

Dorothy P. Watson
Attorney

CIBA-GEIGY Corporation
Summit, New Jersey 07901
Telephone 201 277 5176

CIBA-GEIGY

April 26, 1984

Dr. William Gilbertson
Division of OTC Drug Evaluation (HFN-510)
Document Control Room 12A-55
5600 Fishers Lane
Rockville, Maryland 20857

Dear Dr. Gilbertson:

The enclosed information is submitted for the public record and relates to agency concerns and questions on the subject of phenylpropanolamine, as expressed at "feedback" meetings held on December 2, 1983, and April 11, 1984.

The enclosures consist of the following documents:

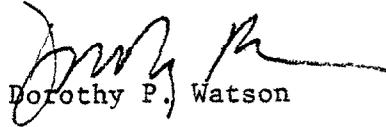
1. ALZA Corporation, Results and Conclusions - Protocol C-81-011, "Evaluation of Phenylpropanolamine Absorption During Oral Administration From Gastrointestinal Therapeutic Systems;" (Attachment 1)
2. Statistics Report (ST-141-83), "Phenylpropanolamine Absorption During Oral Administration From Gastrointestinal Therapeutic Systems - Study 1," September 19, 1983 (Attachment 2);
3. ALZA Corporation, Results and Conclusions - Protocol C-81-011: Study II, "Evaluation of Phenylpropanolamine Absorption During Oral Administration From Gastrointestinal Therapeutic Systems" (Attachment 3);
4. Statistics Report (ST-143-83), "Phenylpropanolamine Absorption During Oral Administration From Gastrointestinal Therapeutic Systems - Study 2," September 22, 1983 (Attachment 4);
5. Statistics Report (ST-144-83), "Phenylpropanolamine Absorption During Oral Administration From Gastrointestinal Therapeutic Systems - Studies 1 and 2," September 26, 1983 (Attachment 5); and
6. Results of an analysis of phenylpropanolamine content uniformity in selected lots of two timed-release appetite control products (Attachment 6).

LET 86

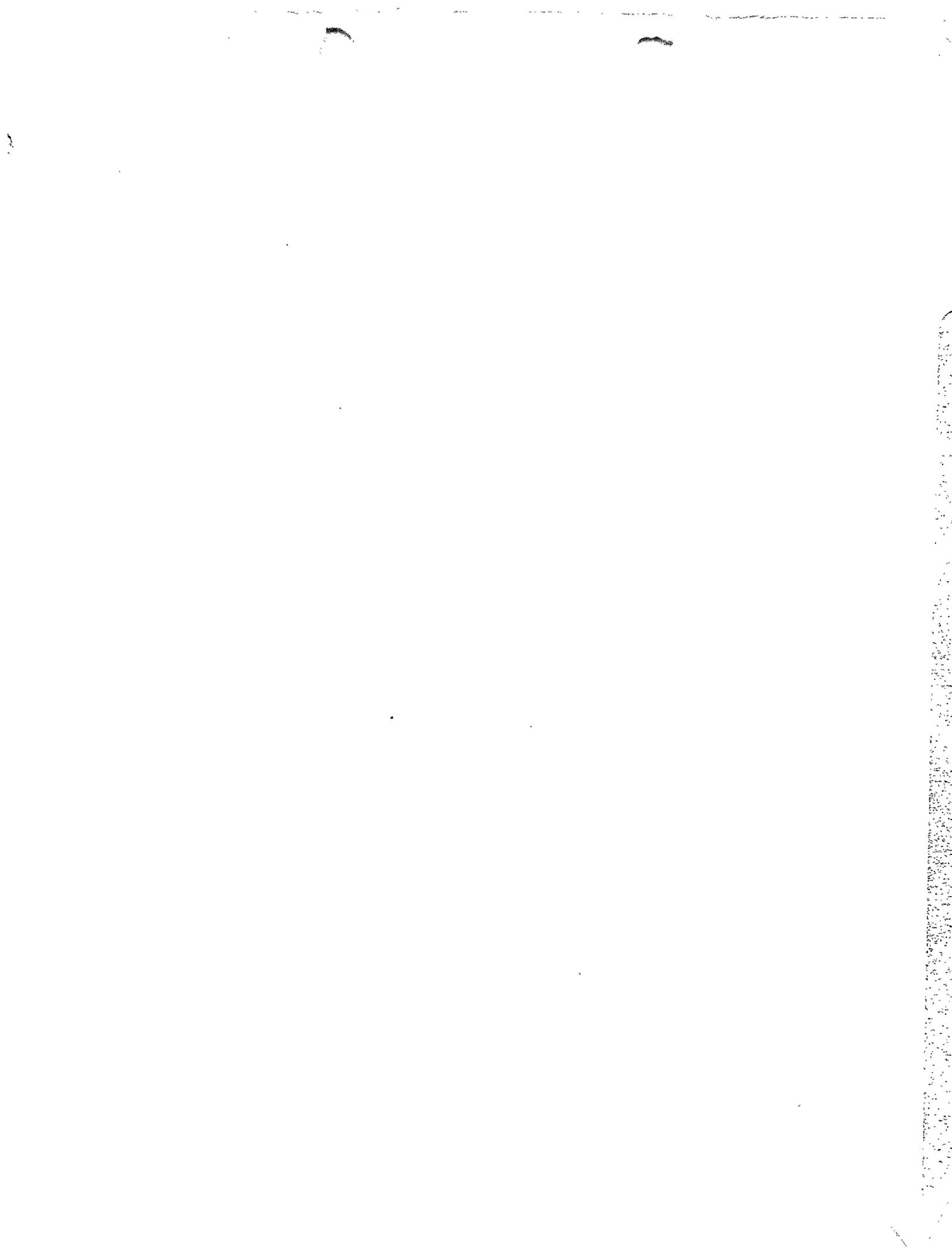
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We trust that this information will prove useful; additional information will be forwarded to you as it becomes available.

Very truly yours,


Dorothy P. Watson

DPW/lt
enclosure



B. STUDY METHODOLOGY

1) Study Objective

The objective of this study was to compare the profile of plasma levels and total urinary excretion of phenylpropanolamine following multiple doses from three oral dosage forms:

- a) The Gastrointestinal Therapeutic System (GITS)
- b) Dexatrim® 12 hr Sustained Release Capsules
- c) Aqueous Solution

2) Study Plan

The study took place over 5 consecutive weeks. During weeks 1, 3 and 5, subjects received 75 mg PPA HCl per day for 4 consecutive days from the dosage form indicated in the table below. (The order of dosage form administration was randomly assigned to the subjects.) Weeks 2 and 4 were rest weeks during which no drugs were given and no biological samples taken. Appendix I contains a detailed schedule of drug administration, blood sampling, urine collection, and food intake for any given week. Blood pressure and pulse measurements were taken just before each dosing cycle and at regular intervals during dosing cycles.

<u>Subject</u>	<u>Week 1</u>	<u>Week 3</u>	<u>Week 5</u>
01	GITS (PPA HCl)	Dexatrim	Solution
02	Solution	Dexatrim	GITS (PPA HCl)
03	Dexatrim	Solution	GITS (PPA HCl)
04	Solution	GITS (PPA HCl)	Dexatrim
05	GITS (PPA HCl)	Solution	Dexatrim
06	Dexatrim	GITS (PPA HCl)	Solution

When subjects took the GITS (PPA HCl) they collected stools, which were then searched to recover the system for assay.

Every subject gave a rating of expected side effects just prior to each blood sample. Subjects were requested to abstain from intake of alcohol and other drugs beginning 24 hours prior to a dosing week and continuing until the last biological sample was taken.

All six subjects completed the study.

3) Collecting and Handling of Biological Samples

a) Blood samples

10 ml of blood were drawn into a heparinized tube. The sample was then centrifuged within 5-7 minutes. 3 to 4 ml of plasma was immediately transferred to labeled 5 ml vials using a fresh disposable pipette for each plasma sample. Samples were stored at -20°C until assayed.

b) Urine samples

Urine was collected by the subjects in brown plastic bottles. At the end of each collection interval the volumes of the collections were measured and recorded and a 15-30 ml aliquot was transferred to a labeled glass vial and stored at -20°C until assayed.

c) Stool search and GITS (PPA HCl) recovery

Stools from subjects taking the GITS (PPA HCl) were methodically searched in an air flow hood. Upon finding a GITS (PPA HCl) in a stool sample, the system was placed in a glass vial and the date & time of defecation and date & time of recovery recorded. Systems were stored at -20°C until assayed.

4) Assay of Plasma and Urine for Phenylpropanolamine Content

The methodology and validation of the phenylpropanolamine assay of plasma and urine is detailed in Appendix II.

5) Assay of Recovered GITS (PPA HCl)

The assay of GITS (PPA HCl) systems recovered from stools for phenylpropanolamine residual content is detailed in Appendix III.

6) Assay of Dosage Forms for PPA HCl Content

Results from PPA HCl assays of aliquots of the solution dosage form, and of samples of the Dexatrim capsules and GITS (PPA HCl) system are presented in Appendix IV.

RESULTS AND CONCLUSIONS

PROTOCOL: C-81-011

TITLE: Evaluation of Phenylpropanolamine Absorption
During Oral Administration From Gastrointestinal
Therapeutic Systems

STUDY DATE: March 1, 1982 - April 2, 1982

STUDY SITE: Clinical Study Unit
Alza Corporation
1274 California Avenue
Palo Alto, CA 94304

PERFORMED BY: C. Ferre, R.N., P. Darley, B.S., V. A. Place, M.D.

REPORTED BY: P. Darley, B.S., D. Swanson, PhD, J. Fara, PhD

MATERIALS:

- 1) Gastrointestinal Therapeutic Systems,
Code #02510, Lot #614982
each containing a total of 75 mg phenylpro-
panolamine HCl, designed to release 20 mg
within the first few minutes and the remaining
55 mg at a nominal rate of 3.5 mg/hr for
approximately 16 hours.
- 2) Dietac® Drops,
(Menley James, lot X908), containing 125 mg/ml
phenylpropanolamine HCl, diluted down to a
concentration of 1.0 mg/ml.
- 3) Dexatrim® Extra Strength 12 hr Time Release
Capsules,
(Thompson Medical Co., Lot # MDF1280A), each
containing 75 mg phenylpropanolamine HCl

SUBJECTS: 6 healthy male volunteers. A description of the
study volunteers is given in Table I.

C. RESULTS

1) Plasma Levels of Phenylpropanolamine HCl

The plasma concentrations of PPA HCl for all six subjects are listed in Tables 2A (GITS), 2B (Dexatrim), and 2C (solution). A level of 6.2 ng/ml (5 ng/ml base) represents the lower limit of detectability of the assay (see Appendix II).

Table 3 lists the average plasma levels for all six subjects at each time of sample. These results are plotted in Figure 1.

Table 4 shows the areas under the plasma concentration curves (AUC's) for both Day 1 and the steady state day (Day 4), for each of the three dosage forms. Note that the low value on Day 1 for Subject 05 while on GITS (PPA HCl) corresponds to an early recovery of the system in the stool, which contained 33% of that day's dose.

2. Residual PPA HCl Content of GITS (PPA HCl) Recovered in the Stool

Table 5 shows the results of the assay of GITS (PPA HCl) recovered in the stool, showing also time and day of defecation as well as time and day of recovery from the stool. A total of 48.7 mg of PPA HCl (2.7% of the total dose) was found in the 22 recovered systems. Half of that amount was found in one system, defecated 10 hours after ingestion.

For six of the systems, it was possible to determine actual gut transit times because of the relative timing of ingestion and system defecation.

3. Urinary Recovery of PPA HCl

The PPA HCl content of all the urine collections is listed in Tables 6A (GITS), 6B (Dexatrim), and 6C (solution).

A. SUMMARY

A study was completed in six male subjects to compare the plasma concentration profiles and the urinary recoveries of phenylpropanolamine HCl (PPA HCl) after oral administration of three different dosage forms: (1) the Gastrointestinal Therapeutic System (GITS), (2) Dexatrim timed-release capsules, and (3) an aqueous solution.

Each subject received each dosage form in a randomized, complete crossover design. Subjects received 75 mg PPA HCl per day for 4 consecutive days during each dosage form dosing cycle, with a week's rest between dosing cycles. Blood samples were drawn at close intervals during Days 1 and 4 of the cycle and the plasma was assayed for phenylpropanolamine HCl. All urine was collected during the entire dosing cycle and assayed for PPA HCl to determine total urinary recovery of drug. The GITS (PPA HCl) were recovered from stools and assayed to determine residual PPA HCl content. (See Appendix I for detailed study schedule.)

The results of the study show that PPA is completely adsorbed when it is administered from a GITS (PPA HCl) or Dexatrim capsule compared to a solution control. Areas under the plasma concentration-time curve (0-24 hr) and amounts excreted in urine are the same for all three dosage forms. Peak plasma concentrations of PPA HCl after administration by Dexatrim capsules and a solution control reach values 2-3 times higher than for GITS (PPA HCl) at both day 1 and day 4. Plasma levels of PPA HCl after GITS (PPA HCl) administration were higher than after Dexatrim after 12 hours post-dose on day 1 and day 4.

The functionality of the Gastrointestinal Therapeutic System is further demonstrated by the fact that less than 3% of the original dose was found in systems recovered in the stool.

None of the subjects experienced any adverse effects during the study and blood pressures remained within normal ranges throughout the study.

Table 7 lists the total amount of PPA HCl excreted by each subject on each dosage form, as well as the total excretion by all subjects for each dosage form, expressed as total amount and as a percent of total dose.

4. Blood Pressure, Pulse, and Side Effects

Table 8 lists the results of blood pressure and pulse monitoring done just before and during each dosing cycle. The schedule of these measurements was reduced somewhat during the second and third dosing cycles, because no clinically significant effects were evident from the first week's monitoring.

Subjects recorded their observations on side effects just before each blood sample. There was no demonstrable change in the subject's observations of these effects throughout the entire study. Neither were there any isolated instances of subjects complaining of adverse effects during the study.

D. CONCLUSIONS

The results of the study show that PPA is completely adsorbed when it is administered from a GITS (PPA HCl) or Dexatrim capsule compared to a solution control. Areas under the plasma concentration-time curve (0-24 hr) and amounts excreted in urine are the same for all three dosage forms. Peak plasma concentrations of PPA HCl after administration by Dexatrim capsules and a solution control reach values 2-3 times higher than for GITS (PPA HCl) at both day 1 and day 4. Plasma levels of PPA HCl after GITS (PPA HCl) administration were higher than after Dexatrim after 12 hours post-dose on day 1 and day 4.

The functionality of the Gastrointestinal Therapeutic System is further demonstrated by the fact that less than 3% of the original dose was found in systems recovered in the stool.

None of the subjects experienced any adverse effects during the study and blood pressures remained within normal ranges throughout the study.

TABLE I

Description of Study Population

<u>Subject Number</u>	<u>Age (yrs)</u>	<u>Body Weight (kgs)</u>		
		<u>Pre-Study (March 1)</u>	<u>Pre-2nd Dosage Form (March 15)</u>	<u>Pre-3rd Dosage Form (March 29)</u>
01	25	76.3	75.4	74.5
02	26	102.3	101.3	102.3
03	32	72.3	71.8	72.7
04	23	80.4	80.0	78.9
05	36	91.8	91.4	91.8
06	21	76.4	76.4	77.2
MEANS	27.1	83.2	82.7	82.9
	±5.1 S.D.	±4.7 S.E.M.	±4.6 S.E.M.	±4.8 S.E.M.

TABLE 2B
 PLASMA LEVELS OF PPA.HCL
 DEXATRIM CAPSULES

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N , NG/ML					
			SUBJECT 01	SUBJECT 02	SUBJECT 03	SUBJECT 04	SUBJECT 05	SUBJECT 06
1	08:00	0	<6.2	<6.2	9.3	<6.2	<6.2	<6.2
1	08:30	0.5	16.4	39.7	<6.2	<6.2	<6.2	12.0
1	09:00	1	55.9	82.0	42.2	53.4	12.5	41.2
1	10:00	2	61.7	126.3	101.8	97.4	63.9	90.2
1	11:00	3	100.2	135.0	131.6	126.6	113.6	93.7
1	12:00	4	151.0	129.9	234.6	164.5	194.2	144.2
1	13:00	5	100.6	113.0	197.7	177.5	163.7	137.1
1	14:00	6	150.4	117.3	179.3	185.6	154.5	125.4
1	16:00	8	126.8	82.9	133.9	162.6	117.4	97.4
1	18:00	10	93.7	64.0	92.5	116.7	94.7	65.5
1	20:00	12	60.3	55.6	79.4	99.5	71.6	41.2
1	24:00	16	32.5	N/A	52.1	66.8	35.4	10.6
2	00:00	24	7.4	11.5	20.5	9.9	20.1	<6.2
3	00:00	24	12.4	<6.2	12.9	10.5	17.9	<6.2
4	00:00	24	6.6	7.2	17.1	12.4	10.6	<6.2
4	00:30	0.5	13.0	41.0	30.0	10.2	20.4	12.2
4	09:00	1	63.9	87.5	60.5	32.3	63.1	51.5
4	10:00	2	113.2	170.9	116.7	103.6	140.9	97.1
4	11:00	3	273.3	100.3	192.4	130.3	310.3	130.1
4	12:00	4	246.4	172.0	232.7	149.0	208.6	103.9
4	13:00	5	106.2	151.4	210.1	157.9	303.6	145.0
4	14:00	6	163.0	130.3	190.6	131.3	253.7	123.5
4	16:00	8	126.0	104.9	152.0	106.2	200.6	93.2
4	18:00	10	86.0	77.0	130.9	70.1	117.3	54.6
4	19:00	11	73.2	67.0	111.7	56.6	80.5	35.7
4	20:00	12	119.0	53.4	96.0	48.4	77.0	37.7
4	22:00	14	45.3	52.7	71.4	37.2	63.9	27.2
4	24:00	16	36.0	37.2	55.2	28.0	52.5	19.9
5	00:00	24	12.4	11.2	22.3	9.3	16.0	<6.2
5	12:00	20	0.9	6.2	9.9	<6.2	7.6	<6.2

PROTOCOL C-81-011

TABLE 2A
 PLASMA LEVELS OF PPA.HCL
 GASTROINTESTINAL THERAPEUTIC SYSTEM

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N, NG/ML					
			SUBJECT 01	SUBJECT 02	SUBJECT 03	SUBJECT 04	SUBJECT 05	SUBJECT 06
1	08:00	0	<6.2	<6.2	<6.2	<6.2	<6.2	<6.2
1	08:30	0.5	72.0	25.4	21.3	41.6	45.7	40.3
1	09:00	1	80.1	N/A	63.9	56.5	75.3	46.5
1	10:00	2	86.3	49.8	92.1	83.2	78.1	69.6
1	11:00	3	74.8	62.1	97.1	73.8	74.1	73.8
1	12:00	4	76.3	54.1	91.3	83.2	74.8	68.5
1	13:00	5	70.2	64.5	102.4	78.9	68.9	71.7
1	14:00	6	74.5	59.8	97.1	86.3	64.9	65.4
1	16:00	8	86.6	62.1	97.4	87.5	68.9	80.0
1	18:00	10	81.9	98.4	92.5	76.8	74.1	66.4
1	20:00	12	69.5	96.2	106.7	86.8	58.9	60.8
1	24:00	16	58.3	71.4	137.5	67.8	26.4	42.2
2	08:00	24	17.4	22.8	97.2	27.9	<6.2	28.5
3	08:00	24	20.1	24.8	38.5	32.8	26.1	28.8
4	08:00	24	6.6	35.6	48.7	43.2	21.5	26.1
4	08:30	0.5	64.5	74.5	46.5	87.5	27.3	39.5
4	09:00	1	77.6	99.3	94.6	116.0	61.4	77.6
4	10:00	2	67.8	95.8	137.8	114.2	72.2	87.5
4	11:00	3	80.9	105.3	136.5	108.6	92.0	85.0
4	12:00	4	73.8	138.4	133.4	109.8	96.2	91.2
4	13:00	5	70.1	116.7	138.8	113.6	86.9	102.4
4	14:00	6	70.1	107.7	145.8	113.3	83.2	79.7
4	16:00	8	77.6	105.5	130.7	89.4	88.7	86.3
4	18:00	10	102.4	122.3	123.5	123.5	101.5	104.9
4	19:00	11	122.3	129.1	122.3	112.3	116.0	93.7
4	20:00	12	96.8	120.4	121.6	126.6	107.7	89.4
4	22:00	14	75.1	125.6	107.8	96.2	80.2	72.9
4	24:00	16	58.6	120.6	94.9	78.2	62.7	53.4
5	08:00	24	15.3	52.4	40.8	37.2	26.9	13.8
5	12:00	20	13.7	34.1	29.4	25.8	<6.2	<6.2

