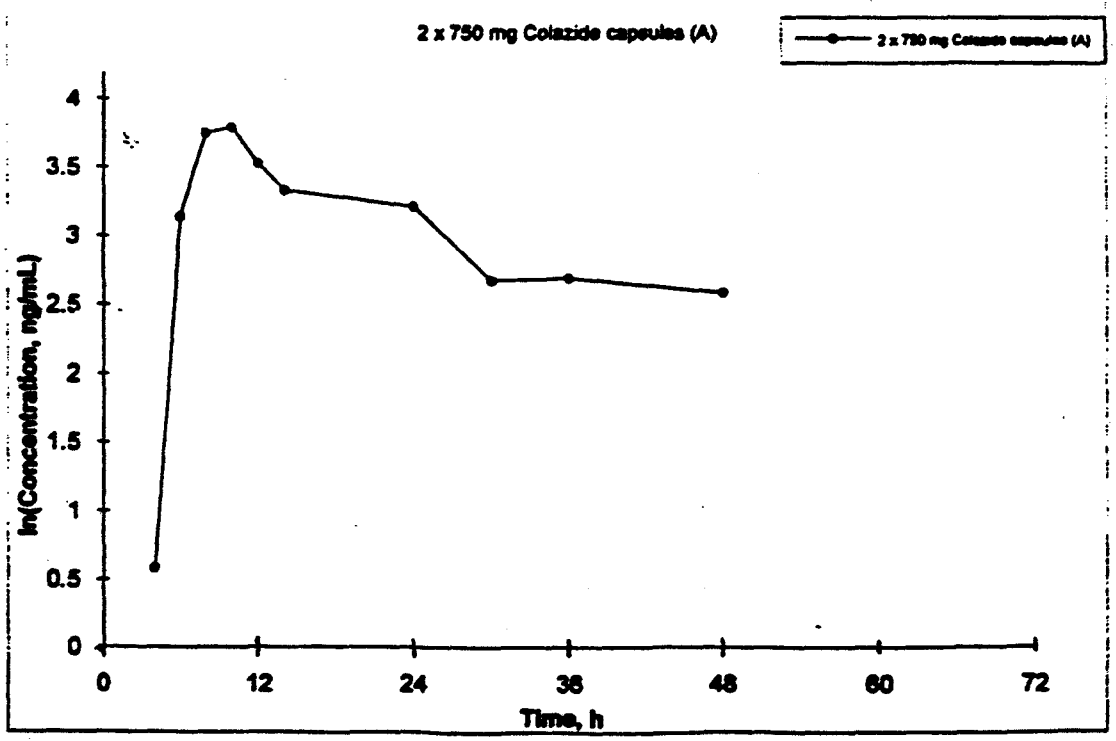


Figure 13B ln(Mean Plasma Concentrations) of N-Ac-4-ABA versus Time Day 1



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TITLE: Pharmacokinetic Study of Balsalazide Disodium (——) in Patients with Ulcerative Colitis Receiving Long-Term Maintenance Treatment

Study Number: GLY01/93

Investigators and Sites:

Table 1 : Investigators and Sites

Investigator	Research Nurse	Site
Dr		

Study Date: June 1993 to October 1993.

Abbreviations used: BSZ = Balsalazide
ASA = 5-aminosalicylic acid
NASA = N-acetyl-5-aminosalicylic acid
ABA = 4-aminobenzoyl-β-alanine
NABA = N-acetyl-4-aminobenzoyl-β-alanine
Cl_{CR} = creatinine clearance
Cl_R = renal clearance

OBJECTIVES: To obtain evidence from blood and urine samples of the pharmacokinetic disposition of BSZ and its metabolites in patients receiving long-term therapy with

BSZ including, if possible, information for patients with mild renal impairment.

METHODS:

Study Design: This was an open, non-randomized, non-comparative, patient monitoring study.

Study Population: 54 patients were studied. Patients were 22 to 69 years of age; 29 were male and 25 were female. 17 patients were studied twice.

Selection criteria: Consenting adult outpatients aged 18 years or over with ulcerative colitis in remission. Patients taking another ASA preparation were excluded. Patients were taking 3-6 gm BSZ bid. BSZ treatment had been continuous for at least one year and at a constant dose for two weeks before the study.

Treatment and Administration:

Eve of Study Day - A twelve-hour urine sample was collected by the patient overnight in the period following the evening dose and immediately preceding the hospital visit.

Study Day - The patient attended clinic and took his/her morning dose of BSZ. Demography, medical history and date of first use of BSZ were recorded, as well as details of current treatment for ulcerative colitis and concomitant medication for other conditions. Dipstick urinalysis and routine laboratory tests were undertaken. Sigmoidoscopy was performed by four investigators either on the study day or within two weeks. Information regarding food intake was not provided.

Study Drug Supplies:

Capsules of BSZ, each containing 750 mg, were prepared, packed and labeled by Biorex Lab. Limited, Enfield, U.K. The to-be-marketed formulation of BSZ was used in this study.

Adverse Events:

Details of adverse events occurring from the start of the urine collection to the end of the clinic visit were recorded.

Biological Sampling:

Blood - Blood samples for pharmacokinetic analysis were collected prior to the morning dose of BSZ and at 1, 2, 4, 6 and 8 hours post-dose or until the patient elected to leave the clinic.

Urine - Urine was collected by the patient after the evening dose of BSZ for 12 hours before the clinic visit.

Pharmacokinetic Analysis:

The following PK parameters were determined for BSZ and it metabolites (ASA, NASA, ABA, and NABA):

1. Cmin - minimum observed plasma concentration
2. Cmax - maximum observed plasma concentration
3. Tmax - time to Cmax

4. AUC_{0-12} - area under the plasma concentration time curve from 0 to 12 hours.
5. Percent dose excreted in the urine - 100*amount excreted in the urine/dose corrected for the molecular weight of the compound
6. Cl_R - renal clearance computed as amount of drug excreted in the urine/plasma AUC_{0-12}.

Data Adjustments:
Actual times were used for analysis of plasma and urine concentrations. Concentrations below the quantification limit (Z disodium dihydrate A in plasma and urine, respectively) were set to zero. Since the 12 hour blood sample was not taken, it was set equal to the predose concentration for estimation of AUC_{0-12}. After admission to the study, two patients were found not to be taking BSZ in a bid regimen. Patient 39, who was studied on two occasions, was taking 1.5 gm BSZ tid up to and during his first study day. His dose was then changed to 3 gm bid. The pharmacokinetic results from his first study day were excluded as they would compromise the analysis. Patient 40 was taking 1.5 gm BSZ four times daily. After discussion, it was decided that this therapy covered two rather than one complete dosage intervals and would not compromise the analysis.

Statistical Analysis:
Plasma concentrations and computed parameters were listed and summarized by dose (mean, standard deviation, minimum, maximum, and number of observations). Mean and by subject plasma concentrations versus time were plotted for each dose on both linear and natural logarithm scales.

Microsoft Excel version 4 was used to calculate the pharmacokinetic parameters, as well as produce the tables and figures. Statistical analyses were done using the SAS procedure, PROC GLM.

While the study included 71 patient study days, it was not possible to include all of these in the statistical analysis, since this would mix within and between subject variation. Thus, two analyses were performed. The first analysis compared the data from patients who had been studied twice and received the same dose on both occasions. The plasma concentration data were normalized to a dose of 1.5 gm prior to analysis. Since this analysis showed no significant differences between the visits for any parameter or any analyte, a second analysis which included all patients, was performed. In this analysis, the data from patients who were studied twice were averaged prior to analyzing dose effects and performing any sub-group analyses. Statistical analysis of the computed kinetic parameters was performed with an analysis of variance model appropriate for a parallel study.

Statistical analysis of the computed kinetic parameters between visits was performed with an analysis of variance model appropriate for a crossover design.

A comparison between males and females was performed using analysis of variance

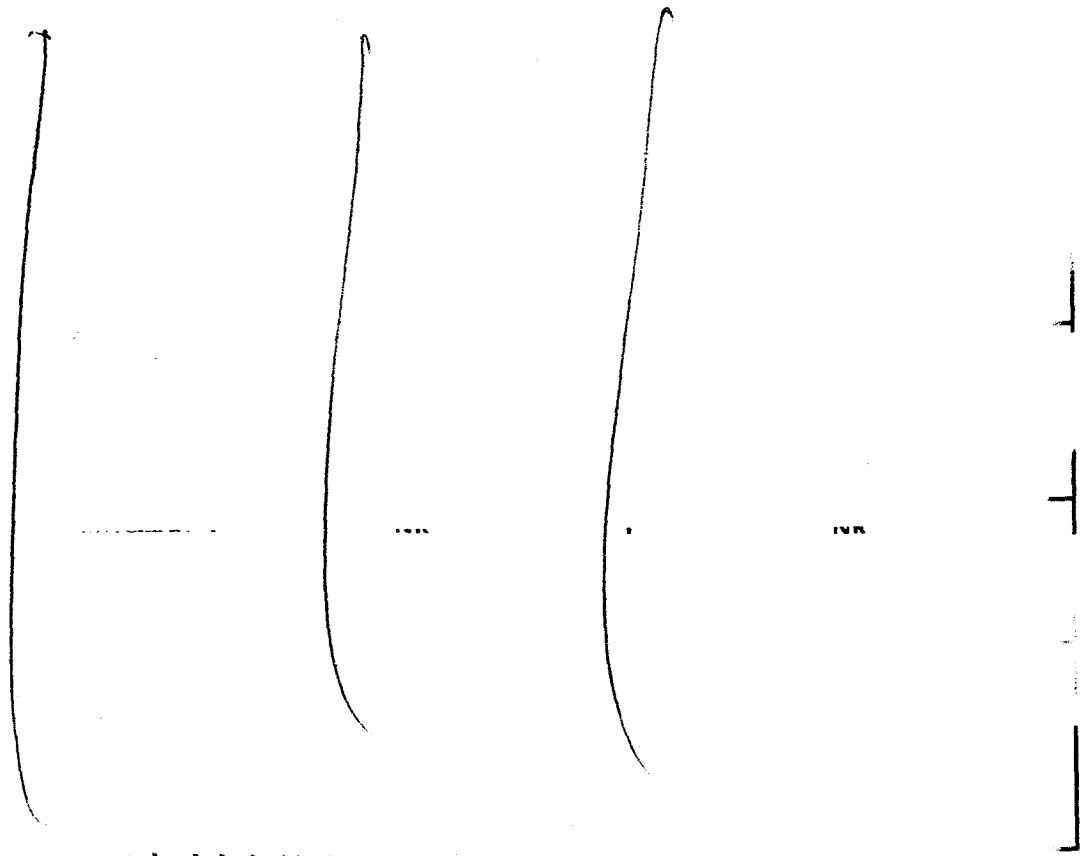
appropriate for a parallel design. The plasma concentration data were normalized to a dose of 1.5 gm prior to analysis.