PROTOCOL C-81-011

TABLE 2C
 PLASMA LEVELS OF PPA.HCL
 AQUEOUS SOLUTION

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N, NG/ML					
			SUBJECT 01	SUBJECT 02	SUBJECT 03	SUBJECT 04	SUBJECT 05	SUBJECT 06
1	08:00	0	<6.2	6.8	<6.2	<6.2	<6.2	<6.2
1	08:30	0.5	28.8	87.5	129.1	48.4	42.2	51.3
1	09:00	1	110.1	97.4	138.4	61.2	68.5	81.3
1	10:00	2	151.2	101.2	129.8	102.4	110.2	77.0
1	11:00	3	117.3	99.7	111.0	117.0	104.3	119.8
1	12:02	4	100.2	73.8	95.8	119.5	81.3	84.6
1	13:00	5	92.1	77.6	85.4	85.0	76.1	64.9
1	14:00	6	78.6	59.0	74.5	77.0	70.6	60.8
1	16:00	8	62.1	57.1	58.1	53.7	52.7	44.1
1	18:00	10	47.8	33.5	42.2	32.0	37.9	27.6
1	20:00	12	33.5	29.2	35.7	24.1	22.7	15.5
1	24:00	4	104.5	96.8	137.0	106.7	115.4	99.3
2	08:00	12	29.2	45.3	36.2	36.6	36.0	25.4
3	08:00	16	18.2	12.9	41.0	18.5	20.7	8.4
4	08:00	16	8.7	13.0	22.3	17.7	23.6	10.4
4	08:30	0.5	43.4	73.0	56.5	49.6	41.7	31.0
4	09:00	1	92.5	78.8	113.6	92.5	85.6	56.5
4	10:00	2	99.3	77.9	130.9	81.3	114.0	67.0
4	11:00	3	81.9	68.3	116.7	80.4	95.1	68.1
4	12:00	4	67.6	55.2	99.5	62.1	90.9	50.9
4	13:00	1	75.3	114.4	136.5	100.2	94.6	111.1
4	14:00	2	112.1	106.1	167.6	102.6	133.4	89.4
4	16:00	4	104.5	72.6	139.0	91.0	119.2	65.2
4	18:00	2	161.6	125.4	191.8	144.0	134.2	89.0
4	19:00	3	125.6	111.1	164.5	134.0	129.9	81.5
4	20:00	4	130.3	94.3	153.3	114.6	N/A	67.5
4	22:00	6	58.7	81.3	124.1	68.3	113.4	44.7
4	24:00	8	49.6	57.1	106.7	64.5	99.7	34.0
5	08:00	16	6.5	16.1	31.6	18.2	38.7	9.9
5	12:00	20	<6.2	11.2	23.0	9.3	21.3	<6.2

TABLE 3
AVERAGE PLASMA LEVELS OF PPA, HCL

DAY OF DOSING CYCLE	HOURS SINCE LAST DOSE TIME OF SAMPLE	GITS & DEXATRIM	SOLN	AVERAGE PLASMA CONCENTRATIONS (NG/ML), & S.E.M., N=6					
				GASTROINTESTINAL THERAPEUTIC SYSTEM		DEXATRIM		SOLUTION	
				MEAN	S.E.M.	MEAN	S.E.M.	MEAN	S.E.M.
1	08:00	0	0	6.2	0.0	6.7	0.5	6.3	0.1
1	08:30	0.5	0.5	41.1	7.3	14.6	5.3	64.5	15.2
1	09:00	1	1	66.1*	7.2	40.0	9.4	92.0	11.7
1	10:00	2	2	76.4	6.3	90.2	10.0	112.0	10.5
1	11:00	3	3	76.0	4.7	110.1	6.5	111.6	3.3
1	12:00	4	4	74.7	5.2	169.7	15.7	93.9	7.1
1	13:00	5	5	77.4	5.5	161.7	12.7	80.2	3.9
1	14:00	6	6	74.7	5.9	153.4	11.3	70.1	3.4
1	16:00	8	8	80.6	5.3	120.2	11.5	54.6	2.5
1	18:00	10	10	81.7	4.9	87.9	0.2	36.0	3.0
1	20:00	12	12	78.4	0.8	60.0	0.3	26.0	3.1
1	24:00	16	4	67.3	15.6	40.9*	0.2	110.1	6.1
2	08:00	24	12	33.2	13.2	12.6	2.5	34.0	2.0
3	08:00	24	16	20.4	2.6	11.0	1.0	10.6	4.9
4	08:00	24	16	30.3	6.3	11.4	2.3	16.0	2.5
4	08:30	0.5	0.5	56.6	9.3	22.6	5.1	49.2	5.9
4	09:00	1	1	87.7	7.9	61.1	7.5	86.6	7.7
4	10:00	2	2	95.0	10.9	125.1	11.7	95.2	9.9
4	11:00	3	3	101.4	8.3	204.1	30.2	85.1	7.5
4	12:00	4	4	107.2	10.3	212.4	21.3	71.0	0.1
4	13:00	5	1	104.6	9.7	192.5	24.3	106.7	0.4
4	14:00	6	2	99.0	11.3	166.9	20.8	110.5	11.4
4	16:00	8	4	96.4	7.0	130.6	16.4	90.7	11.4
4	18:00	10	2	113.0	4.5	89.3	11.9	141.0	14.1
4	19:00	11	3	115.9	5.0	72.1	10.7	124.4	11.2
4	20:00	12	4	110.4	6.1	72.2	12.9	112.0*	14.7
4	22:00	14	6	92.0	0.5	49.6	6.7	81.0	12.0
4	24:00	16	0	70.1	10.5	30.3	5.6	60.6	11.7
5	08:00	24	16	30.0	6.2	12.9	2.3	20.2	5.1
5	12:00	20	20	19.2	5.0	7.5	0.7	12.9	3.0

* N=5

TABLE 4

Areas Under the Plasma Concentration Curve*
for Day 1** and Day 4**

Area Under the Curve, ng/ml-hr

<u>Subject</u>	<u>Gastrointestinal Therapeutic System</u>		<u>Dexatrim</u>		<u>Solution</u>	
	<u>Day 1</u>	<u>Day 4</u>	<u>Day 1</u>	<u>Day 4</u>	<u>Day 1</u>	<u>Day 4</u>
1	1205	1265	1319	1673	1409	1410
2	1196	2019	1222	1430	1281	1347
3	2016	1990	1658	1923	1598	2194
4	1304	1737	1761	1187	1323	1468
5	883***	1391	1380	2202	1319	1871
6	<u>1033</u>	<u>1283</u>	<u>1030</u>	<u>1070</u>	<u>1138</u>	<u>997</u>
Mean	1273	<u>1614</u>	1395	1581	1345	<u>1548</u>
+ S.E.M.	+ 161	+ 142	+ 111	+ 178	+ 62	+ 172

* Computed by trapezoidal approximation

** Day 1: 08:00, Day 1 to 08:00, Day 2
Day 4: 08:00, Day 4 to 08:00, Day 5

*** System taken on Day 1 was recovered in the stool ca. 10 hours from ingestion and contained 23 mg (ca. 33%) of the dose.

TABLE 5

Residual PPA.HCl Content of Gastrointestinal
Therapeutic Systems Recovered from Stools

	Defecation		Recovery from Stool		MG PPA.HCl	Gut Transit Time, hrs
	Day	Time	Day	Time		
Subject 01*	4	14:30	4	17:00	0.07	
	5	07:35	5	09:50	0.73	
Subject 02	2	08:30	2	09:15	3.60	24.5
	3	10:35	3	15:30	2.03	26.5
	5	09:00	5	12:00	0.60	
	5	17:45	5	17:50	1.13	
Subject 03	2	08:45	1	18:00	2.07	24.7
	3	09:00	3	09:30	1.92	25.0
	4	09:15	4	12:00	2.18	25.2
	6	12:15	8	10:30	0.52	
Subject 04	3	14:00	3	16:45	0.75	
	5	07:00**	5	10:00	0.43	
	5	07:00**	5	10:00	1.70	
	6	07:00	6	11:30	1.07	
Subject 05	1	18:15	2	08:25	23.0	10.2
	4	12:00**	4	17:20	0.74	
	4	12:00**	4	17:20	0.74	
	5	06:00	5	10:10	1.46	
Subject 06	3	08:45**	3	17:00	2.90	
	3	08:45**	3	17:00	0.38	
	5	08:45	5	09:30	0.26	
	5	22:00	8	08:30	0.41	
Total Residual PPA.HCl, mg					48.69	

* Subject did not collect stools beyond end of Day 6

** Systems recovered in the same stool

PROTOCOL C-81-011

TABLE 6A
 URINARY EXCRETION OF PPA.HCL
 GASTROINTESTINAL THERAPEUTIC SYSTEM

DAY OF DOSING CYCLE	TIME INTERVAL OF SAMPLE	HOURS IN INTERVAL	MG OF PPA.HCL EXCRETED					
			SUBJECT 01	SUBJECT 02	SUBJECT 03	SUBJECT 04	SUBJECT 05	SUBJECT 06
1	BEFORE 08:00	-	0.0	0.0	0.0	0.0	0.0	0.0
1	08:00 - 12:00	4	11.0	9.5	7.2	0.4	25.6	10.1
1	12:00 - 16:00	4	10.2	10.2	9.0	14.3	10.1	20.9
1	16:00 - 20:00	4	15.5	10.0	16.7*	10.9	9.2	9.7
1	20:00 - 24:00	4	9.7	12.4	-	10.6	7.6	2.2
2	24:00 - 08:00	8	13.4	12.1	10.0	11.0	3.0	14.9
2-3	08:00 - 08:00	24	62.3	47.2	58.0	52.2	47.4	63.9
3-4	08:00 - 08:00	24	66.3	66.7	64.5	55.4	66.4	50.7
4	08:00 - 12:00	4	13.0	10.9	10.1	16.6	9.5	12.0
4	12:00 - 16:00	4	26.0	10.5	10.0	13.0	13.9	13.9
4	16:00 - 20:00	4	7.3	10.6	12.3	14.0	11.0	17.9
4	20:00 - 24:00	4	9.4	10.2	11.9	11.9	13.6	12.0
5	24:00 - 08:00	8	9.4	19.4	13.6	1.0	9.2	0.6
5	08:00 - 12:00	4	1.7	3.4	2.0	4.0	4.0	1.3
5	12:00 - 16:00	4	0.6	2.0	2.0	2.3	1.4	1.2
5-6	16:00 - 08:00	16	0.0	2.2	5.4	0.0	0.0	0.0

* AMOUNT EXCRETED FOR BOTH THIS INTERVAL AND NEXT INTERVAL

PROTOCOL C-81-011

TABLE 6B
 URINARY EXCRETION OF PPA.HCL
 DEXATRIM CAPSULES

DAY OF DOSING CYCLE	TIME INTERVAL OF SAMPLE	HOURS IN INTERVAL	MG OF PPA.HCL EXCRETED					
			SUBJECT 01	SUBJECT 02	SUBJECT 03	SUBJECT 04	SUBJECT 05	SUBJECT 06
1	BEFORE 08:00	-	0.0	0.0	0.0	0.0	0.0	0.0
1	08:00 - 12:00	4	16.5	22.4	0.0	0.4	10.2	15.7
1	12:00 - 16:00	4	27.4	10.9	23.7	24.4	23.1	19.0
1	16:00 - 20:00	4	13.0	9.6	8.7	11.3	9.7	11.2
1	20:00 - 24:00	4	7.9	5.5	5.9	7.9	7.5	2.7
2	24:00 - 08:00	8	5.2	6.7	6.2	6.6	6.1	4.6
2-3	08:00 - 08:00	24	61.0	57.4	61.1	65.2	60.7	26.9
3-4	08:00 - 08:00	24	71.0	56.1	65.6	65.9	60.1	74.3
4	08:00 - 12:00	4	20.2	25.0	13.3	15.9	16.7	15.3
4	12:00 - 16:00	4	20.3	20.3	16.9	22.8	31.6	24.0
4	16:00 - 20:00	4	12.1	10.1	17.9	11.4	4.5	10.0
4	20:00 - 24:00	4	7.4	6.2	8.7	4.9	6.9	4.4
5	24:00 - 08:00	8	5.0	6.4	7.2	4.5	0.0	3.2
5	08:00 - 12:00	4	1.0	1.4	0.6	0.0	2.6	0.0
5	12:00 - 16:00	4	0.5	0.0	0.5	0.7	1.4	0.0
5-6	16:00 - 08:00	16	0.0	0.6	1.0	0.0	0.5	0.0

TABLE 6C
 URINARY EXCRETION OF PPA.HCL
 AQUEOUS SOLUTION

DAY OF DOSING CYCLE	TIME INTERVAL OF SAMPLE	HOURS IN INTERVAL	MG OF PPA.HCL EXCRETED					
			SUBJECT 01	SUBJECT 02	SUBJECT 03	SUBJECT 04	SUBJECT 05	SUBJECT 06
1	BEFORE 08:00	-	0.0	0.1	0.0	0.0	0.0	0.0
1	08:00 - 12:00	4	12.4	13.4	8.5	14.1	9.9	14.9
1	12:00 - 16:00	4	9.0	9.0	7.1	11.5	13.6	7.3
1	16:00 - 20:00	4	6.3	5.0	3.1	3.8	5.0	5.8
1	20:00 - 24:00	4	12.1	12.0	15.1	10.0	17.1	15.0
2	24:00 - 08:00	8	18.0	16.4	16.6	17.4	11.2	13.9
2-3	08:00 - 08:00	24	69.2	67.8	62.8	85.9	69.4	61.8
3-4	08:00 - 08:00	24	67.3	62.2	67.1	69.9	62.5	64.4
4	08:00 - 12:00	4	12.0	9.8	10.5	10.6	10.2	12.0
4	12:00 - 16:00	4	9.2	14.0	13.6	19.0	14.1	10.4
4	16:00 - 20:00	4	23.0	17.7	22.7	20.5	21.6	22.4
4	20:00 - 24:00	4	11.3	9.6	8.4	11.1	4.3	8.9
5	24:00 - 08:00	8	6.0	12.3	4.0	9.0	16.4	6.2
5	08:00 - 12:00	4	1.8	1.9	1.2	2.2	3.4	0.6
5	12:00 - 16:00	4	0.7	1.2	1.0	0.3	3.1	0.5
5-6	16:00 - 08:00	16	0.0	2.0	1.9	1.2	0.4	0.0

Total Urinary Excretion of PPA.HCl

MG PPA.HCl Excreted from
08:00, Day 1 to 12:00, Day 5

<u>Subject</u>	<u>Gastrointestinal Therapeutic System</u>	<u>Dexatrim</u>	<u>Solution</u>
01	257.4	277.2	259.2
02	238.3	247.5	256.1
03	236.1	247.0	244.4
04	226.4	250.7	287.2
05	233.6	249.8	262.0
06	<u>248.1</u>	<u>211.2</u>	<u>245.5</u>
Mean <u>+ S.E.M.</u>	240.0 <u>+ 4.5</u>	247.2 <u>+ 8.6</u>	259.1 <u>+ 6.3</u>
Total Excretion: MG	1440	1483	1554
as % of Dose	82*	82	86

* Based on total dose of 1800 mg minus 49 mg residual found in GITS recovered in stools

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	WEEK	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE			PLASMA LEVEL NG/ML
					SYS-TOLIC	DIAS-TOLIC	PULSE	
1	1	GITS	01MAR82	08:00	110	78	80	5.0
1	1	GITS	01MAR82	10:00	118	78	72	69.5
1	1	GITS	01MAR82	14:00	108	72	76	60.0
1	1	GITS	01MAR82	18:00	102	68	76	66.0
1	1	GITS	03MAR82	08:00	110	74	72	16.2
1	1	GITS	05MAR82	08:00	112	60	72	12.3
1	3	DEXATRIM	15MAR82	08:00	118	70	80	5.0
1	3	DEXATRIM	15MAR82	12:00	112	70	80	121.7
1	3	DEXATRIM	18MAR82	08:00	108	60	70	5.3
1	3	DEXATRIM	18MAR82	11:00	122	70	80	220.2
1	5	SOLUTION	29MAR82	08:00	116	62	72	5.0
1	5	SOLUTION	29MAR82	11:00	106	60	72	94.5
1	5	SOLUTION	01APR82	08:00	118	72	80	7.0
1	5	SOLUTION	01APR82	12:00	122	82	80	54.5
2	1	SOLUTION	01MAR82	08:00	118	70	78	5.5
2	1	SOLUTION	01MAR82	09:00	110	72	70	78.5
2	1	SOLUTION	01MAR82	10:00	124	78	64	81.5
2	1	SOLUTION	01MAR82	12:00	130	78	76	59.5
2	1	SOLUTION	01MAR82	18:00	126	78	76	27.0
2	1	SOLUTION	03MAR82	08:00	122	78	78	10.4
2	1	SOLUTION	05MAR82	08:00	118	72	70	13.0
2	3	DEXATRIM	15MAR82	08:00	128	72	72	5.0
2	3	DEXATRIM	15MAR82	12:00	138	80	78	104.7
2	3	DEXATRIM	18MAR82	08:00	128	72	72	5.8
2	3	DEXATRIM	18MAR82	11:00	126	86	74	151.7
2	5	GITS	29MAR82	08:00	138	82	76	5.0
2	5	GITS	29MAR82	11:00	144	90	80	50.0
2	5	GITS	01APR82	08:00	120	78	78	28.7
2	5	GITS	01APR82	12:00	136	76	80	111.5
3	1	DEXATRIM	01MAR82	08:00	126	80	60	7.5
3	1	DEXATRIM	01MAR82	10:00	130	72	68	82.0
3	1	DEXATRIM	01MAR82	14:00	130	72	68	144.5
3	1	DEXATRIM	01MAR82	18:00	110	64	64	74.5
3	1	DEXATRIM	03MAR82	08:00	128	70	64	10.4
3	1	DEXATRIM	05MAR82	08:00	120	82	68	18.0
3	3	SOLUTION	15MAR82	08:00	124	70	72	5.0
3	3	SOLUTION	15MAR82	12:00	110	60	62	77.2
3	3	SOLUTION	18MAR82	08:00	118	70	64	18.0
3	3	SOLUTION	18MAR82	11:00	124	78	72	94.0
3	5	GITS	29MAR82	08:00	112	70	68	5.0
3	5	GITS	29MAR82	11:00	120	72	64	78.2
3	5	GITS	01APR82	08:00	130	80	72	39.2
3	5	GITS	01APR82	12:00	128	60	76	107.5

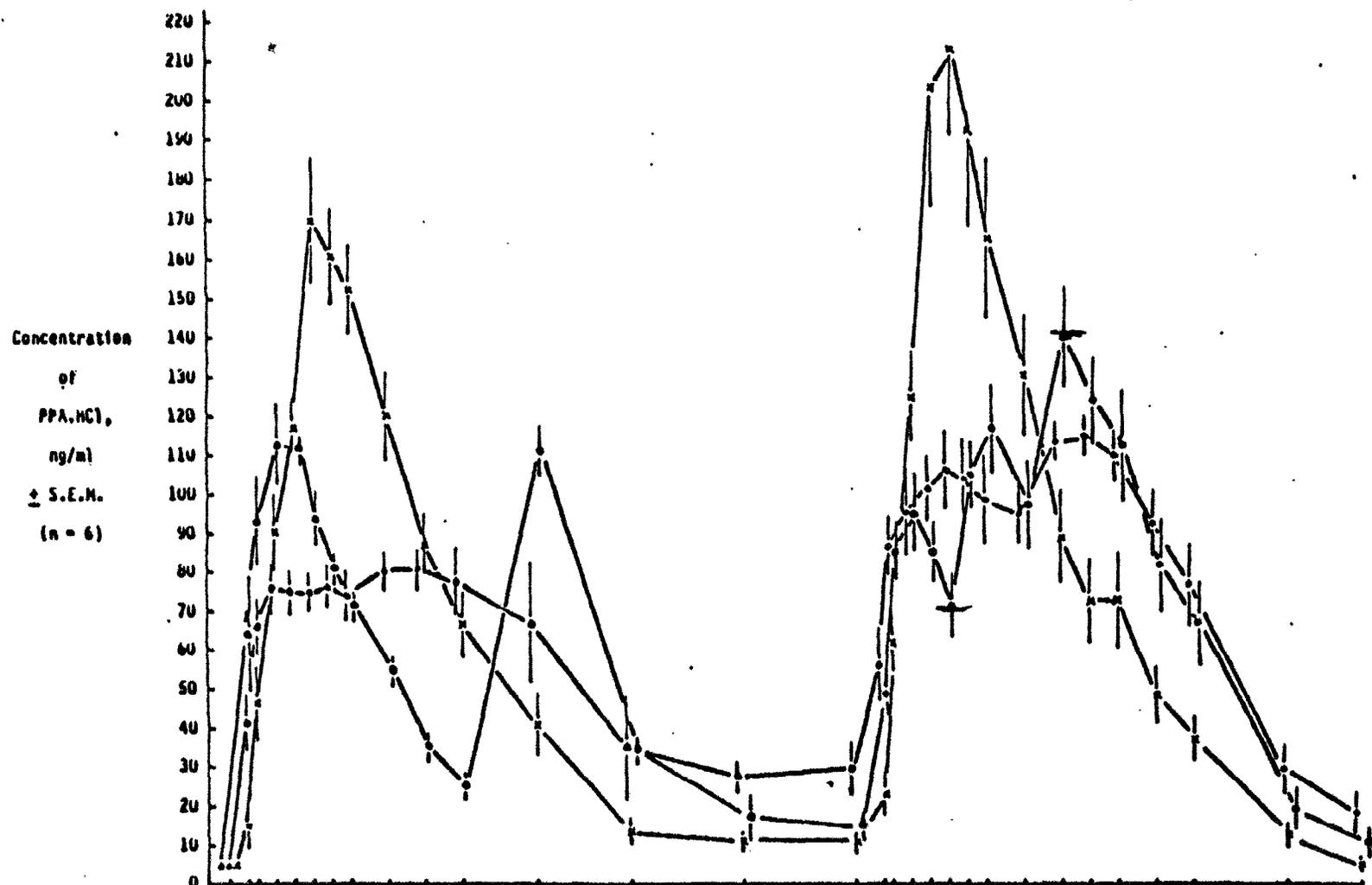
TABLE 8 (CONT.)