A comparison between subjects with mild renal impairment (Cl_{CR}<80 ml/min) and subjects with normal renal function (Cl_{CR}>80 ml/min) was performed using ANOVA appropriate for a parallel design. The plasma concentration data were normalized to a dose of 1.5 gm prior to analysis.

Since only seven patients were greater than 60 years of age, no analysis was attempted examining the effect of age on the pharmacokinetics of BSZ and its metabolites.

Analytical Methods:

An



[a]BSZ values are reported as balsalazide disodium dihydrate.
*NR=not reported

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RESULTS:

Study Population and Demographics:

Fifty-four outpatients, generally in clinical and sigmoidoscopic remission (as evidenced by the last examination) were studied. Seventeen patients were studied twice, therefore, a total of 71 study visits were made by 54 patients.

Of the 54 patients who entered this study, 38 were receiving BSZ 3 gm/day, 6 were receiving 4.5 gm/day, and 10 were receiving 6 gm/day as divided bid doses. Sixteen of the 17 patients who were studied twice remained on the same dose at both visits. However, Patient 39 was taking 1.5 gm BSZ tid at the first visit; this constituted a protocol violation so he was excluded. At the second visit the dose had increased to 6 gm/day taken in two doses. This patient's pharmacokinetic data have only been included from the second study day.

Demographic data are summarized in Table 7. It should be remembered that patients were not randomized to treatment within this study.

Table 7. Summary of Demographic Data

Variable	Parameter	BSZ		
		3gm/day	4.5 gm/day	6 gm/day
Age (years)	N	38	6	10
	Mean (SD)	48 (10.7)	52 (12.5)	45 (9.1)
	Range	22-69	29-64	32-56
Gender	Male	19	2	8
	Female	19	4	2
	Total	38	6	10
Height (cm)	Mean (SD)	168 (8.7)	163 (10.1)	172 (7.1)
	Range	149-181	149-178	158-182
Weight (kg)	Mean (SD)	72 (13.6)	74 (11.5)	83 (9.1)
	Range	47-98	60-93	68-96
Does patient smoke?	No	35	6	10
	Yes	3	0	0
Alcohol consumption (units/week)	0	29	4	4
	1	3	1	3
	2	4	1	2
	3	2	0	1

The patients' ages ranged between 22 and 69 years. Patients receiving BSZ at a dose of 4.5 gm/day tended to be slightly older than the other patients, but this was probably a consequence of small sample numbers. The age ranges for each of the higher dose groups were within the range of those seen in the 3 gm/day dose group.

The overall gender ratio of the patients recruited to this study was 29:25 (M:F). The number of males and females receiving 3 gm/day was identical, slightly more female patients were receiving the 4.5 gm/day dose, while the 6 gm/day dose was mainly taken by male patients.

Patients' weights ranged from 47 to 98 kg and heights ranged from 149 to 182 cm. Those receiving 6 gm/day were heavier and taller than subjects receiving the other doses, probably because this group consisted mainly of male patients.

Seventeen patients drank alcohol, most of whom drank between 1 and 2 units per day. Only three patients smoked, all of whom were taking BSZ 3 gm/day. The amount of tobacco consumed averaged 15 gm/day for these 3 patients.

Current Medication for Ulcerative Colitis:
Three patients were prescribed medication for ulcerative colitis in addition to balsalazide. Patients 34 and 47 both took oral prednisolone and Patient 37 took codeine phosphate and folic acid.

Concomitant Medication for other conditions:
Eleven patients had other conditions which existed on or within one week of the study day that required concomitant medication. The most common conditions were cardiovascular in nature, and asthma. The cardiovascular conditions were treated with a variety of medications. The tables below list the medications taken for both ulcerative colitis and other conditions.

Listing of Current medication for Ulcerative Colitis other than Balsalazide

Hospital	Patient number	Drug name	Daily dose	Route	Start date
1	34	prednisolone	5mg	o	1990
1	37	codeine phosphate	90mg	o	1980
1	37	folic acid	5mg	o	1985
1	47	prednisolone	15mg	o	1992

Listing of Concomitant Medication

Hospital	Patient number	Drug name	Daily dose	Route	Start date	Indication
4	2	salbutamol	4 puffs	i	nk	asthma
4	2	ibuprofen	400mg	o	250693	pulled muscle in back
4	2	Augmentin	1125mg	o	050793	phlebitis (AE)
3	8	prednisolone	15mg	o	210191	asthma/bronchitis
3	8	salbutamol	15mg	i	111191	asthma/bronchitis
3	8	bendrofluazide	2.5mg	o	081292	high BP & oedema
1	11	metformin	2550mg	o	91	diabetes
1	12	Co-proxamol	6 tablets	o	90	headaches
1	14	amiloride 2.5mg/frusemide 20mg	1 tablet	o	0693	oedema associated with menopause
5	25	aspirin	150mg	o	070192	angina
5	25	atenolol	50mg	o	070192	angina
5	25	amlodipine	5mg	o	310392	angina
5	29	atenolol	50mg	o	300992	myocardial infarction
5	29	aspirin	150mg	o	300992	myocardial infarction
5	29	flecainide acetate	200mg	o	200493	myocardial infarction
5	30	Amoxycillin	750mg	o	260893	tooth abscess
1	37	quinine sulphate	300mg	o	85	cramp
1	37	aminophylline	450mg	o	90	asthma
1	37	Lasoride	1 tablet	o	91	hypertension
1	37	dihydrocodeine	30mg	o	0693	arthritis
1	37	glyceryl trinitrate	prn	sl	91	angina
2	40	bendrofluazide	1 tablet	o	260893	fluid in legs
2	43	cod liver oil	1 capsule	o	91	to improve health

*Note: o=oral, i=inhaled, sl=sublingual

Sigmoidoscopy:

The majority of patients had a sigmoidoscopy either on the eve of the study day or on the study day itself. Only Patients 41, 42 and 43 had sigmoidoscopy results recorded from an examination that occurred historically (up to 10 months prior to the study day). Most of the patients had "non-hemorrhagic" or normal findings at sigmoidoscopy. Friable mucosa was detected in seven patients.

Adverse Events:

Five adverse events were described for a total of 71 patient study visits. None of these events was thought to be related to BSZ. Four were probably related to study procedures (insertion of ——— in the patient's arm), and included phlebitis, hand bruising, and lightheadedness. The fifth patient became breathless but had a history of asthma. One patient was prescribed an antibiotic for phlebitis at the site of venepuncture, however, none of the remaining events

required any treatment. There were no serious adverse events.

Analytical Methods:
Assay validation for plasma and urine at the lower limits of the respective calibration curves was inadequate or inaccurate. Few of the assays analyzed QC samples at the respective LOQs. No intraday precision or accuracy data were reported for any of the compounds in either biological matrice. Results of recovery determinations and stability testing, if undertaken, were also not reported.

The plasma assay for BSZ exhibited poor precision at the lower calibration range, and numerous individual samples in the QC runs gave unacceptable accuracy (greater than ±20%). Many of the study samples required dilution to bring them within the calibration curve range for the compound under investigation. Inadequate measures were undertaken to ensure the precision and accuracy of these dilution steps; e.g., BSZ in plasma and urine and NABA in plasma.

Several chromatograms were difficult to interpret due to interfering peaks. In addition, most of the chromatograms contained peaks for the different analytes which were off-scale for the urine determinations.

Pharmacokinetic Results:
In all cases, values are reported as means±SD. Ranges are included in parentheses and are provided because of the high variability in the data. All concentrations that were below assay LOQs were reported as zero and included in calculations. BSZ values are now reported as the free acid (given as the disodium dihydrate for assay validation studies). The plasma concentration vs time curves for most of the analytes exhibited erratic elimination profiles rather than an exponential decline. As a result, the calculation of pharmacokinetic parameters was restricted to Cmin, Cmax, Tmax and AUC_{0-12} for plasma and % recovery in urine, plus renal clearance over 12 hours for urinary data. Mean plasma concentration vs time profiles for BSZ and all metabolites can be located in the Appendix.

Tables 8-12 list the PK parameters as determined from the plasma and urine analysis of BSZ and its metabolites in all patients after the initial visit to the clinic.

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Table 8. BSZ PK Parameters*						
	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-12} (ng*hr/ml)	Percent recovered	Cl_R (L/hr)
Dose A (1.5 gm BSZ bid)	12.1±14.1	131±52.6	1.2±0.5	481±227	0.2±0.2	5.2±3.2
Dose B (2.25 gm BSZ bid)	23.8±15.8	224±124	1.2±0.4	825±374	0.2±0.1	3.8±1.4
Dose C (3 gm BSZ bid)	15±5.3	142±35	1.6±0.9	713±236	0.1±0.1	4.5±1.3
p values from ANOVA	0.427	**0.008**	0.237	0.192	0.337	0.492

*N=30-38 for Dose A, N=6 for Dose B, and N=9 for Dose C.

BSZ was detected in nearly all of the pre-dose plasma samples. Cmax was reached at about 1-2 hours in the majority of subjects. None of the plasma PK parameters were statistically different except for the Cmax value at the intermediate dose; this may be a function of the small number of subjects in this group. There were no significant differences in the AUC_{0-12} values, indicating that the total exposure was not dose-proportional, although this conclusion is tenuous as many of the individual BSZ plasma concentration vs time profiles were erratic and difficult to define. As in other studies, urinary recovery of BSZ was very limited and was <1% in virtually every individual.

Table 9. ASA PK Parameters*						
	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-12} (ng*hr/ml)	Percent recovered	Cl_R (L/hr)
Dose A (1.5 gm BSZ bid)	340±236	676±405	5.1±3	6010±3481	2.3±2.9	1.4±1.7
Dose B (2.25 gm BSZ bid)	755±320	1025±412	5.3±2.7	10864±4609	3.7±2.4	2.4±1.8
Dose C (3 gm BSZ bid)	548±270	889±476	4.5±3.1	8316±4164	3.8±3.1	4.6±5.4
p values from ANOVA	0.151	0.213	0.852	0.163	0.239	**0.014**

*N=33-38 for Dose A, N=6 for Dose B, and N=8-10 for Dose C.

ASA was quantifiable in virtually all of the pre-dose plasma samples. The mean Cmin, Cmax, and AUC_{0-12} were highest for the intermediate dose, but any differences between the groups were not significant. For all doses, the plasma ASA concentration vs time curves exhibited either erratic or plateau profiles, with Cmax values that were only marginally higher than the pre-dose concentrations. Although the mean urinary recoveries were higher for the intermediate and high

dose groups, the difference did not achieve significance. There did, however, appear to be a trend for the renal clearance to increase with dose, from relatively low values at the low and intermediate doses, to rates approaching the creatinine clearance at the top dose. These differences did achieve statistical significance, but the relatively low number of subjects in the intermediate and high dose groups as compared to the low dose group may have affected the outcome of the analysis.

Table 10. NASA PK Parameters*

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-12} (ng*hr/ml)	Percent recovered	Cl_R (L/hr)
Dose A (1.5 gm BSZ bid)	710±344	1065±433	5.0±2.7	10535±4459	15.3±9.0	10±3.7
Dose B (2.25 gm BSZ bid)	1242±309	1507±360	4.5±3.2	16847±3887	14.5±4.9	8.8±2.2
Dose C (3 gm BSZ bid)	1031±371	1427±468	3.1±3.1	14234±4519	12±4.9	12.8±9.4
p values from ANOVA	0.133	0.064	0.215	0.062	0.513	0.262

*N=33-38 for Dose A, N=6 for Dose B, and N=9-10 for Dose C.

All the pre-dose samples contained measurable levels of NASA. As for ASA, the mean Cmin, Cmax, and AUC_{0-12} were highest for intermediate dose, but the differences between the groups were not significant. For all doses the plasma NASA concentration vs time curves exhibited plateau profiles, with Cmax values that were only marginally higher than the pre-dose concentrations. No statistically significant differences were observed for urinary recovery or renal clearance values. As in previous studies with healthy volunteers (#20060 and #20061), mean Cl_R values exceeded the creatinine clearance.

Table 11. ABA PK Parameters*

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-12} (ng*hr/ml)	Percent recovered	Cl_R (L/hr)
Dose A (1.5 gm BSZ bid)	6.5±12.4	20.5±36	1.7±2.7	133±232	0.03±0.15	0
Dose B (2.25 gm BSZ bid)	0	10.8±16.9	2±3.1	59±93	0	0
Dose C (3 gm BSZ bid)	0	25±38.1	1.3±2.2	74±143	0	0
p values from ANOVA	0.141	0.662	0.880	0.321	0.720	-

*N=33-38 for Dose A, N=6 for Dose B, and N=8-10 for Dose C.

Of the 54 patient profiles examined for ABA, 30 exhibited concentrations which were below the assay LOQ (20 ng/ml) for all of the samples collected. This makes comparison of PK parameters between doses difficult, as only 2 subjects from the Dose B group and 3 subjects from the Dose C group had quantifiable levels of ABA in plasma. In the cases where the levels of ABA were apparently measurable, most plasma ABA concentration vs time curves were either erratic or at a plateau. As these patients were on comedication and the method used to quantify ABA in plasma (UV absorption at 260 nm) was relatively non-specific, the few higher results could be artifacts arising from a lack of assay specificity. ABA was detected in the urine of only 2 subjects who received Dose A.

Table 12. NABA PK Parameters*

	C_{min} (ng/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-12} (ng*hr/ml)	Percent recovered	Cl_R (L/hr)
Dose A (1.5 gm BSZ bid)	163±173	296±263	4.2±3	2502±2448	6.7±8.6	17.6±10.5
Dose B (2.25 gm BSZ bid)	418±256	774±426	2.4±2.6	6691±3814	11.1±5.2	22.8±10.3
Dose C (3 gm BSZ bid)	273±220	717±726	1.9±2.1	5825±5650	11.8±10.1	48.1±51.6
p values from ANOVA	0.338	0.217	0.092	0.321	0.169	**0.010**

*N=30-38 for Dose A, N=5-6 for Dose B, and N=7-10 for Dose C.

Although the specificity of the detection method for NABA (UV absorption at 260 nm) was the same as that for ABA, the substantially higher plasma and urine concentrations meant that any effect of non-specific interference from comedication was likely to be less marked. Thus, virtually all the pre-dose plasma samples contained measurable levels of NABA. As for BSZ, ASA, and NASA, the mean Cmin, Cmax, and AUC_{0-12} were highest for the intermediate dose, but the differences between the groups did not achieve statistical significance at the 5% level. All groups exhibited erratic or plateau plasma concentration vs time profiles, with Cmax values which were little higher than the pre-dose concentrations.

The renal clearances increased significantly with higher doses, however, the smaller number of subjects in the intermediate and high dose groups may have confounded the results. As with NASA, mean Cl_R values exceeded normal creatinine clearance values. In any case, the data show that the patients in this study retained a high capacity to clear the acetylated metabolite of ABA in the kidneys, thus minimizing the systemic exposure to the carrier portion of BSZ.

One of the major flaws in the design of this study bears discussion. Blood samples were obtained for only 8 hours after a dose of BSZ, however, AUC determinations for BSZ and all of the metabolites were calculated for a span of 12 hours. Instead of extrapolating drug and

metabolite plasma concentrations from 8 hours to 12 hours using the elimination rate constant, determinations of AUC_{0-12} were made by setting the 12-hour plasma concentration equal to the predose trough concentration. In many subjects, predose plasma concentrations of BSZ and the metabolites were much greater than that observed at 8 hours or even earlier. Therefore, estimations of AUC_{0-12} made in this manner could be overestimated, and this should be kept in mind when interpreting the AUC results in this study. Since renal clearance is determined in part by using the AUC (Au/AUC), many values for this parameter would be underestimated. Again, it should be noted that there were significantly less subjects included in the Dose B and Dose C groups (2.25 gm bid and 3.0 gm bid, respectively) than in Group A.

Patients Studied Twice

There were no statistically significant differences in any of the PK parameters for any of the analytes between the first and second patient visits, however, the caveats listed in the study above apply to the data in this study as well.

Subgroup Analyses of PK Parameters

Two subgroup analyses were performed on the data. One of the original objectives was to determine the effect of renal impairment; gender effects were examined as well. These analyses were performed on the PK data for BSZ, ASA, NASA, ABA, and NABA. Some caution should be used in the interpretation of these analyses, as the sample of patients included were not randomized for renal impairment or gender. Furthermore, groups were not balanced for number of subjects.

Gender

Results of the ANOVA for the plasma and urinary PK parameters comparing females and males are provided in Table 13. The values include subjects from all three dose groups and are dose-normalized to 1.5 gm BSZ.

Table 13. PK parameters from results of gender analysis.[a,b]

	BSZ		ASA		NASA		ABA		NABA	
	M	F	M	F	M	F	M	F	M	F
Cmin (ng/ml)	8.6[c] (5.9)	21.4 (30.8)	323 (216)	415 (234)	635 (313)	751 (313)	3.9 (11.5)	5.1 (9.7)	153 (160)	257 (262)
p value[d]		**0.043**		0.166		0.206		0.693		0.104
Cmax (ng/ml)	97.3 (40)	156 (62.6)	505 (249)	825 (397)	865 (333)	1144 (428)	19 (40.4)	15.4 (15.7)	314 (266)	413 (355)
p value		**<0.001**		**0.002**		**0.014**		0.698		0.281
Tmax (hr)	1.3 (0.6)	1.3 (0.5)	4.6 (3.1)	5.7 (2.6)	4.2 (3.1)	5.4 (2.4)	0.9 (1.8)	2.5 (3.1)	3.9 (3.2)	3.2 (2.7)
p value		0.940		0.198		0.127		**0.035**		0.394
AUC_{0-12} (ng*hr/ml)	389 (136)	570 (263)	4909 (2677)	7366 (3338)	8815 (3844)	11382 (4092)	107 (244)	99 (135)	2289 (1903)	3870 (3658)
p value		**0.004**		**0.008**		**0.030**		0.889		0.076
Percent excreted	0.14 (0.07)	0.22 (0.17)	2.7 (3.4)	2.7 (2.3)	14.9 (9.6)	14.3 (5.9)	0.04 (0.15)	0.03 (0.14)	9.1 (10.1)	7.0 (6.9)
p value		**0.022**		0.997		0.800		0.760		0.385
Cl_R (L/hr)	4.8 (2.9)	4.9 (2.6)	2.6 (3.4)	1.7 (1.5)	11.3 (5.9)	9.2 (3.8)	0 -	0 -	26.9 (31.9)	18.7 (11.1)
p value		0.886		0.273		0.148		-		0.270

[a]All values were normalized to a dose of 1.5 gm.
[b]N was inconsistent and varied from 19-29 for the different values.
[c]Values are presented as means±(SD).
[d]Results of ANOVA comparing dose effects in males vs females.