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	WEEK	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE SYS- TOLIC	DIAS- TOLIC	PULSE	PLASMA LEVEL NG/ML
-----	-----	-----	-----	-----	-----	-----	-----	-----
4	1	SOLUTION	01MAR82	08:00	106	70	68	5.0
4	1	SOLUTION	01MAR82	09:00	120	72	80	49.3
4	1	SOLUTION	01MAR82	10:00	118	70	76	82.5
4	1	SOLUTION	01MAR82	12:00	122	76	76	96.3
4	1	SOLUTION	01MAR82	14:00	122	70	64	62.0
4	1	SOLUTION	01MAR82	18:00	124	70	72	25.8
4	1	SOLUTION	03MAR82	08:00	116	68	74	14.9
4	1	SOLUTION	05MAR82	08:00	110	68	70	14.7
4	3	GITS	15MAR82	08:00	110	60	64	5.0
4	3	GITS	15MAR82	12:00	118	70	72	67.0
4	3	GITS	18MAR82	08:00	118	72	68	34.8
4	3	GITS	18MAR82	11:00	108	68	68	87.5
4	5	DEXATRIM	29MAR82	08:00	122	80	72	5.0
4	5	DEXATRIM	29MAR82	11:00	108	64	64	102.0
4	5	DEXATRIM	01APR82	08:00	122	78	80	10.0
4	5	DEXATRIM	01APR82	12:00	112	60	72	120.7
5	1	GITS	01MAR82	08:00	122	84	72	5.0
5	1	GITS	01MAR82	10:00	120	80	78	62.9
5	1	GITS	01MAR82	14:00	118	72	76	52.3
5	1	GITS	01MAR82	18:00	110	70	78	59.7
5	1	GITS	03MAR82	08:00	122	68	78	21.0
5	1	GITS	05MAR82	08:00	120	78	70	21.7
5	3	SOLUTION	15MAR82	08:00	110	70	78	5.0
5	3	SOLUTION	15MAR82	12:00	118	70	78	65.5
5	3	SOLUTION	18MAR82	08:00	122	70	68	19.0
5	3	SOLUTION	18MAR82	11:00	108	70	68	76.6
5	5	DEXATRIM	29MAR82	08:00	120	72	76	5.0
5	5	DEXATRIM	29MAR82	11:00	126	80	80	91.5
5	5	DEXATRIM	01APR82	08:00	110	70	72	15.0
5	5	DEXATRIM	01APR82	12:00	110	60	72	232.5
6	1	DEXATRIM	01MAR82	08:00	120	78	68	5.0
6	1	DEXATRIM	01MAR82	10:00	120	82	68	72.7
6	1	DEXATRIM	01MAR82	14:00	130	70	72	101.0
6	1	DEXATRIM	01MAR82	18:00	118	78	78	52.8
6	1	DEXATRIM	03MAR82	08:00	108	70	70	5.0
6	1	DEXATRIM	05MAR82	08:00	112	68	70	5.0
6	3	GITS	15MAR82	08:00	122	74	72	5.0
6	3	GITS	15MAR82	12:00	120	70	68	55.2
6	3	GITS	18MAR82	08:00	110	62	68	21.0
6	3	GITS	18MAR82	11:00	128	72	68	68.5
6	5	SOLUTION	29MAR82	08:00	122	72	68	5.0
6	5	SOLUTION	29MAR82	11:00	108	62	60	96.5
6	5	SOLUTION	01APR82	08:00	120	80	68	8.4
6	5	SOLUTION	01APR82	12:00	122	72	68	41.0

Figure 1

Average Plasma Concentrations of PPA.HCl



Hours Since Last Doses:	1										2		3		4										5					
- GITS & Dexatrim -	0	1	2	3	4	5	6	8	10	12	16	24	24	24	0	1	2	3	4	5	6	8	10	11	12	14	16	24	24	
- Solution	0	0	1	1	1	1	1	1	1	2	2	0	0	0	0	0	1	1	1	2	2	2	0	0	0	0	0	0	1	2
* U.S hours	7	8	9	0	1	2	3	4	6	8	0	4	8	8	8	9	0	1	2	3	4	6	8	8	9	0	2	4	8	2
Time of Day	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Way of Using Cycle	----- 1 -----										2	3	----- 4 -----										5							

Legend: ○ GITS × Dexatrim △ Solution

APPENDIX I
Study Schedule

Day of Dosing Cycle	Time of Day	DRUG ADMINISTRATION		Blood Sample	Hours Since Last Dose		Urine Collection Interval	Food Intake
		GITS & Dexatrim	Solution		GITS & Dexatrim	Sol'n		
1	07:30			X	-	-	Control Void Just Prior to 1st Dose Start	Breakfast*
1	08:00	75	37.5	X	0	0	↓ 4 hrs	
1	08:30			X	0.5	0.5		
1	09:00			X	1	1		
1	10:00			X	2	2		
1	11:00			X	3	3	↓ 4 hrs	Lunch*
1	12:00			X	4	4		
1	12:30						↓ 4 hrs	Dinner*
1	13:00			X	5	5		
1	14:00			X	6	6		
1	16:00			X	8	8		
1	18:00			X	10	10	↓ 4 hrs	Dinner*
1	18:30							
1	20:00		37.5	X	12	12	↓ 4 hrs	
1	24:00			X	16	4		
2	07:30						↓ 8 hrs	Breakfast*
2	08:00	75	25	X	24	12		
2	12:00		25					
2	12:30							
2	16:00		25				↓ 24 hrs	Dinner
2	18:30							
3	07:30						↓ 24 hrs	Breakfast*
3	08:00	75	25	X	24	16		
3	12:00		25					
3	12:30							
3	16:00		25				↓ 24 hrs	Dinner
3	18:30							
4	07:30						↓ 4 hrs	Breakfast*
4	08:00	75	25	X	24	16		
4	08:30			X	0.5	0.5		
4	09:00			X	1	1		
4	10:00			X	2	2	↓ 4 hrs	Lunch*
4	11:00			X	3	3		
4	12:00		25	X	4	4	↓ 4 hrs	Dinner*
4	12:30							
4	13:00			X	5	1		
4	14:00			X	6	2		
4	16:00		25	X	8	4	↓ 4 hrs	Dinner*
4	18:00			X	10	2		
4	18:30						↓ 4 hrs	
4	19:00			X	11	3		
4	20:00			X	12	4		
4	22:00			X	14	6		
4	24:00			X	16	8	↓ 8 hrs	Breakfast*
4	24:00							
5	07:30						↓ 4 hrs	Breakfast*
5	08:30			X	24	16		
5	12:00			X	28	20	↓ 4 hrs	
5	16:00				32	24		
5	08:00				48	40	↓ 16 hrs	
							Finish	

* All subjects were served the same menu

The Determination of Phenylpropanolamine
in Plasma by Gas Liquid Chromatography
and Urine by High Pressure Liquid Chromatography

Analysis of the drug in plasma involved extraction of phenylpropanolamine from plasma into toluene, followed by derivatization with trifluoroacetic anhydride to yield an electron-capturing di-trifluoroacetyl derivative, prior to injection into the gas liquid chromatographic system. 2-amino-3-phenyl-1-propanol hydrochloride was used as an internal standard. Quantification was performed by peak area measurement and by use of a standard curve.

Analysis of the drug in urine involved injection of aliquots of urine into the high pressure liquid chromatographic system. Amphetamine sulfate was used as an internal standard. The compounds were chromatographed on a reverse phase column. The drug and internal standard were converted to fluorescent molecules⁽¹⁾ as they eluted from the column by post-column reaction with o-phthalaldehyde. The fluorophores were detected by a fluorescence detector with excitation at 340 nm and an emission cutoff at 418 nm. Quantification was performed by peak area measurement and by use of a standard curve. A detailed description of the high pressure liquid chromatographic system is contained in Exhibit I.

Reference:

- (1) Simons, S.S., Jr. and Johnson, D.F., J. Am. Chem. Soc. 98, pp. 7098-1099, 1976.

Appendix II
(continued)

I. REAGENTS AND EQUIPMENT

Plasma Assay

Varian Model 3700 gas chromatograph; equipped with a Ni⁶³ electron-capture detector and capillary pneumatics.

The gas chromatograph was fitted with a fused silica capillary column (Chrompack-Netherland B.V.) 25 meters x 0.25 mm i.d. coated with OV-101.

Carrier gas was dry, oxygen-free high purity helium at a linear velocity of 30 cm/sec.

Make-up gas was dry, oxygen-free high purity nitrogen, at 30 mL/min.

The temperatures used were: injector 220°C, detector 200°C, oven temperature at 120°C isothermal.

Injections were made in the split mode, with a split ratio of 50:1.

Trifluoroacetic anhydride, Pierce Chemical Company

2-amino-3-phenyl-1-propanol hydrochloride, Aldrich Chemical Company,

4-Dimethylaminopyridine, Aldrich Chemical Company.

Appendix II
(continued)

Urine Assay

Water's Model 6000 A Pump (for mobile phase)

Water's Model 6000 A or Milton Roy Model 196
(for Fluoropa[®] Solution)

Water's Model 710 A WISP Automatic Sample Processor

Coiled teflon tubing (15' x 0.027" i.d.) as a post-
column, in-line reactor

Thermonix 420 BKU water bath, room temperature

Schoeffel FS 970, L.C. Fluorometer

25 cm x 4.6 mm ODS-Hypersil 5 micron column, Shandon
Southern

Spectra-Physics 4100 Integrator-Calculator

Gelman Instrument Co., Glass Fiber Filter type A/E,
47 mm

Fluoropa[®] crystals, Pierce Chemical Co.

Phenylpropanolamine HCl, Sigma

Amphetamine Sulfate

Appendix II
(continued)

II. PROCEDURES

A. Plasma Assay

Standard Solutions Preparation

Phenylpropanolamine HCl (Sigma) was used to prepare standard solutions. 2-amino-3-phenyl-1-propanol hydrochloride (Aldrich) was used to prepare internal standard solutions for the plasma assay. Phenylpropanolamine HCl (25 mg) was accurately weighed, transferred to a 25 ml volumetric flask, and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the phenylpropanolamine HCl stock standard solution (1 mg/ml).

2-amino-3-phenyl-1-propanol hydrochloride (10 mg) was accurately weighed, transferred to a 10 ml volumetric flask and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the 2-amino-3-phenyl-1-propanol hydrochloride stock internal standard solution (1 mg/ml).

Standard Curves, Spiked Plasma

Standard curves were generated by spiking control plasma samples (1 ml) with varying amounts of phenylpropanolamine HCl, and a constant amount of internal standard.

An aliquot (1.0 ml) of the phenylpropanolamine stock solution (1 mg/ml) was transferred to a 1L volumetric flask, and brought to mark with distilled water. Varying microliter aliquots of this dilution (1 µg/ml) were added to control plasma so that final concentrations were 5.23, 20.94, 104.70, 157.05 and 261.75 ng/ml. An aliquot (1.0 ml) of the 2-amino-3-phenyl-1-propanol hydrochloride stock internal standard solution (1 mg/ml) was transferred to a 1L volumetric flask, and brought to mark with distilled water. An aliquot (200 µl) of this dilution was added to each of the five standard plasma solutions for a final concentration of approximately 200 ng/ml. Standard concentrations of phenylpropanolamine HCl in plasma were converted to the equivalent base concentrations by multiplication by 0.3055.

Appendix II
(continued)

Analysis of Plasma

Extraction and derivatization of phenylpropanolamine was carried out in silanized-glass 15 ml Teflon® stoppered centrifuge tubes. Plasma samples (1.0 ml) were spiked with internal standard 2-amino-3-phenyl-1-propanol hydrochloride (200 ng), followed by the addition of KH_2PO_4 buffer (0.2 ml of a 0.5 M solution, pH 11.0), saturated sodium chloride (0.2 ml), and toluene (1.5 ml). The centrifuge tubes were then sealed with teflon stoppers, and shaken by vortex mixing for 1.5 minutes. They were then centrifuged at 2000 RPM for 5 minutes, and the upper organic layer transferred to a second set of silanized 15-ml centrifuge tubes. The remaining aqueous phases were re-extracted with toluene (1.5 ml) by vortex mixing for 1.5 minutes. They were then centrifuged at 2000 RPM for 5 minutes, and the upper organic layer combined with the first toluene extracts.

The combined toluene extracts were then concentrated to approximately 0.5 ml under nitrogen, in a 40°C water bath. 4-dimethylaminopyridine (0.2 mg dissolved in 50 μl toluene) and trifluoroacetic anhydride (70 μl) were then added, and the tubes stoppered with teflon stoppers. The tubes were then heated in a 60°C water bath for 45 minutes. Na_2HPO_4 buffer (2 mls of a 0.5 M solution, pH 6.0) was then added to the tubes, followed by vortex mixing. The toluene layer was then transferred to a third set of silanized centrifuge tubes, followed by injection of microliter aliquots into the chromatographic system. Retention times of the di-trifluoroacetyl derivatives of phenylpropanolamine and 2-amino-3-phenyl-1-propanol were 2.5 and 3.5 minutes respectively.

B. Urine Assay

Standard Solution Preparation

Phenylpropanolamine HCl (Sigma) was used to prepare standard solutions. Amphetamine sulfate (Sigma) was used to prepare internal standard solutions for the urine assay. Phenylpropanolamine HCl (25 mg) was accurately weighed, transferred to a 25 ml volumetric flask, and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the phenylpropanolamine HCl stock standard (1 mg/ml).

Appendix II
(continued)

Amphetamine sulfate (30 mg) was accurately weighed, transferred to a 500 ml volumetric flask and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the amphetamine sulfate stock standard solution (60 µg/ml).

The mobile phase was prepared by adding 6.8 gm KH_2PO_4 , 1.9 gm hexanesulfonate sodium (Regis Chemical Co.), and 1.0 ml triethylamine (Pierce Chemical Co.) to 950 ml distilled water. The pH was adjusted to 3.0 with H_3PO_4 . The solution was then transferred to a 1L volumetric flask and brought to mark with distilled water. Methanol (400 ml) was added to a 1L volumetric flask, which was then brought to mark with the above solution. The mobile phase was vacuum filtered through a 0.3 µ filter before use. The volumetric flow rate of the mobile phase was 1.5 ml/minute. Retention time of phenylpropanolamine and amphetamine were 5.8 minutes and 8.5 minutes respectively.

The eluent from the reverse phase column was mixed with a Fluoropa® solution which was introduced into the solvent stream via a T-fitting (Kel-F) at the rate of 1.5 ml/minute. The Fluoropa® solution was prepared by adding 25 gm boric acid to 950 ml distilled water in a 2L beaker. The pH was adjusted to 10.4 with 50% KOH in water. 2-mercaptoethanol (2.0 ml) was added to the solution, followed by the addition of Fluoropa® (800 mg o-phthalaldehyde) previously dissolved in approximately 10 ml methanol. This solution was vacuum filtered through a 0.3 µ filter before use.

Standard Curves, Spiked Urine

Standard curves were generated by spiking control urine samples (2.0 ml) with varying amounts of phenylpropanolamine HCl, and a constant amount of internal standard.

Varying microliter aliquots of the phenylpropanolamine HCl stock solution were added to control urine so that the final concentrations were 0.955, 3.82, 9.55, 38.2 and 95.5 µg/ml. An aliquot (1.0 ml) of the amphetamine sulfate stock solution was added to each of the five standard urine solutions for a final concentration of approximately 20 µg/ml. Standard concentrations of phenylpropanolamine HCl in urine were converted to the equivalent base concentrations by multiplication by 0.8055.

Analysis of Urine

Urine samples (2.0 ml) were transferred to 15-ml stoppered centrifuge tubes, followed by addition of internal standard amphetamine sulfate (approximately 60 µg). The solution was mixed on a vortex mixer, followed by centrifugation at 2000 RPM for five minutes. The supernatant was transferred to auto-sampler vials by pipet for injection into the chromatographic system.

III.

PLAN OF STUDY

The assay methods described in this report for measuring phenylpropanolamine levels in urine and plasma were evaluated for their precision, accuracy, reproducibility, specificity and linearity. In addition to this, the stability of phenylpropanolamine in urine and plasma was evaluated.

To evaluate the linearity of the methods, a five point standard curve over the expected concentration ranges was constructed. The precision of the method for measurement of drug in plasma and urine was determined as the coefficient of variation of the mean of five replicate assays at each level. Accuracy and reproducibility of the assays were determined by analysis of plasma and urine samples which had been spiked with phenylpropanolamine HCl, split into aliquots, frozen, and assayed on different days. Specificity of the methods for phenylpropanolamine and internal standards was determined.

Stability of the drug in urine was evaluated by analysis of samples which had been spiked with phenylpropanolamine HCl, split into aliquots, and stored at room temperature, 4°C and -20°C. Stability was evaluated over a one month period of time. Stability of the drug in plasma was evaluated in the same manner, except that spiked plasma samples were only stored at -20°C.

IV.

RESULTS AND CONCLUSIONS

Concentrations of phenylpropanolamine in the biological fluids were determined from calibration graphs constructed by plotting the ratio of peak-area measurements of the

Appendix II
(continued)

drug to the internal standard (2-amino-3-phenyl-1-propanol in plasma and amphetamine in urine) against the concentrations of phenylpropanolamine in the standards. The concentration ranges for the standards in plasma and urine were 5.23-261.75 ng/ml and 0.955-95.5 µg/ml, respectively. Plots of peak area ratios (Y) against phenylpropanolamine concentration (X) were linear.

Typical standard curves for phenylpropanolamine in plasma and urine had correlation coefficients of 0.9997 and 0.9999 respectively. Sample standard curves for plasma and urine are shown in Figures 1 and 2 respectively. Sample chromatograms of plasma and urine extracts are shown in Figures 3 and 4 respectively. The methods were specific for drug in that control samples of plasma and urine contained no responses which interfered with either phenylpropanolamine or internal standards.

The precision of the method for measurement of plasma concentrations of phenylpropanolamine, determined as the coefficient of variation of the mean of five replicate assays, was ± 5.63 , 1.80 , ± 6.48 , ± 1.64 , and $\pm 1.63\%$ at 5.23, 20.94 , 104.70 , 157.05 and 261.75 ng/ml respectively. Precision for measurement of urine concentrations of phenylpropanolamine was ± 2.23 , ± 0.55 , 0.41 , ± 0.64 , and $\pm 0.19\%$ at 0.955, 3.82 , 9.55 , 38.2 , and 95.5 µg/ml respectively. The above data are shown in Table I and Ia.

Reproducibility and accuracy of the assays was determined by analysis of blank plasma and urine samples which had been spiked with known amounts of phenylpropanolamine HCl. They were each then divided into aliquots and frozen, followed by analysis on consecutive days. The results of these analyses are shown in Table II and III for plasma and urine respectively. A given extract was injected into the chromatographic systems five successive times to determine the variation of detector response. The results are shown in Table IV. Reproducibility of the plasma assay was $\pm 7.03\%$ (Table II) while that of the urine assay was $\pm 2.69\%$ (Table III). Accuracy of the plasma assay measured as the percent difference between actual amount spiked into the plasma and the average of five assay values was 6.37%. Accuracy of the urine assay measured as the percent difference between the actual amount spiked into urine and the average of eight assay values was 1.58%. The variation of detector response measured as the coefficient of variation of area ratios between standard and internal standard for five successive injections of the same extract was $\pm 0.53\%$ and $\pm 0.75\%$ for plasma and urine respectively (Table IV).

Appendix II.
(continued)

Stability of the drug in plasma was determined by assay of control plasma samples which had been spiked at three levels (approximately 20, 100 and 190 ng/ml), separated into separate aliquots, and stored in silicone coated blood collection tubes at -20°C . Aliquots were assayed periodically over a 33 day period. The results are shown in Table V. The drug in plasma at levels of approximately 100 and 190 ng/ml are stable after 33 days of storage. At approximately 20 ng/ml, however, the level of drug fell off at day 33. It was therefore concluded that at this lower level, samples are stable for at least 28 days, but not 33 days. Samples from clinical studies must therefore be assayed within 28 days of collection.

Stability of the drug in urine was determined by assay of control urine samples which had been spiked at three levels (approximately 2, 25 and 50 $\mu\text{g}/\text{ml}$), separated into separate aliquots, and stored in brown one quart polyethylene bottles at three different temperatures (room temperature, 4°C and -20°C). Aliquots were assayed over a 28 day period. The results are shown in Table VI. The drug in urine at all three levels is only stable for 7 days when stored at room temperature. When stored at 4°C , the drug is stable for 11, 15 and 15 days at levels of approximately 2, 25 and 50 $\mu\text{g}/\text{ml}$ respectively. The drug at all levels in urine was stable for the full 28 days when stored at -20°C .

Appendix II,
(continued)

TABLE I

Precision of the method for measurement of plasma concentrations of phenylpropanolamine determined as the coefficient of variation of the mean of five replicate assays.