There were statistically significant gender effects for Cmax and AUC_{0-12} for BSZ, ASA, and NASA and in Cmin for BSZ, with females exhibiting greater values than males. There were also significantly greater quantities of BSZ recovered in the urine of females. In general, plasma PK parameters were greater for all analytes in females, even though some of the values did not achieve statistical significance. However, these data could not be analyzed for normalized body weight as patient body weights were unavailable.

Renal Impairment

One of the objectives of this study was to look at the effects of renal impairment on PK parameters. To meet this objective, patients were subdivided into mildly impaired (Cl_{CR}<80 ml/min) and normal (Cl_{CR}≥80 ml/min). The two groups were compared with ANOVA. Creatinine clearance was not available for seven patients so these were excluded from any analyses. There were eighteen patients with Cl_{CR}<80ml/min (6M and 12F); the mean Cl_{CR} for this group was 64 ml/min (range=43-78 ml/min). There were 29 patients with Cl_{CR}≥80ml/min (20M and 9F); the mean Cl_{CR} in this group was 104 ml/min (range=80-163 ml/min). The imbalance of males and females confounds the effects to some extent, especially where a gender effect and/or dose effect has been suggested. Not all combinations of gender, dose and renal impairment were examined, therefore, the results must be treated with caution. Subjects from all three dose groups were included and the results dose-normalized to 1.5 gm. None of the PK parameters (Cmin, Cmax, Tmax, AUC_{0-12}, % recovered in urine, and Cl_R) displayed statistically significant differences at a p value of 0.05. The only exception was the Cmin for ABA, with mildly impaired subjects exhibiting greater values (p=0.014). Cmax and AUC_{0-12} values for ABA were not significantly different, however (p=0.886 and 0.280, respectively).

Duration of Treatment

Patients had been using BSZ treatment on average for 3.25 years (with a range of 9 months to 5.5 years), although the dose may have changed during this time for seven patients. It was possible that continued use of BSZ might have an effect on pharmacokinetic parameters. Correlation coefficients were calculated for each analyte for both the plasma PK parameters and the urine parameters (except for ABA since most values were <LOQ) to measure the association with duration of treatment. No linear association between duration of treatment and any PK parameter for any analyte was observed.

SUMMARY:

PK Parameters for Plasma and Urine

An initial evaluation of the comparison within patients showed no significant difference in the results for the first and second visits, however the PK parameters for BSZ, ASA, and NASA were generally more variable than those for NABA, which tended to remain fairly constant on both occasions.

BSZ was detected in almost all of the pre-dose plasma samples. After dosing, there was rapid absorption to give peak plasma concentrations within two hours. Urinary excretion of intact drug was low (<1%) for all subjects and average clearance values were relatively high (~75ml/min), suggesting that systemic exposure should remain low in most subjects.

ASA was quantifiable in virtually all of the pre-dose plasma samples. For all doses the plasma concentration vs time curves exhibited either erratic or plateau profiles. Renal clearance appeared to increase with dose, from relatively low values at the low and intermediate doses, to rates approaching the creatinine clearance at the top dose.

NASA was measurable in all of the pre-dose plasma samples. For all doses, plasma concentration vs time curves exhibited erratic or plateau profiles. Mean Cl_R values (~ 160ml/min) exceeded normal creatinine clearances for all three dosing regimens.

The majority of patient plasma profiles (30 out of 54) contained concentrations of ABA which were below the assay LOQ. In the cases where the levels of ABA were apparently measurable, most profiles showed a plateau plasma concentration vs time curve, generally within the range of 20-50 ng/ml.

All of the pre-dose plasma samples contained measurable levels of NABA. For all doses the concentration vs time curves exhibited erratic or plateau profiles. Cl_R values increased with higher doses and exceeded normal creatinine clearance values. The results indicate that the patients retained a high capacity to clear the acetylated metabolite of ABA in the kidneys, thus minimizing systemic exposure to the carrier moiety.

Of interest is the observation in most of the patients of greater trough concentrations for BSZ in the morning prior to dosing, as compared to plasma concentrations obtained 6 to 8 hours after the morning dose. This could be due to diurnal variation in BSZ absorption; i.e., slower gastrointestinal transit during the night-time hours would allow more time for absorption of the evening dose, resulting in trough plasma concentrations which were higher in the morning as compared to the evening hours.

In general, there did not appear to be any dose-dependency for any of the compounds based on the Cmin values obtained during the three dosing regimens. AUC values were difficult to compare due to the method used to calculate them. Furthermore, the erratic plasma vs concentration profiles observed for most of the compounds did not allow for any half-life determinations. Urinary parameters must be viewed with caution as patients were delegated to their own urine collection. It should also be remembered that a number of the subjects were taking concomitant medication, which could decrease assay sensitivity and/or be a source of potential drug interactions. Finally, the different dosing regimen groups were not balanced for number of subjects.

Sub-group Analysis of Pharmacokinetic Parameters:

Gender:

There were significant gender effects observed for Cmax and AUC_{0-12} for BSZ, ASA, and NASA with females exhibiting greater values than males. More BSZ was also recovered in the urine of females. There were no other gender effects noted in urine parameters. It should be remembered, however, that the data was not analyzed for normalized body weight.

Renal Impairment:

None of the dose-normalized PK parameters in normal vs mildly impaired patients were significantly different for any analyte.

It should be remembered that the patients included in these analyses were not randomized for renal impairment or gender. In addition, the numbers of subjects in each study were not balanced.

CONCLUSION:

It was hypothesized that the potential for absorption of BSZ might be different in patients with ulcerative colitis, and that these patients were likely to have differences in renal function. This study, therefore, investigated the pharmacokinetics of BSZ and its metabolites in patients on maintenance therapy.

Overall, patients were exposed to low systemic concentrations of BSZ. Consistent with the low plasma levels, the mean recoveries of BSZ in urine were <1% for all three dose levels. The mean Cl_R values were also relatively high (~75ml/min) despite the inclusion of a number of subjects with comparatively low creatinine clearance values. These results suggest an efficient renal excretion for the intact drug.

Most of the metabolites exhibited plasma concentration vs time profiles which were erratic or at a plateau. The systemic exposure to the "carrier" metabolite (ABA) was low in the vast majority of patients. ABA and ASA were recovered primarily as their N-acetylated metabolites in urine. The renal clearances of NASA and NABA exceeded normal values for creatinine clearance at all dosage regimens studied.

Patients in this study had received treatment with BSZ for periods ranging from 0.75 to 5.5 years. No association was apparent between the PK parameters for any analyte and duration of treatment. Since no fecal recoveries were determined, it is difficult to assess the release of ASA in the colon, however, long-term use of BSZ appears to be well-tolerated.

APPENDIX

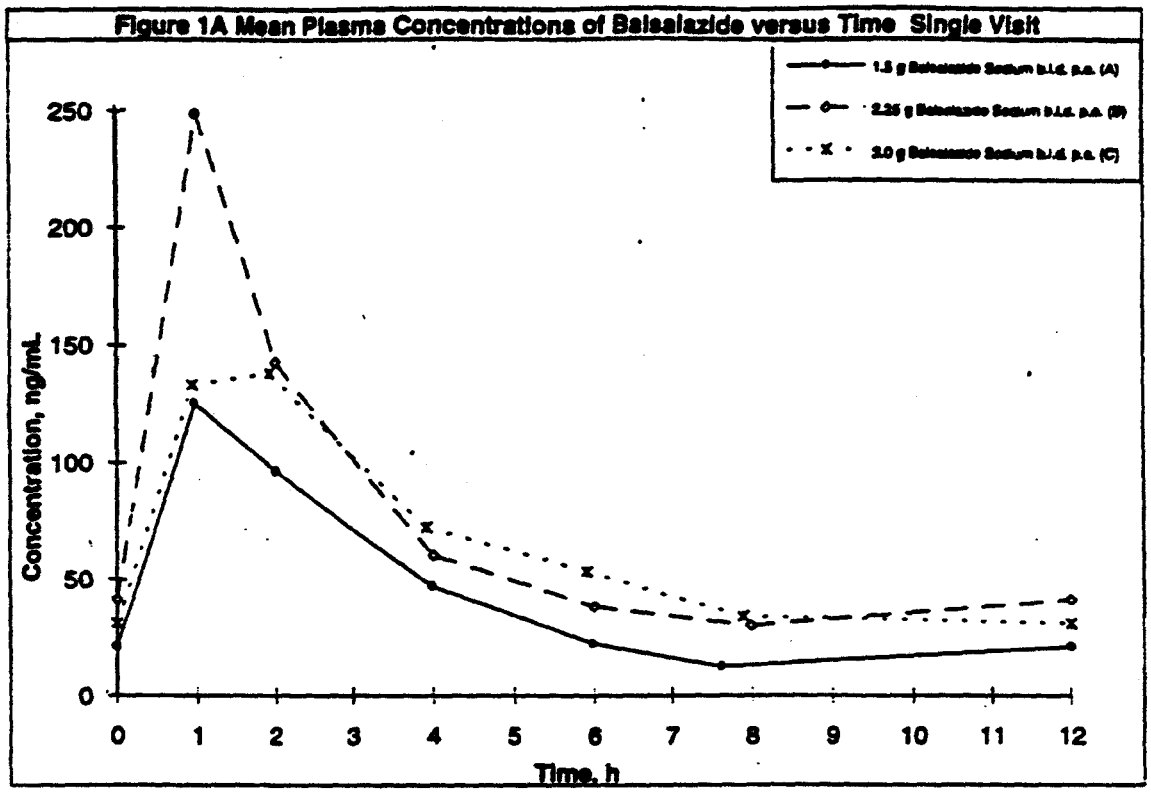


Figure 1A Mean Plasma Concentrations of Balsalazide versus Time Single Visit
1.5 g Balsalazide Sodium b.i.d. p.o. (A)
2.25 g Balsalazide Sodium b.i.d. p.o. (B)
3.0 g Balsalazide Sodium b.i.d. p.o. (C)
Concentration, ng/mL
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Time, h

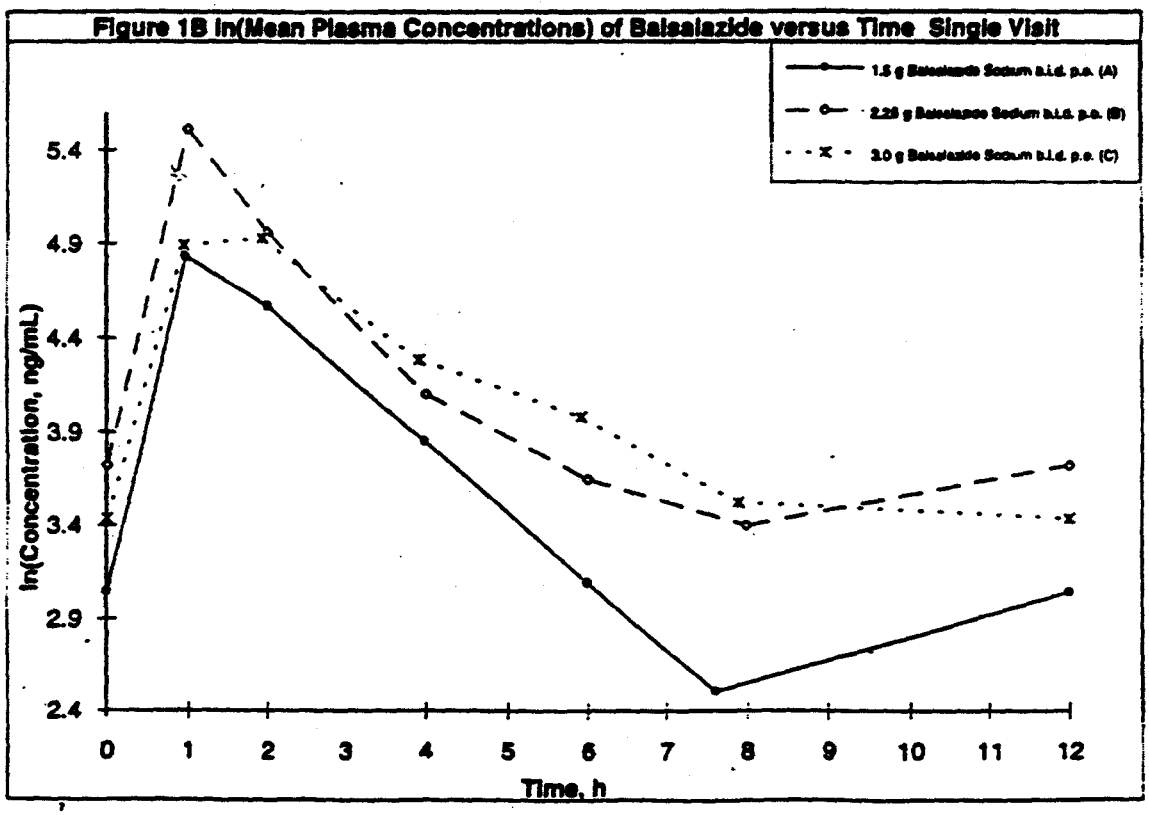


Figure 1B ln(Mean Plasma Concentrations) of Balsalazide versus Time Single Visit
1.5 g Balsalazide Sodium b.i.d. p.o. (A)
2.25 g Balsalazide Sodium b.i.d. p.o. (B)
3.0 g Balsalazide Sodium b.i.d. p.o. (C)
ln(Concentration, ng/mL)
5.4
4.9
4.4
3.9
3.4
2.9
2.4
0 1 2 3 4 5 6 7 8 9 10 11 12
Time, h

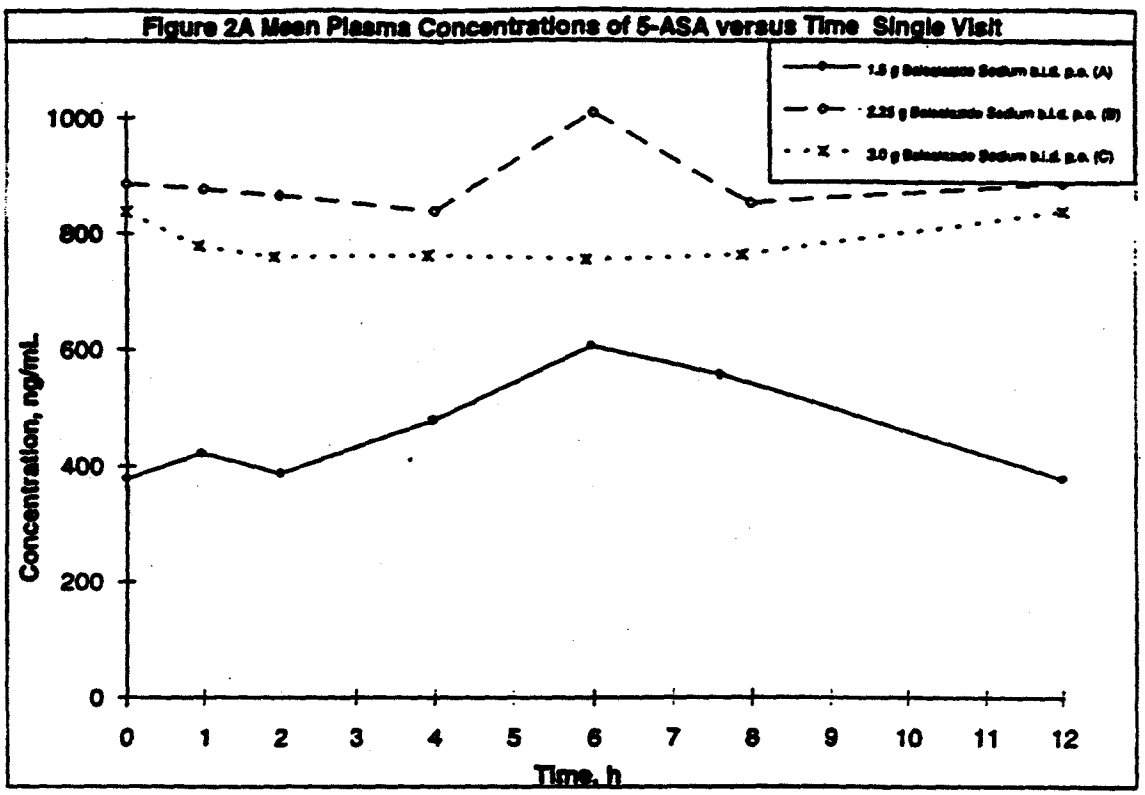


Figure 2A Mean Plasma Concentrations of 5-ASA versus Time Single Visit
1.5 g Balsalazide Sodium b.i.d. p.o. (A)
2.25 g Balsalazide Sodium b.i.d. p.o. (B)
3.0 g Balsalazide Sodium b.i.d. p.o. (C)
Concentration, ng/mL
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Time, h