<u>Plasma</u>	<u>Phenylpropanolamine Concentration (ng/ml)</u>	<u>N</u>	<u>Area Ratio</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Coefficient of Variation</u>
	5.23	4	0.0811	0.0868	0.0049	±5.63%
	5.23		0.0926			
	5.23		0.0850			
	5.23		0.0883			
	20.94	5	0.2808	0.2812	0.0050	±1.80%
	20.94		0.2806			
	20.94		0.2770			
	20.94		0.2778			
	20.94		0.2897			
	104.70	4	1.4860	1.3619	0.0883	±6.48%
	104.70		1.2987			
	104.70		1.364			
	104.70		1.2987			
	157.05	5	1.9904	1.9616	0.0322	±1.64%
	157.05		1.9534			
	157.05		1.9580			
	157.05		1.9139			
	157.05		1.9930			
	261.75	5	3.2536	3.1852	0.0520	±1.63%
	261.75		3.1621			
	261.75		3.1930			
	261.75		3.2039			
	261.75		3.1132			

Appendix II,
(continued)

TABLE Ia

Precision of the method for measurement of urine concentrations of phenylpropanolamine determined as the coefficient of variation of the mean of five replicate assays.

<u>Urine</u>	<u>Phenylpropanolamine Concentration (ug/ml)</u>	<u>Area Ratio</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Coefficient of Variation</u>
	0.955	0.01529			
	0.955	0.01551			
	0.955	0.01502	0.01506	0.00034	±2.23%
	0.955	0.01471			
	0.955	0.01479			
	3.82	0.06072			
	3.82	0.06085			
	3.82	0.06106	0.06094	0.00034	±0.55%
	3.82	0.06060			
	3.82	0.06146			
	9.55	0.1566			
	9.55	0.1557			
	9.55	0.1560	0.1556	0.00064	±0.41%
	9.55	0.1563			
	9.55	0.1546			
	38.2	0.6411			
	38.2	0.6526			
	38.2	0.6475	0.6475	0.0041	±0.64%
	38.2	0.6486			
	38.2	0.6478			
	95.5	1.6369			
	95.5	1.6303			
	95.5	1.6305	1.6324	0.0031	±0.19%
	95.5	1.6345			
	95.5	1.6298			

Appendix II
(continued)

TABLE II

Reproducibility and accuracy of plasma assay. Plasma was spiked with 99.43 ng/ml phenylpropanolamine HCl, then separated into five separate aliquots which were stored at -20 C. These aliquots were assayed on five separate days over a 97 day period.

Phenylpropanolamine HCl
Determined (ng/ml)

97.12
102.34
87.12
87.55
91.33

Average (ng/ml) and
Coefficient of Variation

93.09($\pm 7.03\%$, n = 5)

Percent difference between
actual plasma level and
the average determined
level

6.37

Appendix II
(continued)

Table III

Reproducibility and accuracy of urine assay. Urine was spiked with 22.72 $\mu\text{g/ml}$ phenylpropanolamine HCl, then separated into 8 separate aliquots which were stored at -20°C . These aliquots were assayed two at a time on four different days over a two week period.

<u>Phenylpropanolamine HCl Determined ($\mu\text{g/ml}$)</u>	<u>Average ($\mu\text{g/ml}$) and Coefficient of Variation</u>
22.73	
22.02	
21.65	
21.45	
22.47	
22.93	
23.13	
22.48	
	22.36 ($\pm 2.69\%$, $n = 8$)
	Percent difference between actual urine level and the average determined level
	----- 1.58

Appendix II,
(continued)

Table IV

Reproducibility of detector response. The same extracts of phenylpropanolamine from plasma and from urine were injected into the GLC (for plasma) and the H.P.L.C. (for urine) five times on the same day.

<u>Plasma</u>	<u>Phenylpropanolamine HCl Concentration (ng/ml)</u>	<u>Area Ratio</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Coefficient of Variation</u>
	104.70	1.3605			
	104.70	1.3636			
	104.70	1.3778	1.3652	0.0072	+0.53%
	104.70	1.3605			
	104.70	1.3636			
<u>Urine</u>	<u>Phenylpropanolamine HCl Concentration (µg/ml)</u>	<u>Area Ratio</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Coefficient of Variation</u>
	22.72	0.2676			
	22.72	0.2706			
	22.72	0.2707	0.2708	0.00203	±0.75%
	22.72	0.2727			
	22.72	0.2724			

Appendix II
(continued)

Table V

Stability of phenylpropanolamine HCl in plasma. Plasma was spiked with phenylpropanolamine HCl at three levels (approximately 20, 100, and 190 ng/ml), then separated into separate aliquots which were stored in silicone coated 10 ml blood collection tubes (B-D Vacutainer Brand) at -20°C. Aliquots were assayed periodically over a 33 day period.

Day	Phenylpropanolamine HCl Determined (ng/ml)		
0	21.5	106.2	190.0
1	20.0	102.4	184.1
7	23.3	111.1	183.3
12	26.7	108.9	187.8
15	24.4	118.9	198.9
21	22.0	100.0	177.0
28	23.0	114.0	198.0
33	18.8	106.4	187.1
Average	22.5	108.5	188.3
Standard Deviation	2.5	6.2	6.3
Coefficient of Variation	+11.11%	+5.71%	+3.35%

Appendix II
(continued)

Table VI

Stability of phenylpropanolamine HCl in urine. Urine was spiked with phenylpropanolamine HCl at three levels (approximately 2, 25 and 50 µg/ml), then separated into separate aliquots which were stored in one quart polyethylene bottles (normally used for urine collection in clinical studies) at three different temperatures (room temperature, 4°C and -20°C). Aliquots of these solutions were assayed periodically over a 28 day period.

Phenylpropanolamine HCl Determined (µg/ml)

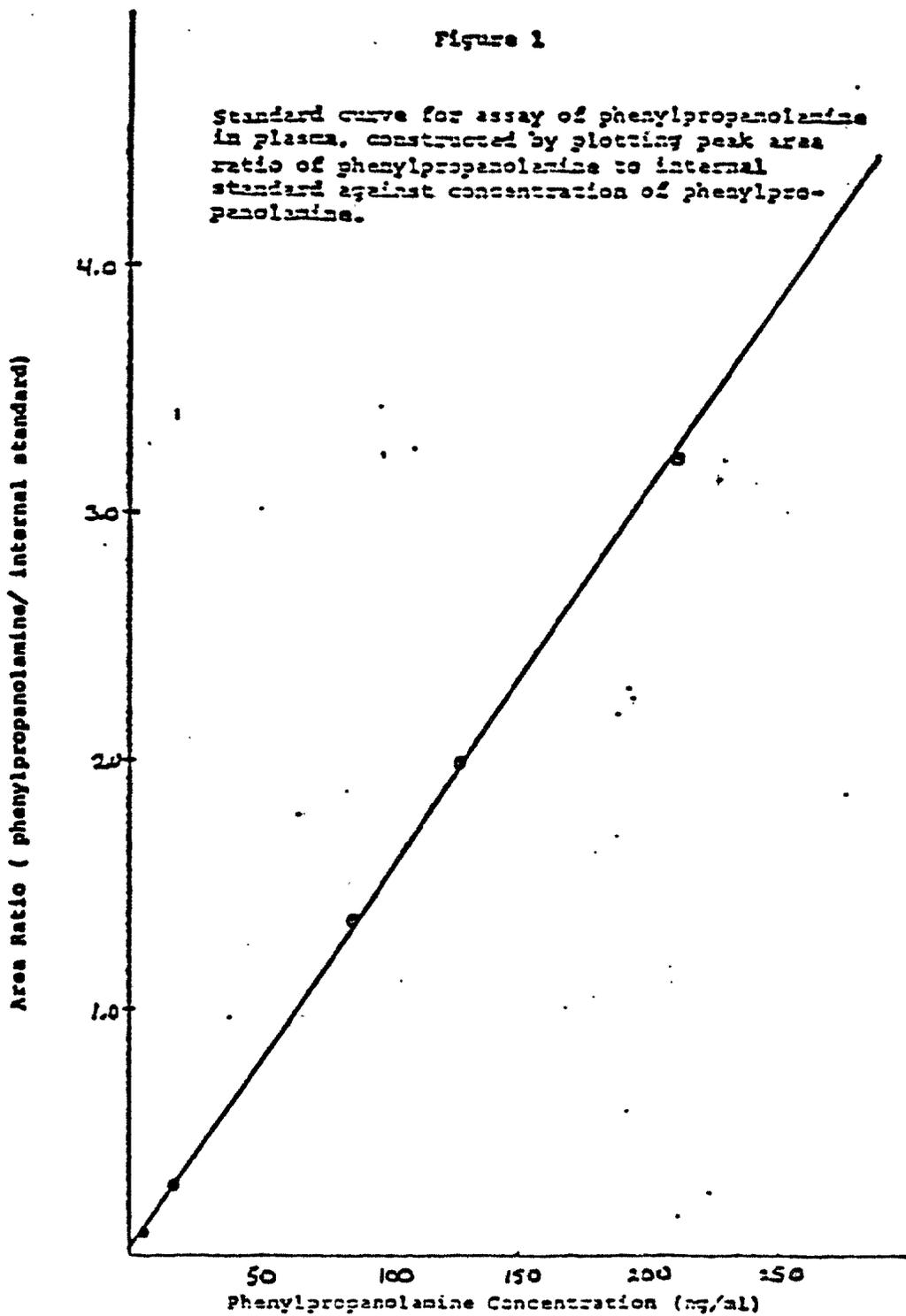
Day	Room Temp	4°C	-20°C	Room Temp	4°C	-20°C	Room Temp	4°C	-20°C
0	1.76	1.81	1.77	21.79	23.82	24.39	49.87	49.39	48.69
3	2.13	2.12	2.10	24.82	25.01	24.87	49.73	50.04	50.11
7	2.23	2.15	2.08	22.52	24.67	24.79	50.22	50.97	50.67
11	1.90	1.96	2.29	18.19	23.51	24.39	44.74	51.60	48.12
15	0.95	1.46	2.10	15.43	24.76	24.69	38.56	50.88	49.77
23	1.03	1.37	2.12	13.01	21.29	22.80	25.53	46.43	50.93
28	1.33	1.68	2.50	14.10	23.48	24.66	25.73	47.43	48.58

4:1615303:1M1CMG
3/24/82

Appendix II
(continued)

Figure 1

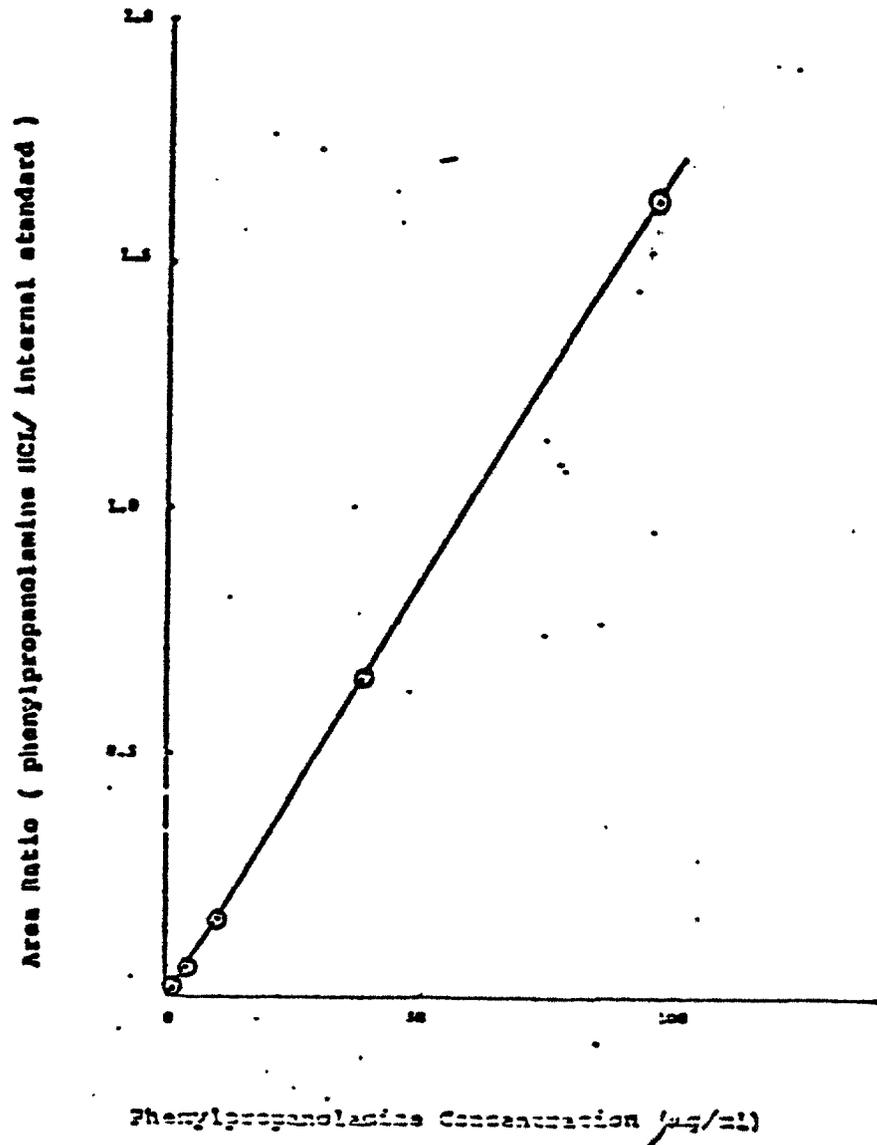
Standard curve for assay of phenylpropanolamine in plasma, constructed by plotting peak area ratio of phenylpropanolamine to internal standard against concentration of phenylpropanolamine.



Appendix II
(continued)

Figure 2

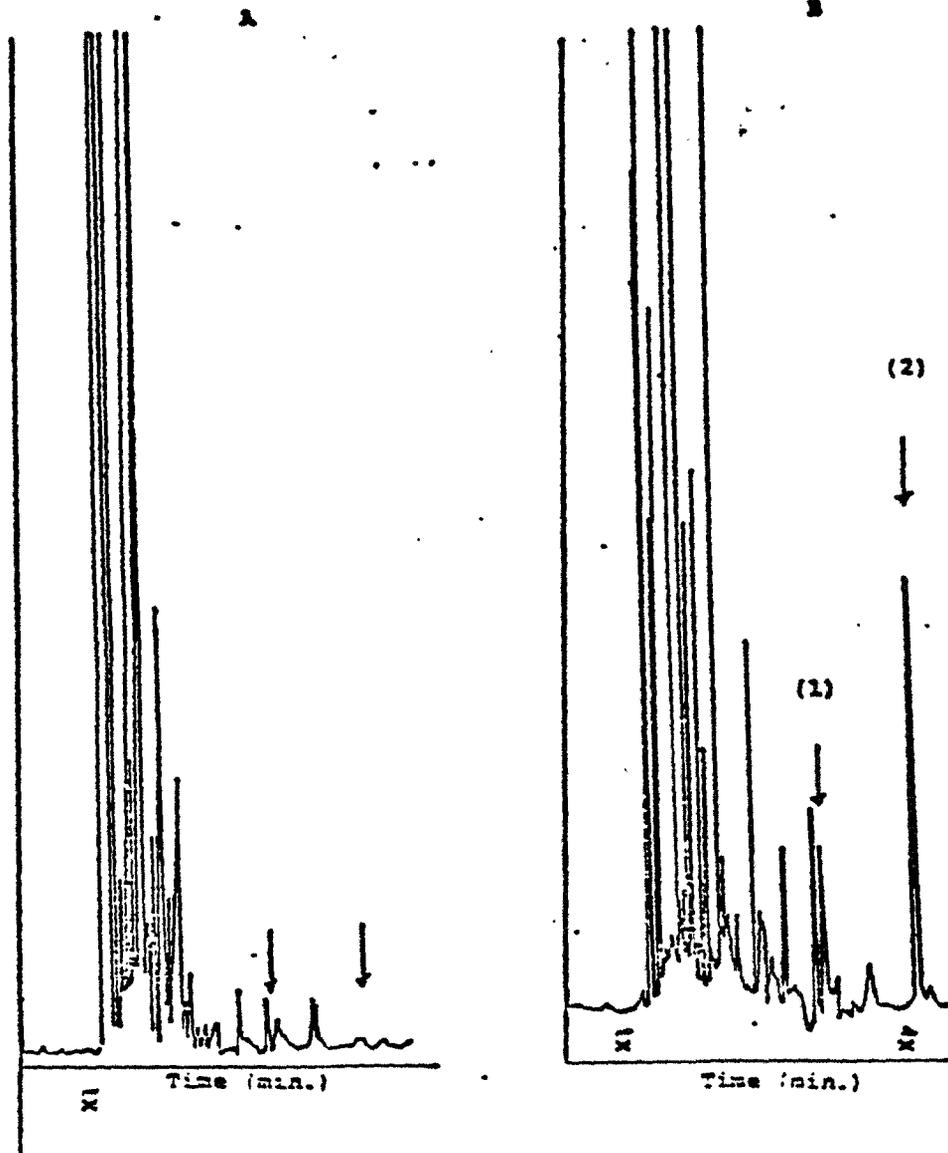
Standard curve for assay of phenylpropanolamine in urine, constructed by plotting peak area ratio of phenylpropanolamine to internal standard against concentration of phenylpropanolamine.



Appendix II
(continued)

Figure 3

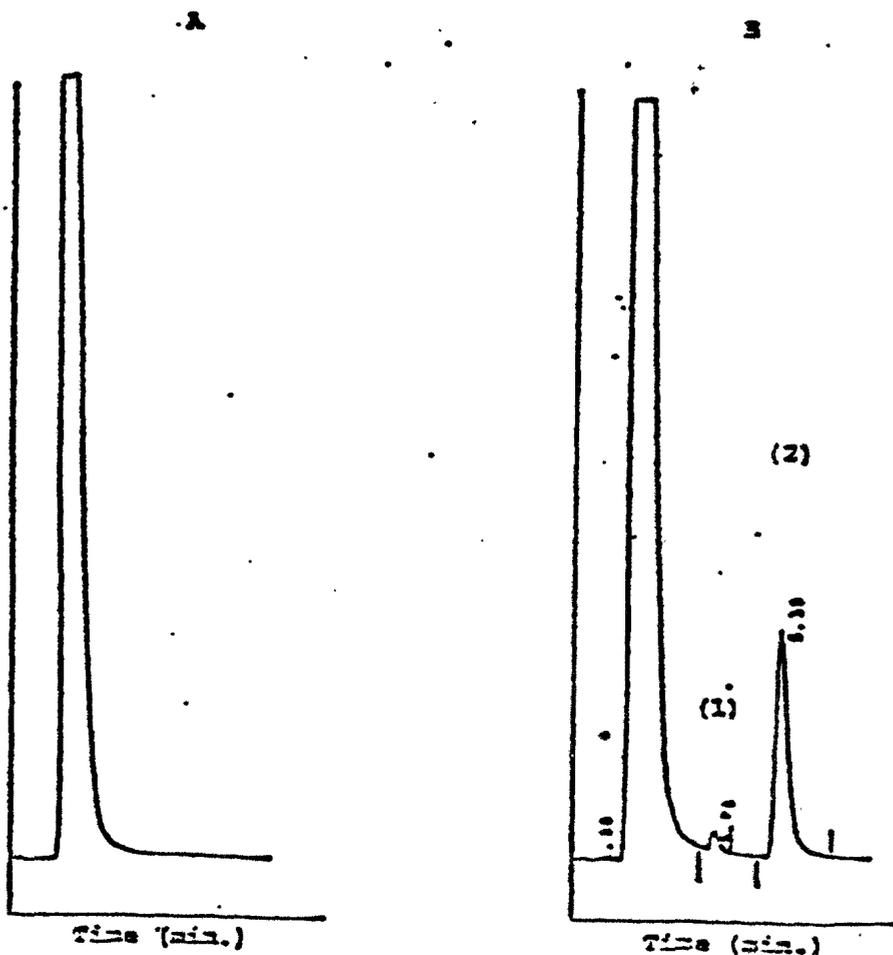
Chromatograms of (A) control extract of 1 ml plasma and (B) extract of 1 ml plasma containing 5.23 ng/ml of phenylpropanolamine HCL (1) and approximately 200 ng 2-amino-3-phenyl-1-propanol hydrochloride (2).



Appendix II.
(continued)

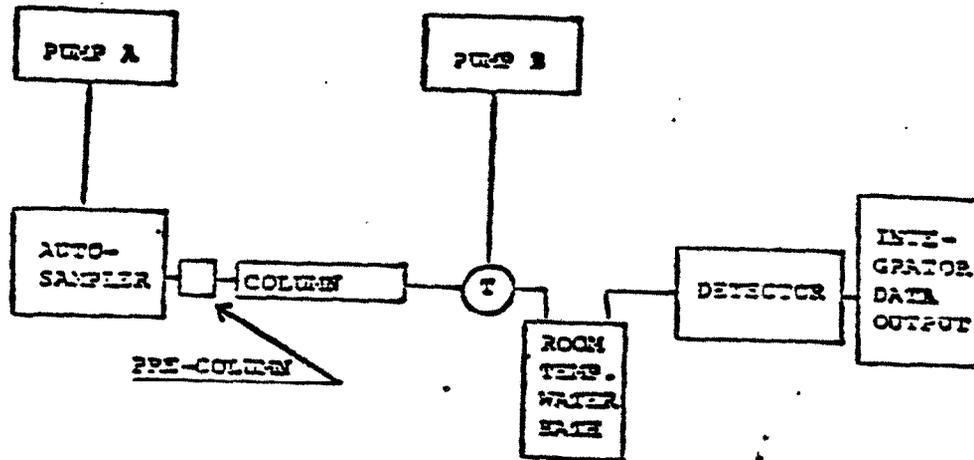
Figure 4

Chromatograms of (A) control sample of urine;
and (B) control sample of urine containing
2.27 μ g/ml phenylpropanolamine HCl (1) and
approximately 60 μ g amphetamine sulfate (2).