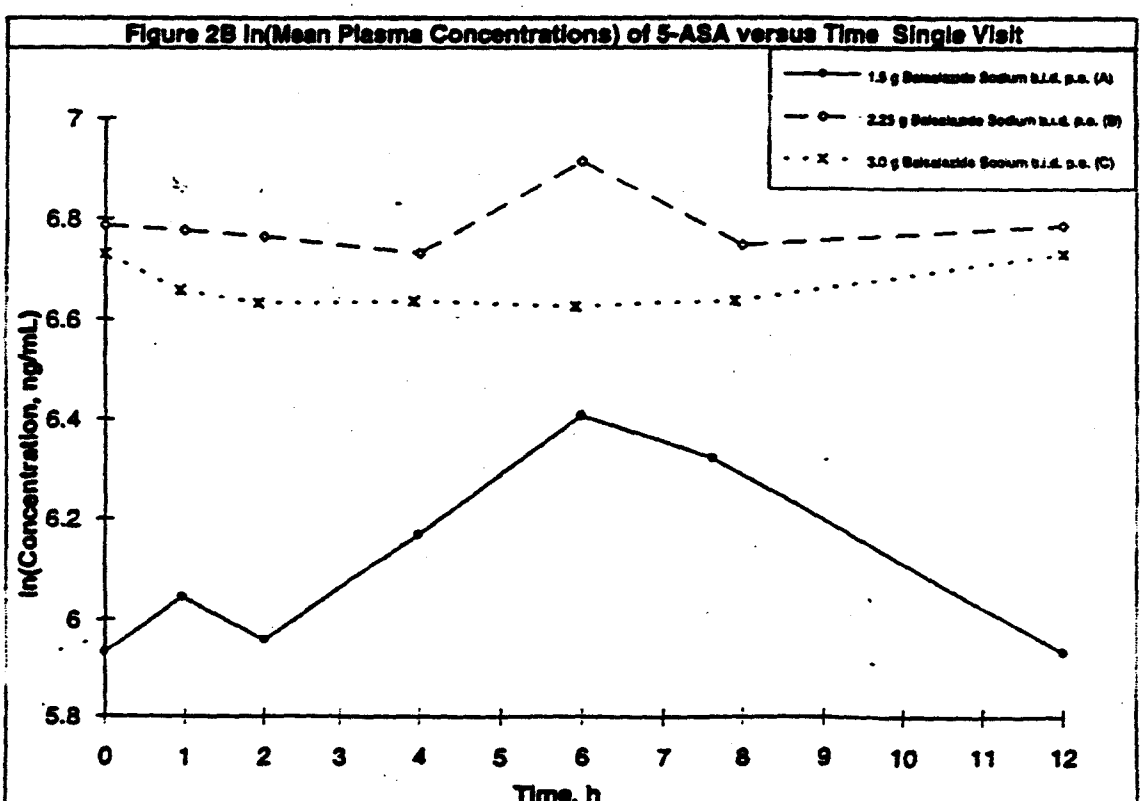


Figure 2B ln(Mean Plasma Concentrations) of 5-ASA versus Time Single Visit
1.5 g Balsalazide Sodium b.i.d. p.o. (A)
2.25 g Balsalazide Sodium b.i.d. p.o. (B)
3.0 g Balsalazide Sodium b.i.d. p.o. (C)
ln(Concentration, ng/mL)
7
6.8
6.6
6.4
6.2
6
5.8
0 1 2 3 4 5 6 7 8 9 10 11 12
Time, h

1.065 028

Figure 3A Mean Plasma Concentrations of NASA versus Time Single Visit

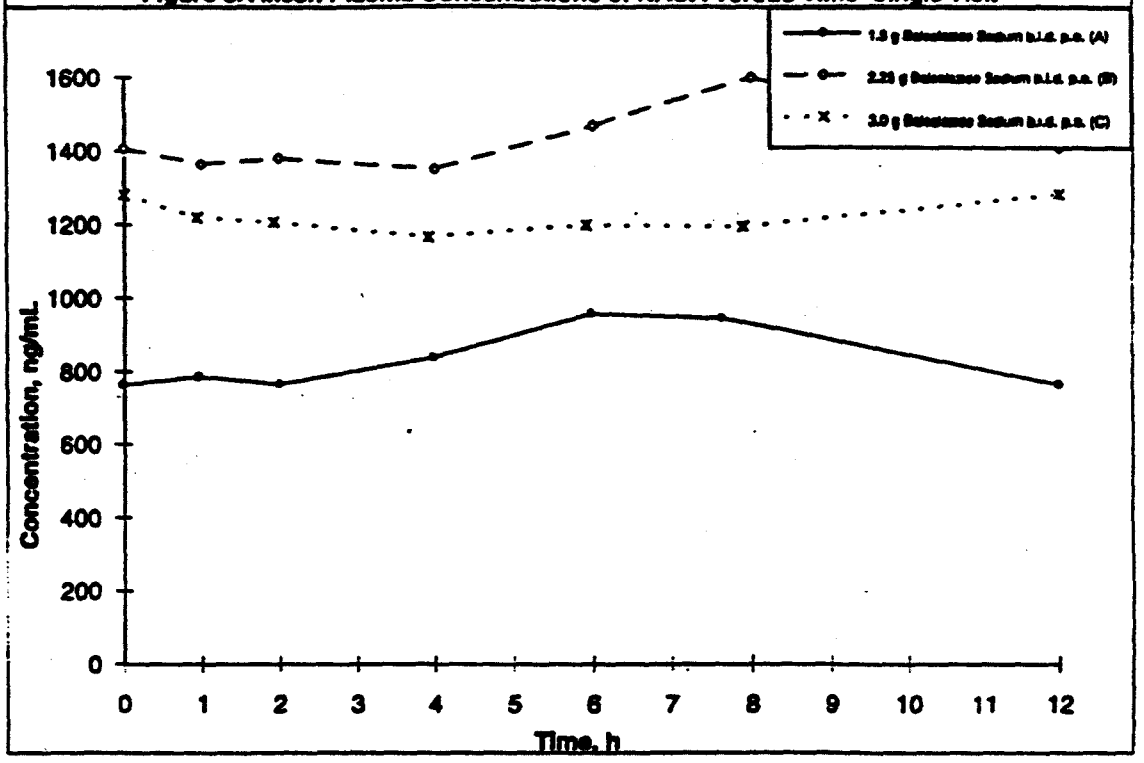
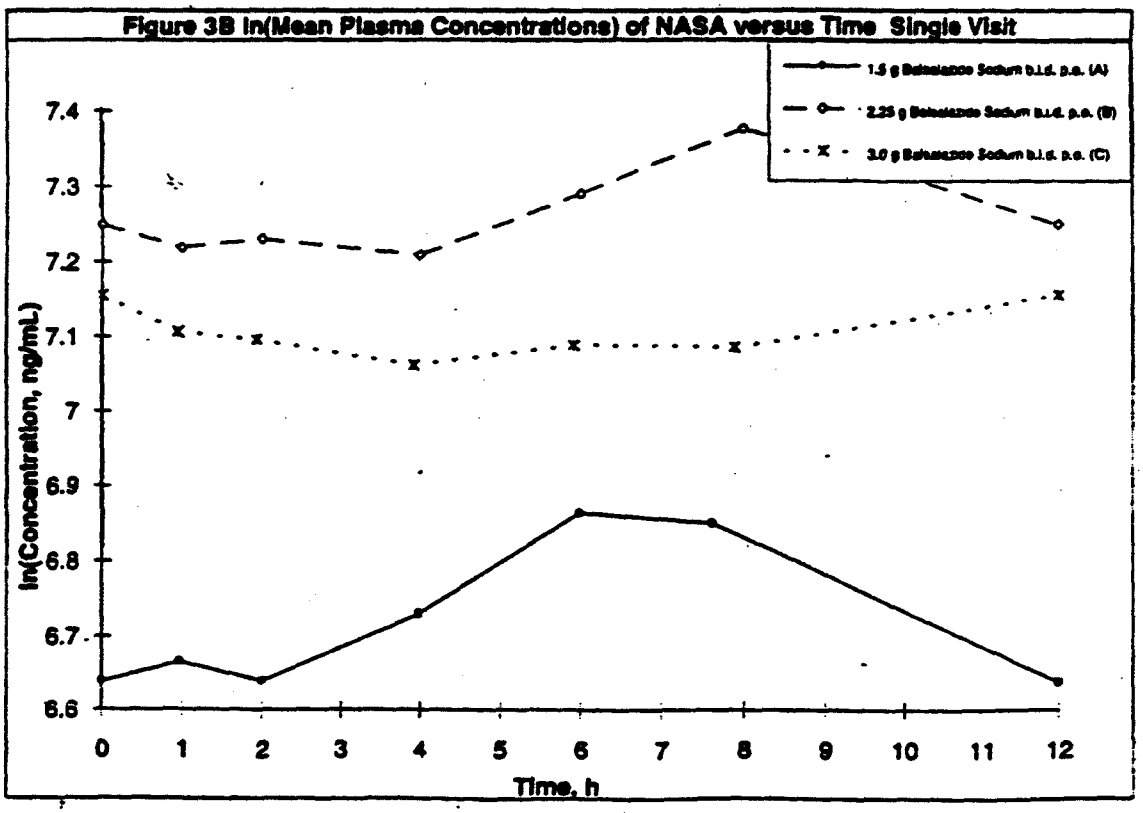


Figure 3B ln(Mean Plasma Concentrations) of NASA versus Time Single Visit



1.065 029

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Figure 4A Mean Plasma Concentrations of 4-ABA versus Time Single Visit

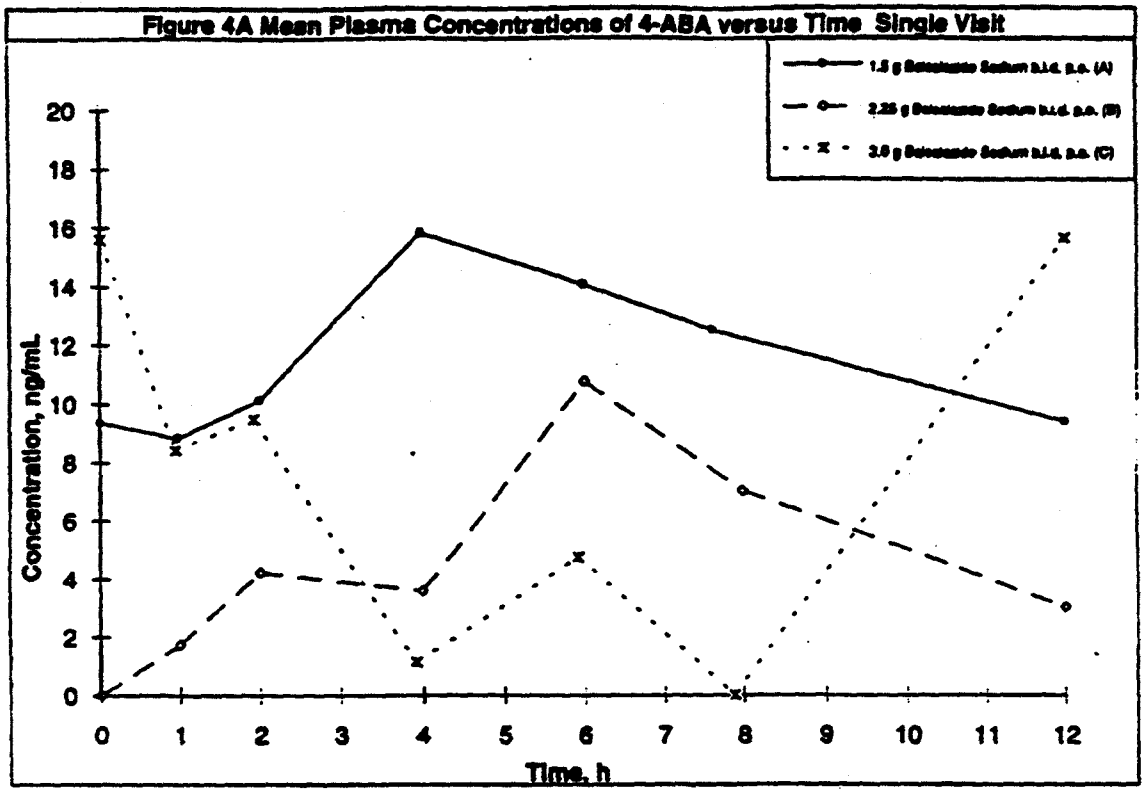
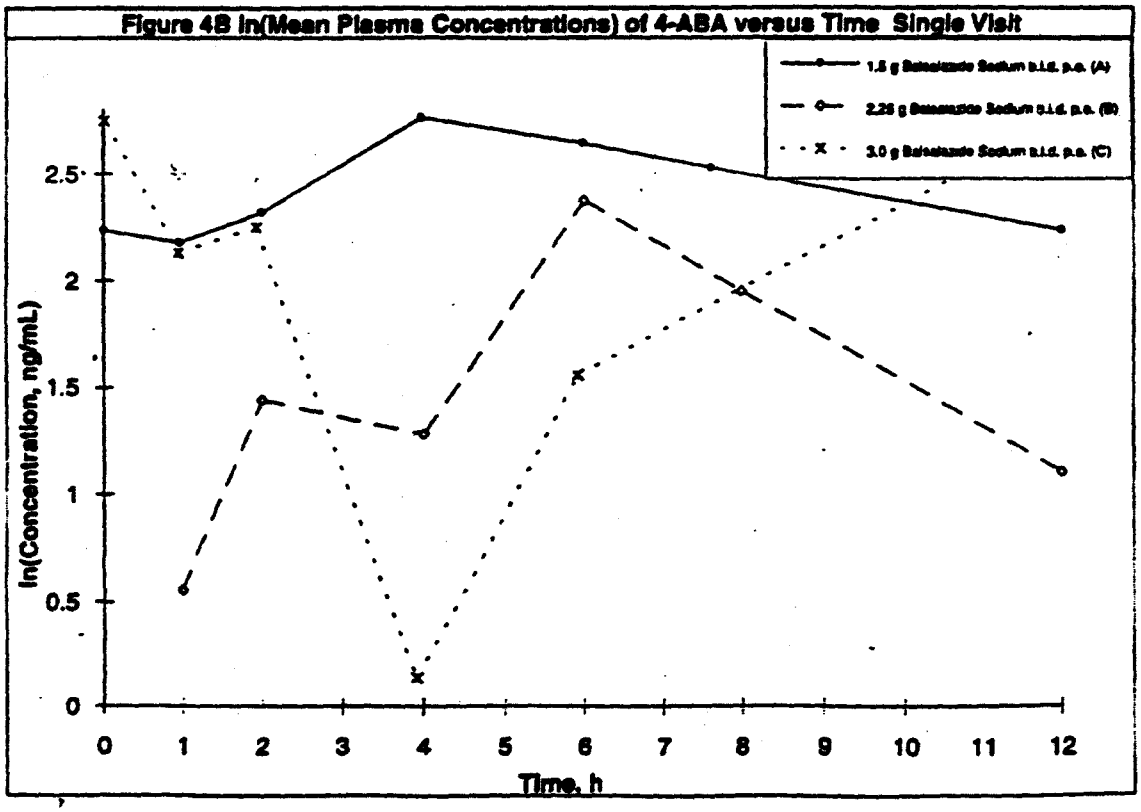


Figure 4B ln(Mean Plasma Concentrations) of 4-ABA versus Time Single Visit



1.065 030

Figure 5A Mean Plasma Concentrations of NABA versus Time Single Visit

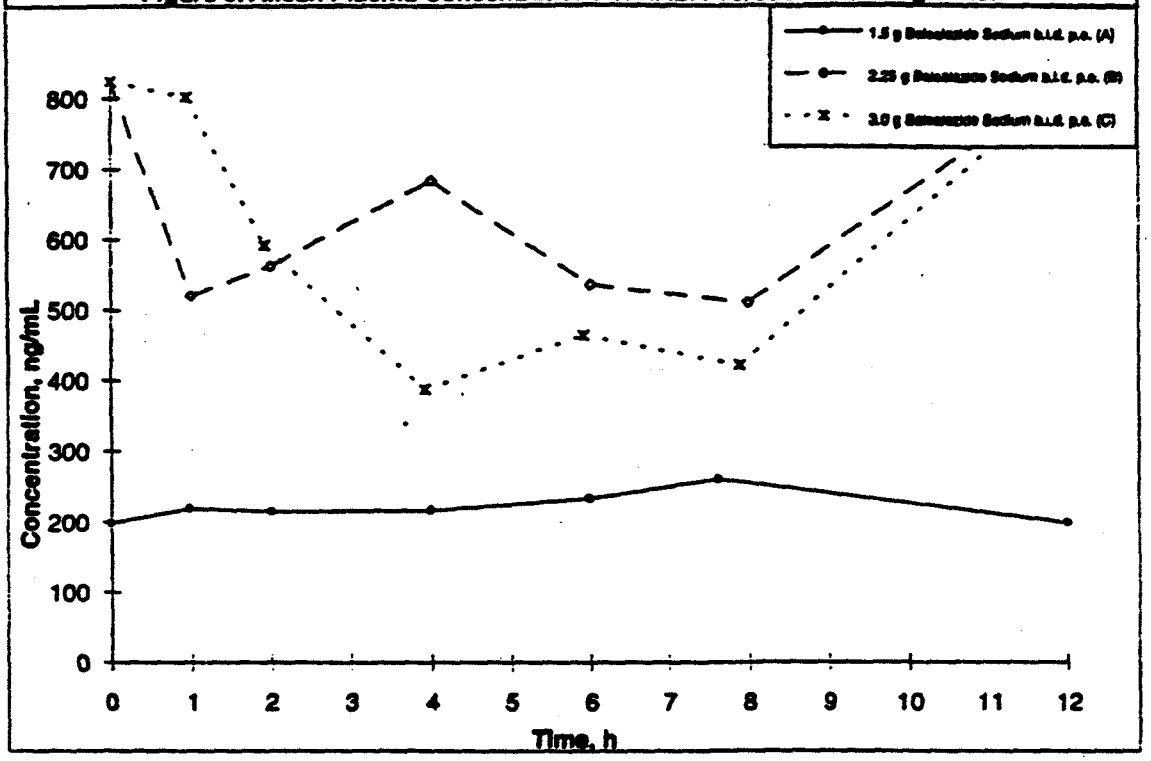
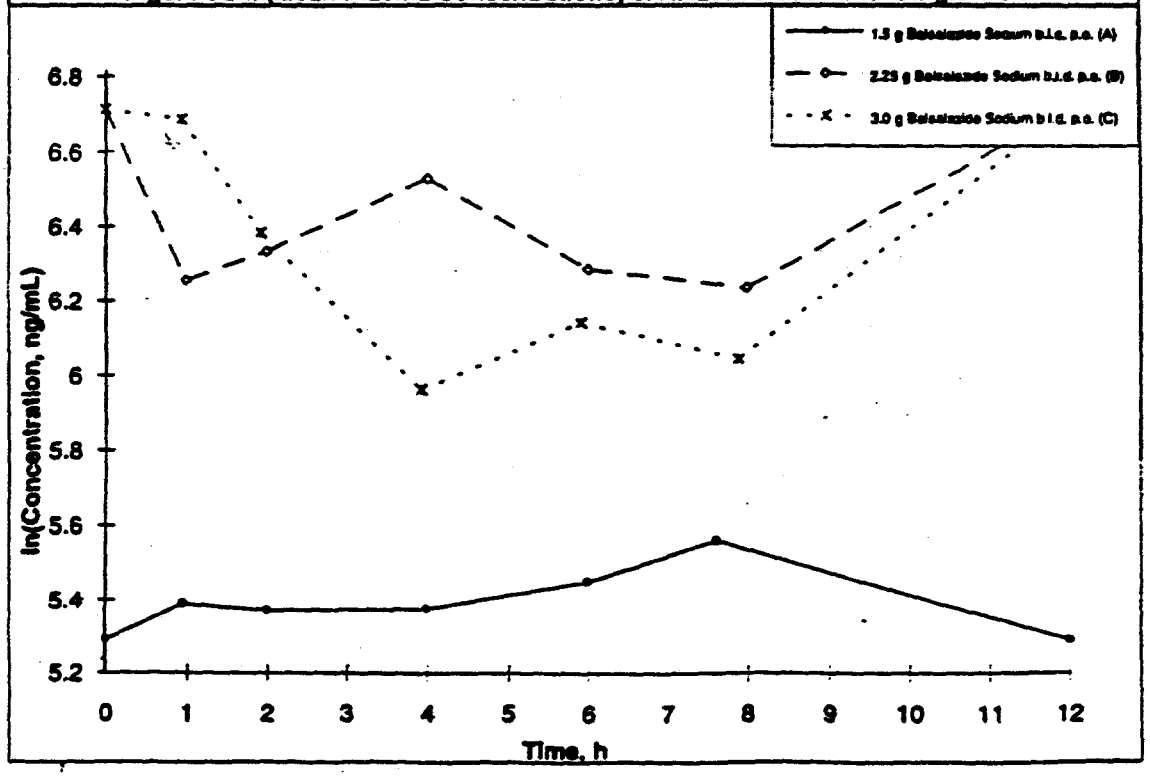