Appendix II
(continued)

EXHIBIT I



- PUMP A: Mobile phase delivery at 1.5 ml/min.
PUMP B: Fluoropa[®] solution delivery at 1.5 ml/min.
AUTOSAMPLER: WISP 710A or equivalent
COLUMN: ODS-Spherasil, Shandon Southern
PPE-COLUMN: Waters's Bondapak C₁₈/Corasil[®]
T: LC Teflon Tee joint
WATER BATH: 15'x0.027" coiled teflon tubing
which serves as the in-line reactor is
immersed in this room-temperature
water bath
DETECTOR: Fluorometer, Schoeffel or equivalent
excitation at 340 nm
emission cutoff at 418 nm
INTEGRATOR: Spectra-Physics 4100 or equivalent

Assay of Residual PPA.HCl Content
of GITS Recovered from Stools

INTRODUCTION

The following is a description of a high pressure liquid chromatographic method for the determination of phenylpropanolamine.HCl (PPA.HCl) content in OROS®. The determination involves crushing the systems and dissolving the particles in distilled water, and injecting a filtrate of this solution into the chromatographic system. The compound is resolved on a reverse phase column and detected by UV absorption at 254 nm. Quantification is obtained by linear regression analysis of peak areas of a standard curve containing at least three standard points. Results will be reported as the HCl salt of PPA. This assay will resolve PPA from α -aminopropiophenone.

SAMPLE PREPARATION

Accurately weigh (mg) each system, then place each system between two plastic weigh boats and crush with a rubber mallet. Quantitatively transfer the crushed system particles to a 250 ml volumetric flask and add about 100 ml distilled, deionized water. Place the volumetric flask in a sonic bath for 10 minutes, to dissolve the drug particles. Cool to room temperature, then fill each flask to volume with H₂O and mix. Filter a portion from each flask and inject 40 μ l into the chromatographic system.

STANDARD PREPARATION

For analysis of systems containing 75 mg of drug, accurately weigh about 60 mg PPA.HCl USP Reference Standard, or equivalent, and transfer quantitatively to a 50 ml volumetric flask.* Fill to volume with H₂O and mix. Prepare working standard dilutions by accurately pipeting the following volumes of PPA.HCl standard stock solution and H₂O into appropriate glass test tubes, and mix. Assuming 60 mg PPA.HCl was used to prepare the standard stock solution, the following calibration standards would be generated:

<u>PPA.HCl Stock</u> <u>(ml)</u>	<u>H₂O</u> <u>(ml)</u>	<u>Final Volume</u> <u>(ml)</u>	<u>PPA.HCl</u> <u>(mg/ml)</u>
1.00	5.00	6.00	0.200
1.00	3.00	4.00	0.300
1.00	2.00	3.00	0.400

Prepare standards daily prior to analysis.

*NOTE: For analysis of systems containing other than 75 mg of drug, divide the expected (labeled) system PPA.HCl content by 75. Then, multiply the product by 60 to get the amount of PPA.HCl needed to prepare a stock solution that, when diluted as suggested above, will bracket the expected sample concentration.

Appendix III
(continued)

ANALYSIS

Assemble a liquid chromatograph employing a controlled volume pumping system, a sample injection device, a UV detector capable of detection at 254 nm and a suitable recorder and/or integrator. Use the chromatographic column as indicated.

EQUIPMENT

Pump: Waters 6000 A or equivalent
Detector: Waters M440 or equivalent
Injector: Waters WISP 710 A or B Automatic Sample Processor, or Rheodyne 7105, or equivalent.
Column: Waters Micro Bondapak C18 10 micron or equivalent
Recorder: mV output matched to detector output
Integrator: Spectra Physics 4100, or equivalent

OPERATING PARAMETERS

Flow Rate: 1.5 ml/min
Pressure: 2500 psig
Detector Wavelength: 254 nm
Chart Speed: 0.2 in/min or 0.5 cm/min
Injection Volume: 40 µl
Column Temp: Ambient
Attenuation: 0.05 AUFS
Retention Time: PPA 7.4 min (nominal)

Appendix III
(continued)

REAGENTS

Mobile Phase: 40:60 MeOH:buffer

Prepare as follows:

To a 1 liter volumetric flask add 700 ml distilled H₂O, 50 ml of 1 M NaH₂PO₄, pH 7, 1.9 g Hexane Sulfonate Na, and 20 ml of 0.25 M triethylammonium phosphate, pH 7.3: Fill to volume with H₂O and mix. Transfer contents to a 2 liter erlenmeyer flask and add 667 ml MeOH. Mix and degas by vacuum filtration.

COLUMN PERFORMANCE

Assemble the specified chromatographic system. To condition the column, set the monitoring wavelength and pass mobile phase through the column at the flow rate to be used for analysis. Equilibrate the system until a steady baseline is obtained and column pressure is stabilized. If repeated sample injections give a stable retention time, proceed to analyze the samples and record the actual conditions used for the analysis.*

*NOTE: If α -aminopropiophenone is to be quantified, inject an aliquot of a test mixture prepared by adding 0.1 ml of α -aminopropiophenone Stock Standard¹ to 9.9 ml of one of the PPA-HCl calibration standards. If a resolution factor of greater than 1 is obtained, proceed to analyze the sample preparations.

CALCULATIONS

IDENTITY

Identify the PPA peak (and, α -aminopropiophenone peak, if present) by comparison of the retention time of the sample preparation(s) with that of the

¹Prepare a Stock Standard of α -aminopropiophenone-HCl as follows:

Weigh 25 mg of α -aminopropiophenone-HCl USP Reference Standard, or equivalent, and quantitatively transfer to a 50 ml volumetric flask. Dissolve and fill to volume with distilled water. If α -aminopropiophenone is detected in sample preparation(s), then dilute this Stock Standard with 0.05 M phosphate buffer, pH 6.5, to obtain 1, 2, 4, and 6 mcg/ml of α -aminopropiophenone-HCl working standards.

Appendix III
(continued)

standard preparation(s). If the retention times match, sample peaks are identified.

CONCENTRATION

Construct a standard curve by plotting concentrations (mg/ml) of PPA·HCl vs. peak area on linear graph paper, or by calculating the best straight line by linear regression analysis. Measure the peak area of the Sample Preparations and determine the concentration of PPA·HCl in the samples from the standard curve. Then calculate:

A. $\text{mg PPA}\cdot\text{HCl in system} = C \times 250 \text{ ml}$

B. $\text{Wt \% PPA}\cdot\text{HCl in system} = \frac{C \times 250 \text{ ml}}{W} \times 100\%$ where

C = concentration of sample solution obtained from standard curve, in mg/ml

W = weight of system, in mg

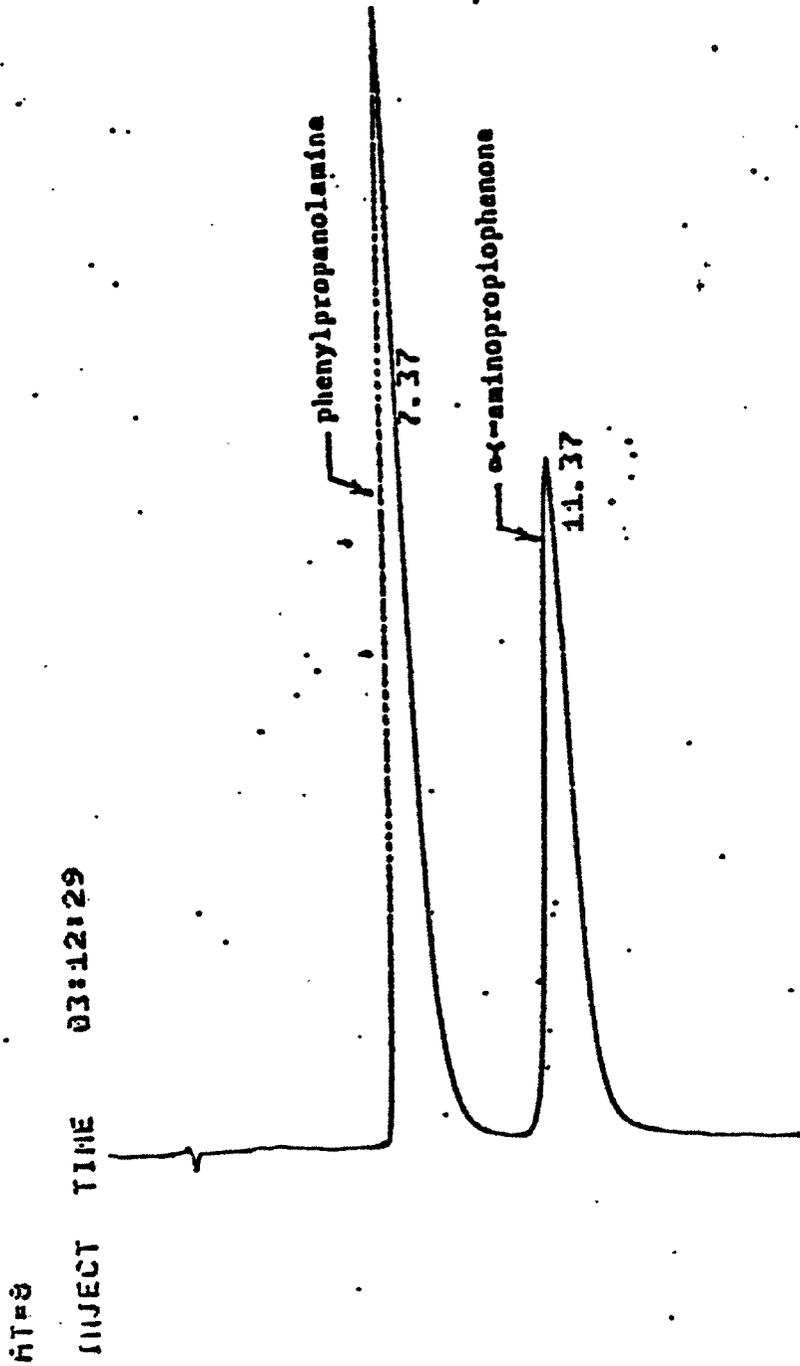
C. From the individual assay results above, calculate the average drug content and standard deviation

NOTE: The same calculation may be used for quantifying α -aminopropiophenone using a standard curve obtained from the working standards suggested on pg. 3. Additional working standards may be prepared to bracket the detected concentration of α -aminopropiophenone in the sample preparation(s).

This method developed by Tom East

Appendix III
(continued)

SAMPLE CHROMATOGRAM



Assay of Dosage Forms for PPA.HCl Content

Gastrointestinal Therapeutic Systems Lot #614982		Dexatrim Capsules Lot #MDF1280A		Aqueous Solution diluted from Dietac Drops Lot #X908			
Sample #	MG PPA.HCl	Sample #	MG PPA.HCl	Week	MG PPA.HCl		
					25 mg Dose	37.5 mg Dose	
1	76.69	1	84.24		1	27.20	37.53
2	75.75	2	68.83		2	28.04	36.15
3	77.51	3	68.72	1	3	28.04	37.53
4	75.30	4	92.00		4	25.13	37.53
5	76.16	5	78.07		5	<u>25.82</u>	<u>36.15</u>
6	76.36	6	82.40		Mean	26.8	37.0
7	75.58	7	71.11		±S.E.M.	0.6	0.3
8	76.48	8	76.76				
9	76.68	9	71.11		1	24.41	37.55
10	<u>76.86</u>	10	<u>71.11</u>		2	24.45	37.73
	76.3		76.4	3	3	24.22	37.70
		±S.E.M.	2.5		4	24.41	37.78
					5	<u>24.82</u>	<u>37.98</u>
					Mean	24.5	37.8
					±S.E.M.	0.1	0.0
					1	25.50	37.33
					2	- *	37.27
				5	3	25.48	37.47
					4	25.14	37.23
					5	<u>26.37</u>	<u>38.01</u>
					Mean	25.6	37.5
					±S.E.M.	0.3	0.2
Mean Total Dose, MG	1831		1834				1833

* Result not included since value is more than 10 standard deviations from mean.

Statistics Report
(ST-141-83)*

PHENYLPROPANOLAMINE ABSORPTION DURING ORAL ADMINISTRATION
FROM GASTROINTESTINAL THERAPEUTIC SYSTEMS - STUDY 1

Elizabeth A. Leszczak

September 19, 1983

Distribution:

Mr. Richard Braun
Dr. Lewis Leeson
Mr. Jerry Ostrov
Dr. Elliot Redalieu
Ms. Elizabeth Leszczak
Statistical Files

*This report corrects results previously reported in Statistics report ST-123-82, which it supersedes.

Statistics Report
(ST-141-83)

PHENYLPROPANOLAMINE ABSORPTION DURING ORAL ADMINISTRATION
FROM GASTROINTESTINAL THERAPEUTIC SYSTEMS - STUDY 1

OBJECTIVE: To compare the bioavailability and the profiles for plasma levels and total urinary excretion for the following three oral dosage forms of phenylpropanolamine:

- (1) The Gastrointestinal Therapeutic System (GITS)
- (2) Dexatrim 12 hour sustained release capsules
- (3) Aqueous solution

DESIGN: Six subjects received 75 mg PPA HCL per day from one of the oral dosage forms indicated above, for four consecutive days during weeks one, three, and five of the study, according to a 3 x 3 Latin square design. Blood samples were drawn during days one and four of the dosing cycle and assayed for phenylpropanolamine HCL. Urine was collected during the entire dosing cycle and the GITS were recovered from stools.

STATISTICAL METHODS: The following parameters were analyzed by analysis of variance (Grizzle)¹:

- Area under the curve for day one
- Area under the curve for day four
- Total urinary excretion.

In addition, Westlake's confidence intervals² were calculated for each pair of dosage forms for these three parameters.

Plasma levels were also analyzed by a repeated measures analysis of variance (ANOVA)³. This analysis tests the null hypothesis of equality of all formulation means, as well as parallelism of the

response curves over time (formulation by time interaction). Comparisons between formulations at each time point were made using Student's t tests.

Since the ANOVA table for the repeated measures analysis contains three "error" terms (main plot error, subplot error, and the subject by time interaction), appropriate error terms for performing the tests at each time point were constructed as linear combinations of the main plot and subplot mean squares^{4, 5}.

For plasma levels below the level of sensitivity of the assay method (6.2), the value of 6.2 was used in the calculation of means and in the analysis of variance.

RESULTS AND CONCLUSIONS: There were no significant differences among the three oral dosage forms for bioavailability as measured by area under the curve at either day one ($p=0.38$), or day four ($p=0.92$), or in total urinary excretion ($p=0.22$). Significant differences in the shapes of the plasma concentration time curves are indicated by the highly significant formulation by time interaction (Table 2) and the plot of mean plasma levels (Figures 1 and 2). These differences can also be seen from the comparisons of the three formulations at each time point as presented in Table 1.

Elizabeth A. Leszczak 9-22-83
Elizabeth A. Leszczak, M.S. Date
Statistician I

Approved: *Murray Selwyn* 9/22/83
Murray R. Selwyn, Ph.D. Date
Director,
Statistics and Data Systems

Records are on file and available for inspection in the offices of Research Statistics in Summit, New Jersey.

REFERENCES AND NOTES:

1. Grizzle, James E. "The Two-Period Changeover Design and Its Use in Clinical Trials", *Biometrics* 21, (June, 1965), pp. 467-480.
2. Westlake, W.J. "Use of Confidence Intervals in Analysis of Comparative Bioavailability Trials". *J. Pharm. Sci.* (1972) 61, pp. 1340-1341.
3. Westlake, W.J. "The Use of Balanced Incomplete Block Designs in Comparative Bioavailability Trials". *Biometrics* 30, (June, 1974). pp. 319-327.
4. Cochran, W.G. and Cox, G.M. *Experimental Designs*. Wiley (1957). pp. 298-299.
5. In the construction of tests of formulation means at each time point, the subject by time interaction and subplot error were pooled.

Table 1

Mean Plasma Concentration by Time*

<u>Hour</u>	<u>Gastrointestinal Therapeutic System</u>	<u>Dexatrim</u>	<u>Aqueous Solution</u>
0	<6.2 a	<6.7 a	<6.3 a
0.5	41.1 a	14.6 b	64.6 a
1	66.1 a	48.0 a	92.8 b
2	76.4 a	90.2 a, b	112.0 b
3	76.0 a	118.1 b	111.7 b
4	74.7 a	169.7 b	93.9 a
5	77.4 a	161.7 b	80.2 a
6	74.7 a	153.4 b	70.1 a
8	80.6 a	120.2 c	54.6 b
10	81.7 a	87.9 a	36.8 b
12	78.4 a	67.9 a	26.8 b
16	67.3 a	40.9 b	110.1 c
24	33.2 a	12.6 a	34.8 a
48	28.4 a	11.0 a	18.6 a
72	30.3 a	11.4 a	16.0 a
72.5	56.6 a	22.6 b	49.2 a
73	87.8 a	61.1 b	86.6 a
74	95.8 a	125.1 b	95.2 a
75	101.4 a	204.1 b	85.1 a
76	107.1 a	212.4 c	71.0 b
77	104.6 a	192.5 b	106.7 a
78	99.8 a	166.9 b	118.5 a
80	96.4 a	130.6 b	98.7 a
82	113.0 a	89.3 b	141.0 c
83	116.0 a	72.1 b	124.4 a
84	110.4 a	72.2 b	112.0 a
86	92.8 a	49.6 b	81.8 a
88	78.1 a	38.3 b	68.6 a
96	30.8 a	12.9 a	20.2 a
100	19.2 a	7.5 a	12.9 a

*Means labeled with a common letter at each time point are not significantly different ($p > 0.05$).

Table 2
Statistical Analysis for Blood Concentrations

<u>Source</u>	<u>df</u>	ANOVA <u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Subjects	5	61862	12372	7.25	0.01
Periods	2	8883	4442	2.60	0.13
Formulations	2	17229	8615	5.05	0.04
Main plot error	8	13660	1708		
Time	29	795508	27431	64.25	0.0001
Subject x Time	145	61907	427		
Formulation x Time	58	348016	6000	16.06	0.0001
Period x Time	58	23762	410	1.10	0.31
Subplot error	229	85555	374		

Table 3

Statistical Analysis for Area Under the Curve - Day 1

<u>Source</u>	<u>df</u>	ANOVA <u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Subject	5	891935			
Period	2	205758	102879	5.02	0.039
Formulation	2	45236	22618	1.10	0.38
Error	8	164059	20507		

	<u>Mean</u>
GITS	1273
Dexatrim	1395
Aqueous Solution	1345

95% Westlake Confidence Limits

GITS vs. Dexatrim	+21.7%
GITS vs. Solution	+18.0%
Dexatrim vs. Solution	+15.2%

Table 4

Statistical Analysis for Area Under the Curve - Day 4

<u>Source</u>	<u>df</u>	ANOVA <u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Subject	5	1534274			
Period	2	273175	136587	1.72	0.24
Formulation	2	13200	6600	0.08	0.92
Error	8	635490	79436		

	<u>Mean</u>
GITS	1614
Dexatrim	1581
Aqueous Solution	1548

95% Westlake Confidence Limits

GITS vs. Dexatrim	<u>+23.6%</u>
GITS vs. Solution	<u>+24.5%</u>
Dexatrim vs. Solution	<u>+24.6%</u>

Table 5

Statistical Analysis for Total Urinary Excretion

<u>Source</u>	<u>df</u>	<u>ANOVA</u> <u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Subject	5	1558.7			
Period	2	56.3	28.2	0.09	0.91
Formulation	2	1113.5	556.8	1.84	0.22
Error	8	2420.6	302.6		