Figure 5B ln(Mean Plasma Concentrations) of NABA versus Time Single Visit



1.065 031

APPENDIX II

1. **Formulation/Manufacturing Information**

2. **Results of Dissolution Testing**

3. **Assay Validation Consult**

—) Formulations Used in Human Pharmacokinetic and Clinical Studies for Acute Ulcerative Colitis

Study Number	Lot Number	Dosage Form and Strength	Batch Size (capsules)	Manufacturer (Date Mfd)	Formulation
20060	7H467	750 mg capsules		Biorex (8/92)	Formulation A[1]
20061	7H467	750 mg capsules		Biorex (8/92)	Formulation A
GLY01/93	2501H	750 mg capsules			Formulation B[2]
	2863H	750 mg capsules			Formulation B
	2935H	750 mg capsules			Formulation B
	0642J	750 mg capsules			Formulation B
	4178J	750 mg capsules			Formulation B
CP069101	M6272R02	750 mg capsules		Anabolic (5/93)	Formulation B
CP099301	N6272B01	750 mg capsules		Anabolic (3/94)	Formulation B
57-3001	0863J	750 mg capsules			Formulation B
	1007J	750 mg capsules			Formulation B
	1048J	750 mg capsules			Formulation B
	1150J	750 mg capsules			Formulation B
	1219J	750 mg capsules			Formulation B
0028/011	262034	750 mg capsules		Biorex (6/88)	Formulation A
	262040	750 mg capsules		Biorex (10/88)	Formulation A
	262041	750 mg capsules		Biorex (11/88)	Formulation A
	262047	750 mg capsules		Biorex (11/88)	Formulation A
0028/017	262034	750 mg capsules		Biorex (6/88)	Formulation A
	262041	750 mg capsules		Biorex (11/88)	Formulation A
	262047	750 mg capsules		Biorex (11/88)	Formulation A

[1] Formulation A contains 750 mg balsalazide disodium — ng colloidal silicon dioxide and — ng magnesium stearate

[2] Formulation B contains 750 mg balsalazide disodium — mg colloidal silicon dioxide and — magnesium stearate, the formulation proposed for marketing

Fig.1

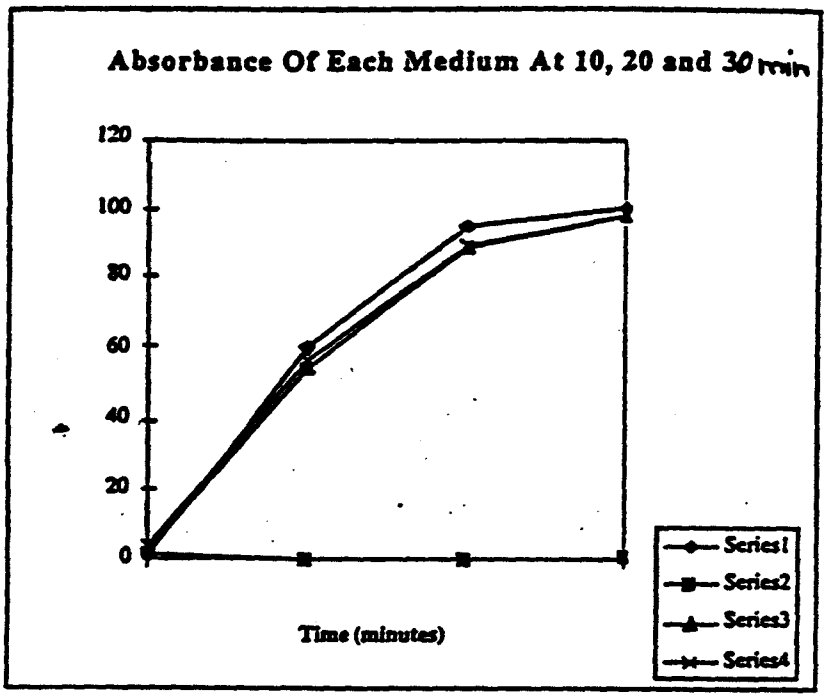


Fig.1
KEY : Series 1 Water (deionized)
Series 2 0.1M Hydrochloric Acid
Series 3 Phosphate buffer pH 7.4
Series 4 Phosphate buffer pH 6.4

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a) Dissolution in Water (deionized)

	Absorbance	% Dissolved	Average
After 10 minutes	0.506		
	0.656		
	0.523		
	0.634		
	0.524		
	0.610		60.79
After 20 minutes	0.873		
	0.964		
	0.972		
	0.904		
	0.809		
	0.896		95.38
After 30 minutes	0.923		
	0.965		
	1.015		
	0.972		
	0.889		
	0.932		100.27
Ref Std	0.818		

Reference standard contains 28.8 mg Balsalazide Sodium

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d) Phosphate buffer pH 6.4

	Absorbance	% Dissolved Average	
After 10 minutes	0.571		
	0.537		
	0.604		
	0.504		
	0.576		
	0.550		
After 20 minutes	0.913		
	0.912		
	0.892		
	0.835		
	0.838		
	0.825		
After 30 minutes	0.970		
	0.964		
	0.943		
	0.969		
	0.951		
	0.948		
Ref Std	0.874		

Reference standard contains 29.9 mg Balsalazide Sodium

c) Phosphate buffer pH 7.4

	Absorbance	% Dissolved	Average
After 10 minutes	0.552		
	0.273		
	0.540		
	0.590		
	0.606		
	0.540		
After 20 minutes	0.796		
	0.729		
	0.852		
	0.820		
	0.950		
	0.905		
After 30 minutes	0.887		
	0.977		
	0.915		
	0.911		
	0.957		
	0.910		
Ref Std	0.843		

Reference standard contains 29.7 mg Balsalazide Sodium

b) 0.1M Hydrochloric Acid

	Absorbance	% Dissolved	Average
After 10 minutes	0.000		
	-0.001		
	0.001		
	0.000		
	0.000		
	0.000		
After 20 minutes	0.002		
	0.003		
	0.002		
	0.001		
	0.003		
	0.005		
After 30 minutes	0.003		
	0.003		
	0.003		
	0.002		
	0.003		
	0.005		
Ref Std	0.935		

Reference standard contains 31.3 mg Balsalazide Sodium

Table 1 : Dissolution Test Data

		Percent (%) Dissolved Mean Test Results [Specification, Q		
		Time (minutes)		
Lot Number	Study Number	10 minutes	20 minutes	30 minutes
N6272B01	CP099301[a]			
N6272B02				
1048J	57-3001[a]			
1150J				
0863J				
1219J				
1007J				
2501H	GLY01/93[c]	These lots were manufactured prior to implementation of the dissolution test.		
2863H				
2935H				
0642J				
4178J				

a. Phase III clinical study.
b. Data from 600 cc container stability study (NDA Volume 1.004 page 151)
c. Pharmacokinetic study.

The dissolution test for balsalazide disodium was developed in 1993 based on the following criteria:

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Confidential,
Commercial Information

Comments on NDA 20-610:

1. Sponsor's comments concerning Internal Standard / External Standard methods should be viewed with caution. Internal Standard methods should always result in at least as good accuracy and precision and usually much better than an external method. The three articles provided included by Sponsor (see 1.045 120 to 1.045 143) discuss caution in choosing an internal standard and show examples where lower accuracy and precision was observed when employing an internal standard. It is true that for certain methods more caution is required in choosing an internal standard such as [redacted] for analysis of phenobarbital (page 1.045 25). This should have been obvious to a good analyst since barbiturates and hydantoins are very different molecules (note: I'm well aware of this example since we quantified phenobarbital and other anticonvulsant drugs in human plasma when I was on the staff of the Clinical Pharmacology Center at Northwestern University in Chicago).

2. The Sponsor "Shoots himself in the foot" when extolling the virtues of the external standard method (see 1.046 220 and 221) for variation in extraction efficiency.

3. Based on the validation data provided for BSZ I would conclude that the Sponsor's [redacted]). The authors indicate they would carefully monitor samples in the 5 µg/mL and 10 µg/mL range by adding QC standards to the individual [redacted] We always add QC samples to our runs. I would think this would be especially important for an external standard method. It appears the Sponsor decided that an internal standard might be important to increase the [redacted] for quantifying BSZ plasma concentrations but their validation data was not much better than that provided in 1067/35-1010. Also troubling is that the Sponsor only provided intra-day accuracy and precision data for the 1067/50-1010 method (see tables).

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The firm has indicated that information on drug abuse is not applicable.

/S/ 5/22/98

Melodi McNeil, Regulatory Health Project Manager

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