	<u>Mean</u>
GITS	240.0
Dexatrim	247.2
Aqueous Solution	259.1

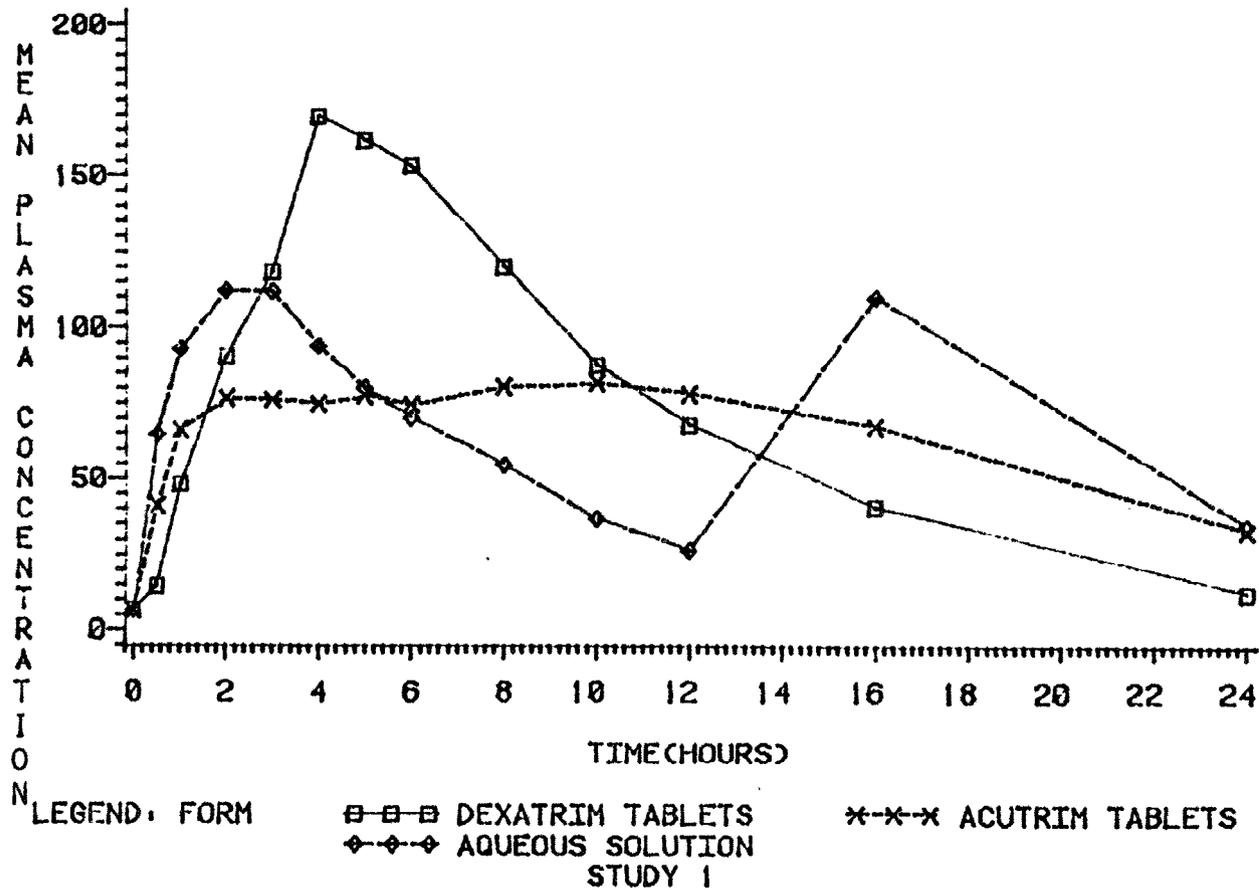
95% Westlake Confidence Limits

GITS vs. Dexatrim	<u>+11.1%</u>
GITS vs. Solution	<u>+15.7%</u>
Dexatrim vs. Solution	<u>+12.4%</u>

(ST-141-83)

Figure 1

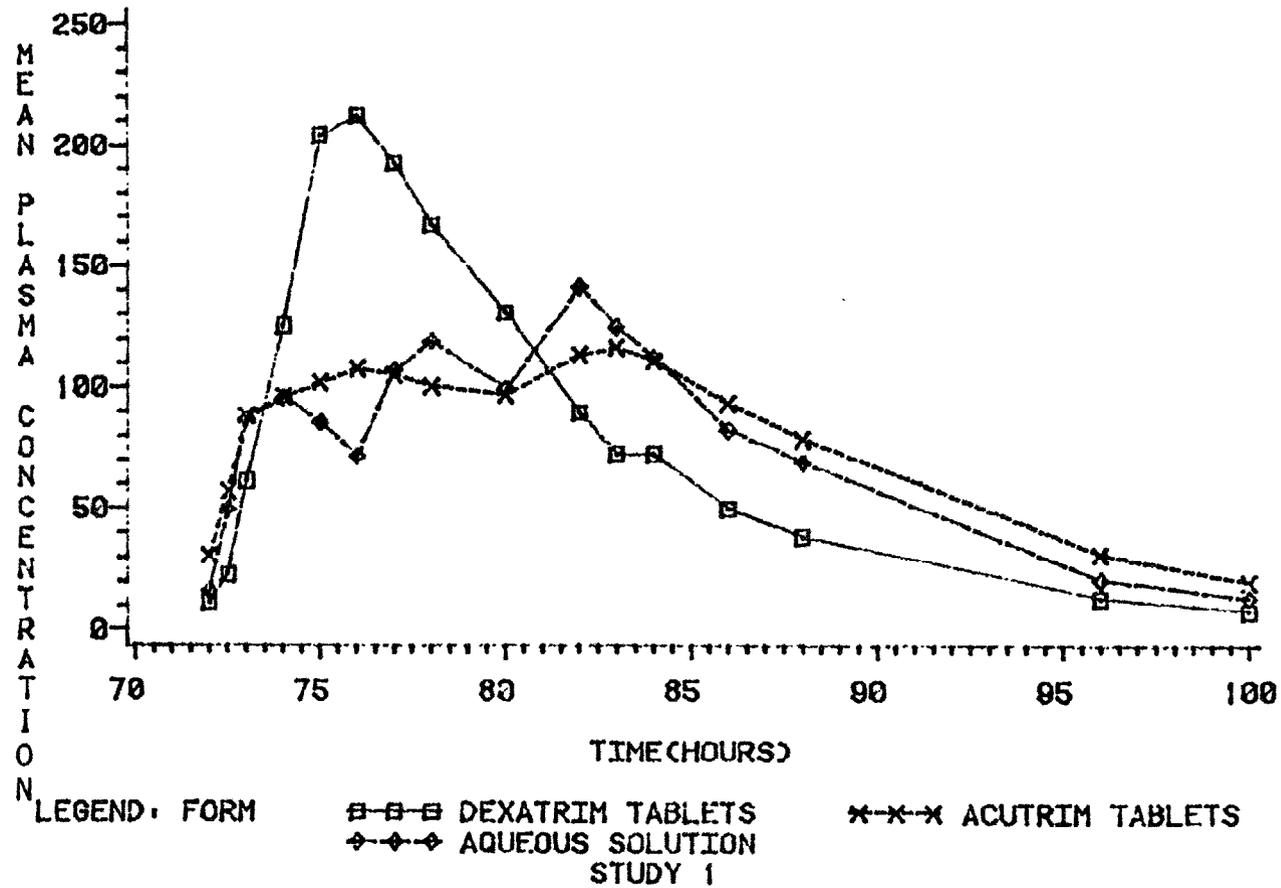
MEAN PLASMA CONCENTRATION OF PHENYLPROPANOLAMINE DAY 1



(ST-141-83)

Figure 2

MEAN PLASMA CONCENTRATION OF PHENYLPROPANOLAMINE DAY 4



RESULTS AND CONCLUSIONS

PROTOCOL: C-81-011: Study II

TITLE: Evaluation of Phenylpropanolamine Absorption
During Oral Administration From Gastrointestinal
Therapeutic Systems

STUDY DATE: October 18, 1982 - December 10, 1982

STUDY SITE: Clinical Study Unit
Alza Corporation
1274 California Avenue
Palo Alto, CA 94304

PERFORMED BY: J. Balestra, R.N., D. Thorn, B.S.,
P. Darley, B.S., V. A. Place, M.D.

REPORTED BY: P. Darley, B.S., D. Thorn, B.S., D. Swanson, Ph.D.,
J. Fara, Ph.D.

MATERIALS:

- 1) Gastrointestinal Therapeutic Systems, _____
Code #02510, Control 154982
each containing a total of 75 mg phenylpro-
panolamine HCl, designed to release 20 mg
within the first few minutes and the remaining
55 mg at a nominal rate of 3.5 mg/hr for
approximately 16 hours.
- 2) Phenylpropanolamine Solution
Code #80070, Lot #146082
Concentration of 1.0 mg/ml
- 3) Dexatrim® Extra Strength 12 hr Time Release
Capsules,
(Thompson Medical Co., Lot #SDF282E), each
containing 75 mg phenylpropanolamine HCl

SUBJECTS: 12 healthy male volunteers. A description of the
study volunteers is given in Table I.

PROTOCOL C-81-011: Study II

A. SUMMARY

A study was completed in 12 male subjects to compare the plasma concentration profiles of phenylpropanolamine HCl (PPA HCl) after oral administration of three different dosage forms: (1) the Gastrointestinal Therapeutic System (GITS), (2) Dexatrim timed-release capsules, and (3) an aqueous solution.

Each subject received each dosage form for 4 consecutive days with two weeks rest between dosing cycles in a randomized, complete crossover design. During the GITS and Dexatrim dosing cycles subjects received one unit (75 mg GITS or 75 mg capsule) per day at 8 A.M. During the solution dosing cycle subjects received two 37.5 mg doses, on Day 1, one at 8:00 A.M., the other at 8:00 P.M., and three 25.0 mg doses on Day 2, 3 and 4, one at 8 A.M., one at 12 noon and the third at 4 P.M. Subjects ate a standard breakfast one-half hour prior to the 8 A.M. dose during all dosing cycles. Blood samples were drawn at close intervals during Days 1 and 4 of the cycle and the plasma was assayed for phenylpropanolamine HCl. All urine was collected during the entire dosing cycle as a backup should the plasma assay be inadequate. The GITS (PPA HCl) were recovered from stools and assayed to determine residual PPA HCl content. (See Appendix I for detailed study schedule.)

The results of the study show that PPA is absorbed to an equal extent from the GITS dosage form and the Dexatrim capsules on the first and fourth day of dosing, since areas under the plasma concentration curve are not significantly different (one sample t-test, $p > 0.05$). Peak plasma concentrations of PPA HCl after administration by Dexatrim capsules and a solution control reach values up to 2 times higher than for GITS (PPA HCl) during both Day 1 and Day 4. Plasma levels of PPA HCl after GITS (PPA HCl) administration were higher than after Dexatrim, at 16 hours post-dose on Day 1 and after 12 hours post-dose on Day 4.

The functionality of the Gastrointestinal Therapeutic System is further demonstrated by the fact that less than 2% of the original dose was found in systems recovered in the stool.

None of the subjects experienced any adverse effects during the study and blood pressures remained within normal ranges throughout the study.

PROTOCOL C-81-011: Study II

B. STUDY METHODOLOGY

1) Study Objective

The objective of this study was to compare the profile of plasma levels following multiple doses from three oral dosage forms:

- a) The Gastrointestinal Therapeutic System (GITS)
- b) Dexatrim® 12 hr Sustained Release Capsules
- c) Aqueous Solution

2) Study Plan

The study took place over 8 consecutive weeks. During weeks 1, 4, and 7 (Group I) and weeks 2, 5, and 8 (Group II) subjects received 75 mg PPA HCl per day for 4 consecutive days from the dosage form indicated in the table below. (The order of dosage form administration was randomly assigned to the subjects.) Weeks 3 and 6 were rest weeks during which no drugs were given and no biological samples taken. Appendix I contains a detailed schedule of drug administration, blood sampling, urine collection, and food intake for any given week. Blood pressure and pulse measurements were taken just before each dosing cycle and at regular intervals during dosing cycles.

<u>Group I</u>			
<u>Subject</u>	<u>Week 1</u>	<u>Week 4</u>	<u>Week 7</u>
07	Dexatrim	Solution	GITS (PPA HCl)
08	GITS (PPA HCl)	Solution	Dexatrim
10	Solution	GITS (PPA HCl)	Dexatrim
11	GITS (PPA HCl)	Dexatrim	Solution
12	Solution	Dexatrim	GITS (PPA HCl)

<u>Group II</u>			
<u>Subject</u>	<u>Week 2</u>	<u>Week 5</u>	<u>Week 8</u>
09*	Dexatrim	GITS (PPA HCl)	Solution
13	Dexatrim	Solution	GITS (PPA HCl)
14	Dexatrim	GITS (PPA HCl)	Solution
15	GITS (PPA HCl)	Dexatrim	Solution
16	Solution	GITS (PPA HCl)	Dexatrim
17	Solution	Dexatrim	GITS (PPA HCl)
18	GITS (PPA HCl)	Solution	Dexatrim

* Subject 09 was originally scheduled to be in Group I, but due to illness was started in Group II.

When subjects took the GITS (PPA HCl) they collected stools, which were then searched to recover the system for assay.

Every subject was asked if they were experiencing any side effects just prior to each blood sample. Subjects were requested to abstain from intake of alcohol and other drugs beginning 24 hours prior to a dosing week and continuing until the last biological sample was taken.

All 12 subjects completed the study.

3) Collecting and Handling of Biological Samples

a) Blood samples

10 ml of blood were drawn into a heparinized tube. The sample was then centrifuged within 5-7 minutes. 3 to 4 ml of plasma was immediately transferred to labeled 10 ml tubes using a fresh disposable pipette for each plasma sample. Samples were stored at -20°C until assayed.

b) Urine samples

Urine was collected by the subjects in brown plastic bottles. At the end of each collection interval the volumes of the collections were measured and recorded and a 15-30 ml aliquot was transferred to a labeled glass vial and stored at -20°C for possible future assay.

c) Stool search and GITS (PPA HCl) recovery

Stools from subjects taking the GITS (PPA HCl) were methodically searched in an air flow hood. Upon finding a GITS (PPA HCl) in a stool sample, the system was placed in a glass vial and the date & time of defecation and date & time of recovery recorded. Systems were stored at -20°C until assayed.

4) Assay of Plasma and Urine for Phenylpropanolamine Content

The methodology and validation of the phenylpropanolamine assay of plasma and urine is detailed in Appendix II.

PROTOCOL C-81-011: Study II

5) Assay of Recovered GITS (PPA HCl)

The assay of GITS (PPA HCl) systems recovered from stools for phenylpropanolamine residual content is detailed in Appendix III.

6) Assay of Dosage Forms for PPA HCl Content

Results from PPA HCl assays of aliquots of the solution dosage form, and of samples of the Dexatrim capsules and GITS (PPA HCl) system are presented in Appendix IV.

C. RESULTS

1) Plasma Levels of Phenylpropanolamine HCl

The plasma concentrations of PPA HCl for all 12 subjects are listed in Table 2A (GITS), 2B (Dexatrim), and 2C (Solution). Missing values for subject 16 during the Dexatrim treatment are due to subject accidentally ingesting incorrect dosage form on morning of Day 1; this subject received correct dosage form beginning on Day 2. All other missing values are due to phlebotomy failures.

Table 3 lists the average plasma levels for all 12 subjects at each time of sample. These results are plotted in Figures 1 and 2.

Table 4 shows the areas under the plasma concentration curves (AUC's) for both Day 1 and the steady state day (Day 4), for each of the three dosage forms. Note that the low value on Day 1 for Subject 09 while on GITS (PPA HCl) corresponds to an early recovery of the system in the stool, which contained 33% of that day's dose.

Comparisons of AUC's between dosage forms for significant differences (one sample t-test, 0.05 significance levels) are shown below.

Not Significant

Significant

- | | |
|--------------------------------|--------------------------------|
| 1) GITS vs Dexatrim, Day 1 | 5) GITS vs Solution, Day 4 |
| 2) GITS vs Dexatrim, Day 4 | 6) Dexatrim vs Solution, Day 1 |
| 3) GITS vs Solution, Day 1 | |
| 4) Dexatrim vs Solution, Day 4 | |

2) Residual PPA HCl Content of GITS (PPA HCl) Recovered in the Stool

Table 5 shows the results of the assay of GITS (PPA HCl) recovered in the stool, showing also time and day of defecation as well as time and day of recovery from the stool. A total of 45 were recovered, 44 with an average residual content of 1.15 mg (1.5% of dose) and one system containing >25mg which was defecated in 8.7 hours.

PROTOCOL C-81-011: Study II

For 12 of the systems, it was possible to determine actual gut transit times because of the relative timing of ingestion and system defecation.

.3. Blood Pressure, Pulse, and Side Effects

Table 8 lists the results of blood pressure and pulse monitoring done just before and during each dosing cycle.

Subjects recorded their observations on side effects just before each blood sample. There was no demonstrable change in the subjects' observations of effects throughout the entire study. Neither were there any isolated instances of subjects complaining of adverse effects during the study.

D. CONCLUSIONS

The results of the study show that PPA is absorbed equally from the GITS dosage form and the Dexatrim capsules. Peak plasma concentrations of PPA HCl after administration by Dexatrim capsules and a solution control reach values up to 2 times higher than for GITS (PPA HCl) at both Day 1 and Day 4. Plasma levels of PPA HCl after GITS (PPA HCl) administration were higher than after Dexatrim after 12 hours post-dose on Day 1 and Day 4.

The functionality of the Gastrointestinal Therapeutic System is demonstrated by the fact that less than 3% of the original dose was found in systems recovered in the stool. Also, it is evident from the graphical display of the plasma concentrations vs time that the GITS maintains a plasma profile that is devoid of the peaks and valleys found in the profiles for Dexatrim capsules and the solution.

None of the subjects experienced any adverse effects during the study and blood pressures remained within normal ranges throughout the study.

TABLE I

Description of Study Population

<u>Subject Number</u>	<u>Age (yrs)</u>	<u>Body Weight (kgs)</u>	
		<u>Pre-Study</u>	<u>Post-Study</u>
07	37	90.5	92.0
08	27	74.0	74.5
09	36	70.5	71.5
10	32	93.5	95.5
11	24	76.0	76.5
12	28	104.0	101.5
13	26	70.0	70.0
14	38	91.5	89.0
15	27	85.5	83.5
16	39	92.5	92.5
17	35	82.0	82.5
18	<u>35</u>	<u>73.5</u>	<u>75.5</u>
Means	32.0 ±5.3 S.D.	83.6 ±3.16 S.E.M.	83.7 ±2.98 S.E.M.

PROTOCOL C-81-011: Study II

TABLE 2A
PLASMA LEVELS OF PPA HCL

GASTROINTESTINAL THERAPEUTIC SYSTEMS

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N, NG/ML					
			SUBJECT 07	SUBJECT 08	SUBJECT 09	SUBJECT 10	SUBJECT 11	SUBJECT 12
1	08:00	0	0.0	0.0	0.0	0.0	0.0	0.0
1	08:30	0.5	30.7	27.1	0.0	12.7	32.5	4.2
1	09:00	1	63.4	53.7	5.0	41.3	65.0	13.3
1	10:00	2	66.5	07.4	40.7	57.3	62.1	31.4
1	11:00	3	73.8	99.9	74.8	52.3	73.0	71.1
1	12:00	4	70.7	83.4	73.8	48.4	69.6	75.6
1	14:00	6	-	82.8	66.0	49.1	-	62.4
1	16:00	8	68.4	70.5	71.4	44.6	87.3	76.6
1	20:00	12	72.6	85.1	42.0	52.0	77.9	76.3
1	24:00	16	63.8	67.3	28.9	56.2	73.0	54.9
2	08:00	24	15.9	13.4	1.5	26.9	13.0	19.4
3	08:00	24	1.9	0.0	0.0	15.9	10.1	12.9
4	08:00	24	31.5	13.0	23.2	22.6	26.3	15.1
4	09:00	1	44.6	69.8	56.2	71.2	54.1	41.0
4	10:00	2	94.1	76.6	79.2	70.6	99.5	61.6
4	12:00	4	106.2	66.3	66.4	62.3	80.2	60.1
4	13:00	5	119.2	77.0	86.5	63.7	93.0	67.1
4	14:00	6	97.4	69.9	84.2	61.2	101.7	59.9
4	16:00	8	111.6	73.2	83.2	70.2	100.2	73.6
4	17:00	9	107.4	98.3	80.1	69.3	86.3	70.2
4	18:00	10	102.8	59.2	74.6	62.4	77.7	74.2
4	20:00	12	117.9	82.2	70.9	70.5	77.7	68.4
4	24:00	16	60.5	57.0	55.9	53.1	76.3	59.0
5	08:00	24	25.4	5.1	15.0	21.3	23.0	21.6
5	12:00	28	14.4	0.0	6.1	11.9	9.4	7.0

PROTOCOL C-81-011: Study II

TABLE 2A (CONT.)

PLASMA LEVELS OF PPA HCL

GASTROINTESTINAL THERAPEUTIC SYSTEMS

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N, NG/ML					
			SUBJECT 13	SUBJECT 14	SUBJECT 15	SUBJECT 16	SUBJECT 17	SUBJECT 18
1	08:00	0	0.0	0.0	0.0	0.0	0.0	0.0
1	08:30	0.5	26.1	7.7	66.2	1.7	76.2	4.0
1	09:00	1	67.1	35.5	70.4	19.0	72.4	32.1
1	10:00	2	83.7	46.8	75.7	54.4	66.5	103.9
1	11:00	3	81.5	49.0	72.1	55.2	52.0	100.0
1	12:00	4	77.0	45.4	63.5	54.5	50.1	80.4
1	14:00	6	72.0	133.0	81.3	52.4	71.9	101.4
1	16:00	8	73.0	114.4	70.0	46.0	54.2	104.6
1	20:00	12	81.3	60.0	67.0	51.0	-	104.0
1	24:00	16	82.0	37.5	56.2	51.6	65.0	99.4
2	08:00	24	30.0	7.9	23.7	15.5	17.9	37.4
3	08:00	24	77.3	36.1	16.6	22.8	-	26.7
4	08:00	24	40.2	29.7	16.6	19.7	26.6	40.2
4	09:00	1	51.0	90.1	76.0	75.7	89.7	45.7
4	10:00	2	69.4	94.7	96.0	70.2	146.3	55.7
4	12:00	4	119.3	94.3	77.9	70.2	72.0	114.1
4	13:00	5	120.0	99.3	85.6	79.2	75.0	144.1
4	14:00	6	104.1	95.8	86.8	92.1	83.9	143.0
4	16:00	8	84.4	105.4	81.4	59.5	70.4	151.0
4	17:00	9	95.0	100.0	75.7	73.1	86.1	139.9
4	18:00	10	97.6	110.6	76.5	71.4	89.1	154.0
4	20:00	12	117.3	103.5	91.2	62.7	-	171.4
4	24:00	16	84.4	57.6	56.0	46.7	53.7	107.2
5	08:00	24	31.4	15.5	29.0	11.4	-	39.6
5	12:00	20	13.0	7.2	16.8	8.3	2.9	24.2

PROTOCOL C-81-011: Study II

TABLE 2B
 PLASMA LEVELS OF PPA HCL
 DEXATRIM CAPSULES

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N, NG/ML					
			SUBJECT 07	SUBJECT 08	SUBJECT 09	SUBJECT 10	SUBJECT 11	SUBJECT 12
1	08:00	0	0.0	0.0	0.0	0.0	0.0	0.0
1	00:30	0.5	0.0	0.0	0.0	17.7	6.3	0.9
1	09:00	1	26.0	31.4	27.1	44.6	70.6	31.6
1	10:00	2	09.9	115.2	88.6	77.2	104.8	46.9
1	11:00	3	99.8	110.8	117.2	98.9	100.2	79.6
1	12:00	4	122.3	134.9	132.9	123.9	112.8	67.4
1	14:00	6	133.4	146.1	148.9	117.3	110.7	78.2
1	16:00	8	101.2	112.8	91.1	106.6	106.1	68.5
1	20:00	12	40.9	73.8	52.0	67.6	-	34.1
1	24:00	16	26.0	31.8	29.4	47.2	31.8	17.6
2	08:00	24	0.0	4.2	3.0	10.9	5.8	2.1
3	08:00	24	1.9	3.1	5.6	4.7	3.4	7.8
4	08:00	24	4.5	5.7	3.4	7.4	1.4	10.8
4	09:00	1	5.6	55.6	19.2	21.0	57.2	104.5
4	10:00	2	95.3	101.4	66.8	71.8	94.8	125.9
4	12:00	4	119.0	145.1	110.1	106.5	80.6	141.5
4	13:00	5	138.4	150.3	164.0	104.9	93.3	160.1
4	14:00	6	99.9	141.7	129.9	96.0	102.3	160.7
4	16:00	8	97.1	135.2	94.8	118.8	84.0	127.0
4	17:00	9	79.0	140.6	75.5	104.9	47.2	139.1
4	18:00	10	67.9	109.5	74.5	91.3	60.6	131.0
4	20:00	12	44.4	67.0	60.9	86.1	71.5	84.4
4	24:00	16	20.0	34.0	25.4	44.7	20.4	51.1
5	08:00	24	1.5	5.7	5.0	11.3	0.0	10.7
5	12:00	20	0.0	0.0	0.0	2.5	0.0	4.2

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TABLE 2B (CONT.)
 PLASMA LEVELS OF PPA HCL
 DEXATRIM CAPSULES

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	CONCENTRATION, NG/ML					
			SUBJECT 13	SUBJECT 14	SUBJECT 15	SUBJECT 16	SUBJECT 17	SUBJECT 18
1	08:00	0	0.0	0.0	0.0	-	0.0	0.0
1	08:30	0.5	18.9	0.0	10.2	-	0.0	0.0
1	09:00	1	79.0	27.3	70.9	-	63.5	10.3
1	10:00	2	130.1	91.1	117.3	-	100.0	77.2
1	11:00	3	164.3	99.0	132.0	-	158.1	149.9
1	12:00	4	133.7	130.3	156.5	-	269.1	164.0
1	14:00	6	173.0	137.0	147.4	-	321.7	100.7
1	16:00	8	170.5	101.3	164.6	-	102.0	156.4
1	20:00	12	109.0	74.0	132.4	-	109.5	95.7
1	24:00	16	50.9	10.5	103.4	-	47.2	50.1
2	08:00	24	11.9	0.4	13.4	-	7.2	16.3
3	08:00	24	15.0	12.7	10.3	0.0	19.4	24.0
4	08:00	24	13.5	12.7	12.3	0.0	11.0	21.6
4	09:00	1	40.2	13.0	105.6	33.3	99.3	35.4
4	10:00	2	73.5	91.0	116.0	99.9	135.2	141.9
4	12:00	4	141.5	132.0	136.4	141.7	175.4	204.3
4	13:00	5	170.7	130.0	145.1	130.4	212.7	217.6
4	14:00	6	166.9	140.3	149.0	135.0	239.7	223.0
4	16:00	8	160.6	116.5	143.2	105.9	104.3	205.3
4	17:00	9	149.7	99.7	127.5	98.4	179.7	266.5
4	18:00	10	130.3	90.7	107.7	01.0	157.3	145.1
4	20:00	12	93.5	71.5	00.9	62.3	-	124.4
4	24:00	16	60.6	30.6	-	30.7	67.9	00.3
5	08:00	24	20.0	0.3	11.3	1.9	-	-
5	12:00	20	5.1	1.4	2.5	3.5	6.7	10.1

PROTOCOL C-81-011: Study II

TABLE 2C
 PLASMA LEVELS OF PPA HCL
 AQUEOUS SOLUTION

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N, NG/ML					
			SUBJECT 07	SUBJECT 08	SUBJECT 09	SUBJECT 10	SUBJECT 11	SUBJECT 12
1	08:00	0	0.0	0.0	0.0	0.0	0.0	0.0
1	08:30	0.5	61.3	58.2	9.3	46.2	24.3	41.2
1	09:00	1	90.2	88.7	38.8	79.9	90.2	90.1
1	10:00	2	121.3	116.7	109.5	62.9	119.5	74.7
1	11:00	3	87.6	85.9	114.9	64.8	114.4	71.9
1	12:00	4	62.4	84.3	81.5	62.2	102.1	62.6
1	14:00	6	55.7	63.2	53.1	46.4	69.9	45.4
1	16:00	8	42.0	47.2	31.5	38.1	44.2	24.6
1	20:00	12	20.0	22.5	12.9	24.5	18.9	13.4
1	24:00	4	104.3	100.3	94.0	102.9	88.1	69.0
2	08:00	12	31.3	28.8	18.6	30.7	29.8	12.9
3	08:00	16	5.1	4.2	6.3	21.3	10.3	10.4
4	08:00	16	9.4	4.2	5.8	17.6	10.5	11.5
4	09:00	1	39.5	74.5	73.0	53.0	52.7	73.5
4	10:00	2	77.2	79.9	94.0	76.8	76.5	77.7
4	12:00	3	69.9	63.4	61.2	53.4	70.7	65.5
4	13:00	1	121.4	114.0	142.2	110.2	104.9	122.1
4	14:00	2	118.0	101.5	116.3	101.5	92.2	132.9
4	16:00	4	95.7	75.5	75.0	74.8	93.6	100.4
4	17:00	2	123.2	131.2	130.2	137.5	141.9	136.0
4	18:00	3	124.0	129.7	126.2	119.5	134.5	120.3
4	20:00	4	103.0	109.7	99.3	105.0	103.0	94.1
4	24:00	0	54.7	40.4	45.9	52.6	59.0	40.0
5	08:00	16	9.3	7.1	11.5	13.3	10.3	11.9
5	12:00	20	3.5	4.1	0.0	10.5	3.4	6.0

PROTOCOL C-81-011: Study II

TABLE 2C (CONT.)
 PLASMA LEVELS OF PPA HCL
 AQUEOUS SOLUTION

DAY OF DOSING CYCLE	TIME OF SAMPLE	HOURS SINCE LAST DOSE	C O N C E N T R A T I O N , NG/ML					
			SUBJECT 13	SUBJECT 14	SUBJECT 15	SUBJECT 16	SUBJECT 17	SUBJECT 18
1	08:00	0	0.0	0.0	0.0	0.0	0.0	0.0
1	08:30	0.5	0.0	0.4	63.7	0.7	46.5	49.4
1	09:00	1	40.2	13.2	98.7	66.0	81.8	125.7
1	10:00	2	121.1	58.1	108.5	182.9	121.6	131.4
1	11:00	3	131.8	89.1	96.3	79.6	107.5	118.4
1	12:00	4	117.0	88.2	79.6	68.8	79.4	92.2
1	14:00	6	96.4	64.7	55.0	45.3	54.7	73.0
1	16:00	8	59.8	41.7	41.2	27.9	44.8	60.3
1	20:00	12	31.8	20.5	18.2	7.1	23.6	23.3
1	24:00	4	92.5	81.3	84.3	78.7	89.6	193.9
2	08:00	12	58.5	25.8	24.5	18.5	34.4	38.5
3	08:00	16	31.8	15.8	15.3	8.0	6.7	28.5
4	08:00	16	44.2	18.7	9.2	3.8	8.6	33.8
4	09:00	1	61.9	27.2	98.5	28.5	32.3	41.3
4	10:00	2	96.3	83.9	94.9	62.3	87.6	94.5
4	12:00	3	187.2	84.6	73.4	55.7	66.4	123.1
4	13:00	1	261.4	164.9	154.9	129.1	111.2	178.5
4	14:00	2	235.7	151.8	125.6	99.5	118.0	223.7
4	16:00	4	197.0	162.1	104.1	74.3	91.8	166.3
4	17:00	2	277.0	154.5	137.9	131.9	145.8	248.9
4	18:00	3	298.6	143.8	153.5	117.3	138.4	221.7
4	20:00	4	222.9	97.8	105.9	88.8	118.2	167.2
4	24:00	8	115.9	58.3	52.4	36.1	49.1	100.3
5	08:00	16	28.9	15.8	9.4	2.4	7.8	23.8
5	12:00	20	15.1	7.8	8.8	2.2	8.8	14.8

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TABLE 3
AVERAGE PLASMA LEVELS OF PPA HCL

DAY OF DOSING CYCLE	HOURS SINCE LAST DOSE	GITS & DEXATRIM	SOLN	AVERAGE PLASMA CONCENTRATIONS (NG/ML), & S.E.M., N=12					
				GASTROINTESTINAL THERAPEUTIC SYSTEM		DEXATRIM		SOLUTION	
				MEAN	S.E.M.	MEAN	S.E.M.	MEAN	S.E.M.
1	00:00	0	0	0.0	0.0	0.0**	0.0	0.0	0.0
1	00:30	0.5	0.5	24.1	7.2	4.9**	2.2	34.8	6.7
1	09:00	1	1	45.5	7.1	44.0**	7.0	75.3	8.9
1	10:00	2	2	64.7	6.0	95.1**	7.0	104.0	7.2
1	11:00	3	3	71.3	5.0	119.9**	8.4	96.9	5.9
1	12:00	4	4	66.7	4.1	141.4**	14.8	81.0	5.1
1	14:00	6	6	77.3*	7.9	154.1**	10.0	60.2	4.2
1	16:00	8	8	73.5	6.0	117.2**	10.3	41.9	3.2
1	20:00	12	12	70.7**	5.4	79.7*	9.9	19.7	1.9
1	24:00	16	4	61.4	5.4	42.1**	7.3	98.4	9.2
2	00:00	24	12	10.5	2.0	7.6**	1.5	20.7	3.3
3	00:00	24	16	20.0**	6.6	9.1	2.2	13.6	2.9
4	00:00	24	16	26.1	3.0	0.7	1.0	14.0	3.6
4	09:00	1	1	63.9	5.0	49.2	10.4	54.7	6.4
4	10:00	2	2	84.6	7.0	101.2	7.1	83.5	3.0
4	12:00	4	3	84.5	5.7	136.9	8.9	80.4	11.1
4	13:00	5	1	92.7	7.0	152.9	10.6	143.6	12.6
4	14:00	6	2	90.0	6.4	149.5	13.0	134.7	13.7
4	16:00	8	4	89.4	7.2	131.1	10.7	109.2	12.1
4	17:00	9	2	90.0	5.9	125.7	16.6	158.0	14.4
4	18:00	10	3	87.6	7.6	104.0	9.1	152.2	15.6
4	20:00	12	4	94.0**	9.7	77.0**	6.1	116.6	11.3
4	24:00	16	0	64.6	4.9	43.1**	6.0	60.1	6.8
5	00:00	24	16	21.0**	3.0	7.6*	1.9	12.5	2.1
5	12:00	20	20	10.2	1.9	3.0	0.9	5.5	1.5

* N=10
** N=11

TABLE 4

AREA* UNDER THE PLASMA CONCENTRATION CURVE
FOR DAY 1** AND DAY 4 **

SUBJECT	AREA UNDER THE CURVE, HR-NG/ML					
	GASTROINTESTINAL THERAPEUTIC SYSTEM		DEXATRIM		SOLUTION	
	DAY 1	DAY 4	DAY 1	DAY 4	DAY 1	DAY 4
7	1390	1922	1320	1204	1356	1693
8	1541	1378	1591	1727	1475	1630
9	923***	1423	1393	1331	1214	1677
10	1105	1321	1556	1533	1120	1640
11	1525	1735	1460	1180	1554	1710
12	1294	1338	864	2056	1040	1702
13	1652	1984	2236	2100	1789	3625
14	1335	1782	1437	1595	1178	1994
15	1408	1604	2535	1999	1400	1893
16	1027	1309	-	1495	1032	1373
17	1293	1591	2419	2707	1448	1700
18	2045	2599	2108	2868	1762	2928
MEAN	1378	1665	1720	1816	1364	1963
± S.E.M.	87	109	158	159	74	187

* COMPUTED BY TRAPEZOIDAL APPROXIMATION

** DAY 1: 08:00, DAY 1 TO 08:00, DAY 2 EXCEPT FOR SOLUTION,
WHERE 2 TIMES (08:00, DAY 1 TO 20:00, DAY 1)

DAY 4: 08:00, DAY 4 TO 08:00, DAY 5

*** SYSTEM TAKEN ON DAY 1 WAS DEFECATED 8.7 HOURS AFTER
INGESTION AND CONTAINED 25 MG (33%) OF THE DOSE.

TABLE 5

RESIDUAL PPA HCL CONTENT OF GASTROINTESTINAL
THERAPEUTIC SYSTEMS RECOVERED FROM STOOLS

SUBJECT	DEFECATION		RECOVERY FROM STOOLS		MG PPA.HCL	GUT TRANSIT TIME, HRS*
	DAY	TIME	DAY	TIME		
7	2	06:00	2	08:30	1.10	22.0
	2	18:00	4	08:00	2.47	10.0
	4	06:30	4	08:45	1.23	22.5
	5	21:30	6	15:20	1.21	37.5
8**	3	07:00	3	11:15	1.30	
	4	06:45	4	08:15	0.75	
9	1	16:40	2	11:20	>25	8.7
	2	16:00	3	09:15	6.8	8.0
	4	08:20	4	09:50	0.39	
	5	08:45	5	09:00	0.55	
10	3	06:55***	3	08:10	0.44	
	3	06:55***	3	08:10	2.64	
	4	13:25	4	22:00	1.09	
	5	06:30	5	15:00	2.65	
11**	3	09:00	3	11:30	0.73	
	5	07:22	5	10:45	1.54	
	5	14:30	7	08:40	0.30	
12	2	10:15	3	08:30	0.65	26.3
	4	12:00	4	13:00	0.62	
	5	08:55***	5	11:00	0.66	
	5	08:55***	5	11:00	1.02	
13	3	13:30	3	14:45	0.76	
	4	11:30***	4	12:45	0.64	
	4	11:30***	4	12:45	0.29	
	5	08:30	5	09:00	2.04	

* WHEN ABLE TO DETERMINE UNAMBIGUOUSLY

** SUBJECT 8 COLLECTED STOOLS THROUGH DAY 7 WITHOUT RECOVERY OF LAST TWO SYSTEMS.
SUBJECT 11 COLLECTED STOOLS THROUGH DAY 6 WITHOUT RECOVERY OF LAST SYSTEM.

*** SYSTEMS RECOVERED IN THE SAME STOOL

RESIDUAL PPA HCL CONTENT OF GASTROINTESTINAL
THERAPEUTIC SYSTEMS RECOVERED FROM STOOLS

SUBJECT	DEFECATION		RECOVERY FROM STOOLS		MG PPA.HCL	GUT TRANSIT TIME, HRS*
	DAY	TIME	DAY	TIME		
14	1	19:00	2	08:30	1.58****	11.0
	3	12:15	3	16:00	0.56	
	4	13:20	4	17:10	0.68	
	4	20:15	5	09:00	1.93	
15	3	13:00	3	15:30	0.31	
	4	09:45	4	10:35	0.20	
	5	11:00	5	15:00	0.37	
	6	15:00	6	15:45	0.74	
16	3	09:30***	3	15:30	0.74	
	3	09:30***	3	15:30	1.44	
	5	09:50***	5	10:00	0.84	
	5	09:50***	5	10:00	0.59	
17	2	08:30	2	11:15	0.78	24.5
	3	10:00	3	14:30	0.91	26.0
	4	11:30	4	12:30	1.30	27.5
	5	08:30	5	08:35	1.13	24.5
18	2	13:00	2	15:10	1.82	
	3	06:30	3	09:00	0.83	
	4	07:00	4	08:30	0.85	
	5	07:00	5	08:30	1.23	

* WHEN ABLE TO DETERMINE UNAMBIGUOUSLY

*** SYSTEMS RECOVERED IN THE SAME STOOL

**** AT RECOVERY SYSTEM WAS 'BALLOONED' INTO A SHPERE.

TABLE 6

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE SYS- TOLIC	DIAS- TOLIC	PULSE	PLASMA LEVEL, (NG/ML)
7	DEXATRIM	18OCT82	08:00	130	78	58	0.0
7	DEXATRIM	18OCT82	12:00	120	74	58	122.3
7	DEXATRIM	19OCT82	08:00	116	50	66	0.0
7	DEXATRIM	20OCT82	08:00	126	70	68	1.9
7	DEXATRIM	21OCT82	08:00	114	64	62	4.5
7	DEXATRIM	21OCT82	12:00	110	70	76	119.0
7	DEXATRIM	22OCT82	08:00	116	70	68	1.5
7	SOLUTION	08NOV82	08:00	118	78	60	0.0
7	SOLUTION	08NOV82	12:00	130	82	72	62.4
7	SOLUTION	09NOV82	08:00	124	78	76	31.3
7	SOLUTION	10NOV82	08:00	122	74	84	5.1
7	SOLUTION	11NOV82	08:00	128	78	74	9.4
7	SOLUTION	11NOV82	12:00	130	78	84	69.9
7	SOLUTION	12NOV82	08:00	114	80	66	9.3
7	GITS	29NOV82	08:00	130	88	60	0.0
7	GITS	29NOV82	12:00	114	78	80	70.7
7	GITS	30NOV82	08:00	114	78	68	15.9
7	GITS	01DEC82	08:00	112	78	80	1.9
7	GITS	02DEC82	08:00	112	80	72	31.5
7	GITS	02DEC82	12:00	114	80	72	106.2
7	GITS	03DEC82	08:00	106	76	72	25.4
8	GITS	18OCT82	08:00	122	68	78	0.0
8	GITS	18OCT82	12:00	108	68	72	83.4
8	GITS	19OCT82	08:00	110	66	84	7.1
8	GITS	20OCT82	08:00	110	62	72	13.4
8	GITS	21OCT82	08:00	110	62	72	0.0
8	GITS	21OCT82	08:00	100	60	80	13.0
8	GITS	22OCT82	12:00	112	66	78	66.3
8	SOLUTION	08NOV82	08:00	108	68	62	5.1
8	SOLUTION	08NOV82	08:00	112	74	76	0.0
8	SOLUTION	09NOV82	12:00	118	72	74	84.3
8	SOLUTION	10NOV82	08:00	112	68	90	28.8
8	SOLUTION	11NOV82	08:00	118	68	90	4.2
8	SOLUTION	11NOV82	08:00	114	78	88	4.2
8	SOLUTION	12NOV82	12:00	110	68	80	53.4
8	DEXATRIM	29NOV82	08:00	108	58	80	0.0
8	DEXATRIM	29NOV82	12:00	104	78	80	134.9
8	DEXATRIM	30NOV82	08:00	108	72	80	4.2
8	DEXATRIM	01DEC82	08:00	110	70	80	3.1
8	DEXATRIM	02DEC82	08:00	114	70	96	5.7
8	DEXATRIM	02DEC82	12:00	120	78	92	145.1
8	DEXATRIM	04DEC82	08:00	128	68	96	5.7

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE SYS- TOLIC	DIA- TOLIC	PULSE	PLASMA LEVEL, (NG/ML)
9	DEXATRIM	25OCT82	08:00	126	72	62	0.0
9	DEXATRIM	25OCT82	12:00	130	74	60	132.9
9	DEXATRIM	26OCT82	08:00	124	70	62	3.0
9	DEXATRIM	27OCT82	08:00	128	60	64	5.6
9	DEXATRIM	28OCT82	08:00	124	64	66	3.4
9	DEXATRIM	28OCT82	12:00	122	74	60	110.1
9	DEXATRIM	29OCT82	08:00	120	66	64	5.0
9	GITS	15NOV82	08:00	134	74	72	0.0
9	GITS	15NOV82	12:00	134	68	66	73.8
9	GITS	16NOV82	08:00	130	68	70	1.5
9	GITS	17NOV82	08:00	126	74	76	0.0
9	GITS	18NOV82	08:00	124	60	70	23.2
9	GITS	18NOV82	12:00	118	72	66	66.4
9	GITS	19NOV82	08:00	122	72	72	15.0
9	SOLUTION	06DEC82	08:00	130	68	64	0.0
9	SOLUTION	06DEC82	12:00	126	76	64	81.5
9	SOLUTION	07DEC82	08:00	118	68	64	18.6
9	SOLUTION	08DEC82	08:00	124	68	68	6.3
9	SOLUTION	09DEC82	08:00	134	58	84	5.8
9	SOLUTION	09DEC82	12:00	126	76	72	61.2
9	SOLUTION	10DEC82	08:00	120	62	72	11.5
10	SOLUTION	18OCT82	08:00	130	80	68	0.0
10	SOLUTION	18OCT82	12:00	118	80	66	62.2
10	SOLUTION	19OCT82	08:00	118	70	62	30.7
10	SOLUTION	20OCT82	08:00	116	70	62	21.3
10	SOLUTION	21OCT82	08:00	112	70	66	17.6
10	SOLUTION	21OCT82	12:00	108	70	76	53.4
10	SOLUTION	22OCT82	08:00	114	68	68	13.3
10	GITS	08NOV82	08:00	122	80	76	0.0
10	GITS	08NOV82	12:00	112	76	56	48.4
10	GITS	09NOV82	08:00	128	78	64	26.9
10	GITS	10NOV82	08:00	118	76	66	15.9
10	GITS	11NOV82	08:00	118	78	68	22.6
10	GITS	11NOV82	12:00	112	80	68	62.3
10	GITS	12NOV82	08:00	124	70	70	21.3
10	DEXATRIM	29NOV82	08:00	124	80	72	0.0
10	DEXATRIM	29NOV82	12:00	114	80	72	123.9
10	DEXATRIM	30NOV82	08:00	118	78	68	10.9
10	DEXATRIM	01DEC82	08:00	110	72	76	4.7
10	DEXATRIM	02DEC82	08:00	124	80	72	7.4
10	DEXATRIM	02DEC82	12:00	106	78	76	106.5
10	DEXATRIM	04DEC82	08:00	116	80	72	11.3

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE SYS- TOLIC	DIAS- TOLIC	PULSE	PLASMA LEVEL, (NG/ML)
11	GITS	18OCT82	08:00	122	68	70	0.0
11	GITS	18OCT82	12:00	126	84	60	69.6
11	GITS	19OCT82	08:00	110	48	68	13.0
11	GITS	20OCT82	08:00	116	62	64	18.1
11	GITS	21OCT82	08:00	106	60	68	26.3
11	GITS	21OCT82	12:00	110	68	68	88.2
11	GITS	22OCT82	08:00	116	60	60	23.8
11	DEXATRIM	08NOV82	08:00	128	76	74	0.0
11	DEXATRIM	08NOV82	12:00	120	68	70	112.8
11	DEXATRIM	09NOV82	08:00	130	70	72	5.8
11	DEXATRIM	10NOV82	08:00	120	58	74	3.4
11	DEXATRIM	11NOV82	08:00	120	66	70	1.4
11	DEXATRIM	11NOV82	12:00	110	78	62	88.6
11	DEXATRIM	12NOV82	08:00	128	78	68	0.0
11	SOLUTION	29NOV82	08:00	128	76	68	0.0
11	SOLUTION	29NOV82	12:00	116	74	64	102.1
11	SOLUTION	30NOV82	08:00	118	68	76	29.8
11	SOLUTION	01DEC82	08:00	118	68	68	10.3
11	SOLUTION	02DEC82	08:00	120	60	68	10.5
11	SOLUTION	02DEC82	12:00	118	74	68	70.7
11	SOLUTION	04DEC82	08:00	128	60	72	10.3
12	SOLUTION	18OCT82	08:00	138	78	70	0.0
12	SOLUTION	18OCT82	12:00	144	88	74	62.6
12	SOLUTION	19OCT82	08:00	150	80	80	12.9
12	SOLUTION	20OCT82	08:00	134	84	66	18.4
12	SOLUTION	21OCT82	08:00	134	82	70	11.5
12	SOLUTION	21OCT82	12:00	130	80	82	65.5
12	SOLUTION	22OCT82	08:00	136	82	78	11.9
12	DEXATRIM	08NOV82	08:00	142	78	70	0.0
12	DEXATRIM	08NOV82	12:00	140	80	58	67.4
12	DEXATRIM	09NOV82	08:00	132	82	64	2.1
12	DEXATRIM	10NOV82	08:00	132	78	70	7.8
12	DEXATRIM	11NOV82	08:00	130	84	70	10.8
12	DEXATRIM	11NOV82	12:00	136	86	64	141.5
12	DEXATRIM	12NOV82	08:00	132	78	72	10.7
12	GITS	29NOV82	08:00	138	78	80	0.0
12	GITS	29NOV82	12:00	134	84	76	75.6
12	GITS	30NOV82	08:00	138	82	72	19.4
12	GITS	01DEC82	08:00	128	78	68	12.9
12	GITS	02DEC82	08:00	134	80	84	15.1
12	GITS	02DEC82	12:00	142	88	68	68.1
12	GITS	03DEC82	08:00	128	76	80	21.6

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE SYS- TOLIC	DIAS- TOLIC	PULSE	PLASMA LEVEL, (NG/ML)
13	DEXATRIM	25OCT82	08:00	116	70	58	0.0
13	DEXATRIM	25OCT82	12:00	108	70	66	133.7
13	DEXATRIM	26OCT82	08:00	110	74	64	11.9
13	DEXATRIM	27OCT82	08:00	114	66	72	15.0
13	DEXATRIM	28OCT82	08:00	114	70	68	13.5
13	DEXATRIM	28OCT82	12:00	106	72	68	141.5
13	DEXATRIM	29OCT82	08:00	104	68	58	20.0
13	SOLUTION	15NOV82	08:00	114	74	62	0.0
13	SOLUTION	15NOV82	12:00	98	78	80	117.0
13	SOLUTION	16NOV82	08:00	98	74	80	58.5
13	SOLUTION	17NOV82	08:00	94	72	76	31.8
13	SOLUTION	18NOV82	08:00	98	72	76	44.2
13	SOLUTION	18NOV82	12:00	104	78	76	187.2
13	SOLUTION	19NOV82	08:00	114	78	70	28.9
13	GITS	06DEC82	08:00	108	78	64	0.0
13	GITS	06DEC82	12:00	98	58	76	77.0
13	GITS	07DEC82	08:00	98	72	72	30.0
13	GITS	08DEC82	08:00	108	78	64	77.3
13	GITS	09DEC82	08:00	102	76	68	40.2
13	GITS	09DEC82	12:00	98	76	76	119.3
13	GITS	10DEC82	08:00	112	68	64	31.4
14	DEXATRIM	25OCT82	08:00	122	78	76	0.0
14	DEXATRIM	25OCT82	12:00	122	80	78	138.3
14	DEXATRIM	26OCT82	08:00	122	72	76	8.4
14	DEXATRIM	27OCT82	08:00	120	64	80	12.7
14	DEXATRIM	28OCT82	08:00	122	74	74	12.7
14	DEXATRIM	28OCT82	12:00	124	78	62	132.8
14	DEXATRIM	29OCT82	08:00	118	74	72	8.3
14	GITS	15NOV82	08:00	120	80	58	0.0
14	GITS	15NOV82	12:00	118	80	84	45.4
14	GITS	16NOV82	08:00	114	80	82	7.9
14	GITS	17NOV82	08:00	114	70	86	36.1
14	GITS	18NOV82	08:00	118	74	88	29.7
14	GITS	18NOV82	12:00	118	78	88	94.3
14	GITS	19NOV82	08:00	122	72	90	15.5
14	SOLUTION	06DEC82	08:00	124	78	84	0.0
14	SOLUTION	06DEC82	12:00	116	76	84	88.2
14	SOLUTION	07DEC82	08:00	118	78	84	25.8
14	SOLUTION	08DEC82	08:00	118	76	88	15.8
14	SOLUTION	09DEC82	08:00	122	80	84	10.7
14	SOLUTION	09DEC82	12:00	118	80	80	84.6
14	SOLUTION	10DEC82	08:00	122	74	80	15.0

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE SYS- TOLIC	DIAS- TOLIC	PULSE	PLASMA LEVEL, (NG/ML)
15	GITS	25OCT82	08:00	116	62	72	0.0
15	GITS	25OCT82	12:00	112	60	72	63.5
15	GITS	26OCT82	08:00	128	70	68	23.7
15	GITS	27OCT82	08:00	124	70	74	16.6
15	GITS	28OCT82	08:00	120	64	70	16.6
15	GITS	28OCT82	12:00	120	78	64	77.9
15	GITS	29OCT82	08:00	118	68	72	29.8
15	DEXATRIM	15NOV82	08:00	130	78	72	0.0
15	DEXATRIM	15NOV82	12:00	122	82	68	156.5
15	DEXATRIM	16NOV82	08:00	130	68	84	13.4
15	DEXATRIM	17NOV82	08:00	132	80	78	10.3
15	DEXATRIM	18NOV82	08:00	124	76	76	12.3
15	DEXATRIM	18NOV82	12:00	124	78	74	136.4
15	DEXATRIM	19NOV82	08:00	128	76	84	11.3
15	SOLUTION	06DEC82	08:00	122	84	68	0.0
15	SOLUTION	06DEC82	12:00	118	84	64	79.6
15	SOLUTION	07DEC82	08:00	114	76	76	24.5
15	SOLUTION	08DEC82	08:00	126	74	80	15.3
15	SOLUTION	09DEC82	08:00	126	74	76	9.2
15	SOLUTION	09DEC82	12:00	128	84	84	73.4
15	SOLUTION	10DEC82	08:00	128	68	80	9.4
16	SOLUTION	25OCT82	08:00	124	78	74	0.0
16	SOLUTION	25OCT82	12:00	120	84	64	60.8
16	SOLUTION	26OCT82	08:00	116	80	78	18.5
16	SOLUTION	27OCT82	08:00	112	74	76	0.0
16	SOLUTION	28OCT82	08:00	112	74	74	3.0
16	SOLUTION	28OCT82	12:00	116	70	68	55.7
16	SOLUTION	29OCT82	08:00	112	74	70	2.4
16	GITS	15NOV82	08:00	126	78	60	0.0
16	GITS	15NOV82	12:00	124	84	68	54.5
16	GITS	16NOV82	08:00	118	88	76	15.5
16	GITS	17NOV82	08:00	138	84	80	22.8
16	GITS	18NOV82	08:00	128	84	84	19.7
16	GITS	18NOV82	12:00	124	84	68	78.2
16	GITS	19NOV82	08:00	120	88	78	11.4
16	DEXATRIM	06DEC82	08:00	140	88	80	-
16	DEXATRIM	06DEC82	12:00	-	-	-	-
16	DEXATRIM	07DEC82	08:00	138	88	84	-
16	DEXATRIM	08DEC82	08:00	140	88	84	0.0
16	DEXATRIM	09DEC82	08:00	148	86	76	0.0
16	DEXATRIM	09DEC82	12:00	136	88	68	141.7
16	DEXATRIM	10DEC82	08:00	136	88	84	1.9

BLOOD PRESSURE AND PULSE MEASUREMENTS

SUBJECT	DOSAGE FORM	DATE	TIME	BLOOD PRESSURE SYS- TOLIC	DIAS- TOLIC	PULSE	PLASMA LEVEL, (NG/ML)
17	SOLUTION	25OCT82	08:00	112	74	68	0.0
17	SOLUTION	25OCT82	12:00	108	68	68	79.4
17	SOLUTION	26OCT82	08:00	116	70	68	34.4
17	SOLUTION	27OCT82	08:00	118	76	64	6.7
17	SOLUTION	28OCT82	08:00	118	76	68	8.6
17	SOLUTION	28OCT82	12:00	109	78	60	66.4
17	SOLUTION	29OCT82	08:00	114	74	66	7.0
17	DEXATRIM	15NOV82	08:00	110	72	60	0.0
17	DEXATRIM	15NOV82	12:00	118	78	70	269.1
17	DEXATRIM	16NOV82	08:00	118	74	68	7.2
17	DEXATRIM	17NOV82	08:00	116	78	68	19.4
17	DEXATRIM	18NOV82	08:00	116	74	68	11.0
17	DEXATRIM	18NOV82	12:00	118	72	74	175.4
17	DEXATRIM	19NOV82	08:00	108	78	78	-
17	GITS	06DEC82	08:00	116	82	68	0.0
17	GITS	06DEC82	12:00	116	82	72	50.1
17	GITS	07DEC82	08:00	122	72	72	17.9
17	GITS	08DEC82	08:00	124	78	72	-
17	GITS	09DEC82	08:00	118	78	76	26.6
17	GITS	09DEC82	12:00	116	78	76	72.0
17	GITS	10DEC82	08:00	116	78	64	-
18	GITS	25OCT82	08:00	110	72	62	0.0
18	GITS	25OCT82	12:00	110	68	66	88.4
18	GITS	26OCT82	08:00	116	80	72	37.4
18	GITS	27OCT82	08:00	104	68	68	26.7
18	GITS	28OCT82	08:00	116	74	60	48.2
18	GITS	28OCT82	12:00	114	78	64	114.1
18	GITS	29OCT82	08:00	110	72	62	39.6
18	SOLUTION	15NOV82	08:00	112	80	62	0.0
18	SOLUTION	15NOV82	12:00	116	80	84	92.2
18	SOLUTION	16NOV82	08:00	116	80	70	30.5
18	SOLUTION	17NOV82	08:00	116	82	68	28.5
18	SOLUTION	18NOV82	08:00	118	82	62	33.0
18	SOLUTION	18NOV82	12:00	124	84	58	123.1
18	SOLUTION	19NOV82	08:00	120	82	72	23.8
18	DEXATRIM	06DEC82	08:00	124	86	68	0.0
18	DEXATRIM	06DEC82	12:00	118	84	72	164.0
18	DEXATRIM	07DEC82	08:00	116	82	64	16.3
18	DEXATRIM	08DEC82	08:00	114	84	96	24.8
18	DEXATRIM	09DEC82	08:00	114	82	72	21.6
18	DEXATRIM	09DEC82	12:00	116	82	64	204.3
18	DEXATRIM	10DEC82	08:00	116	84	84	-

FIGURE 1
PPA HCl Plasma Concentrations
Day 1

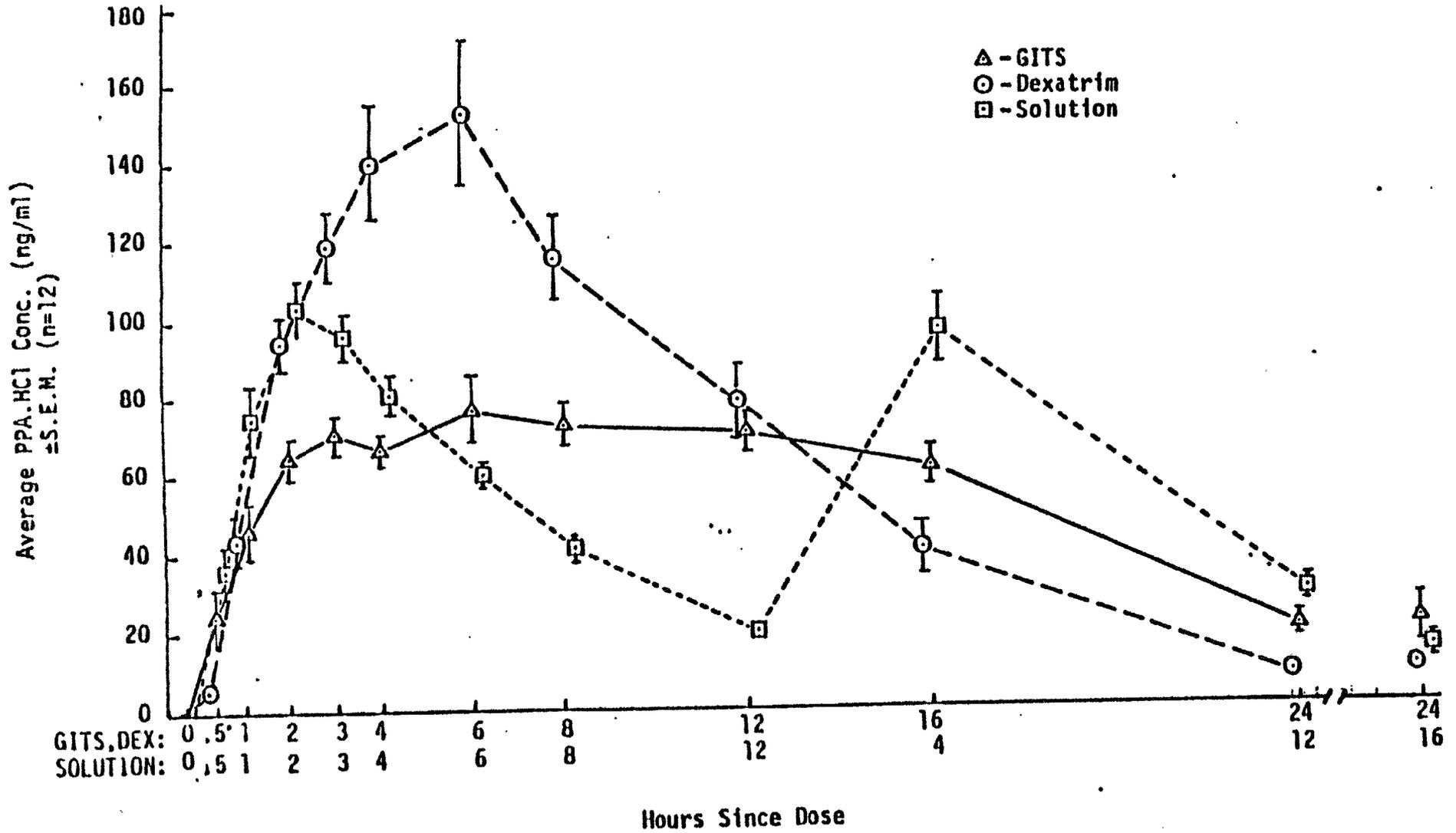
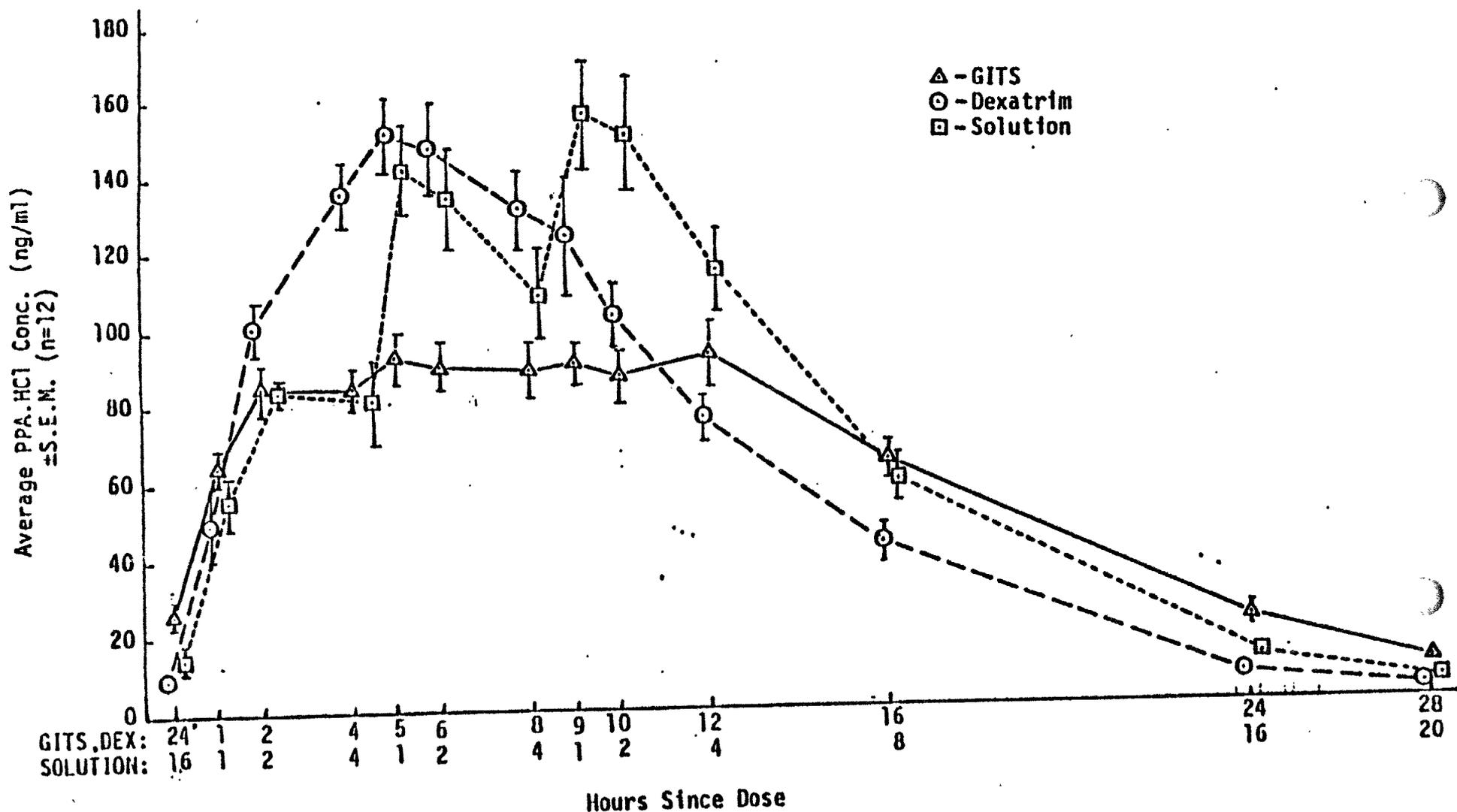


FIGURE 2
PPA HCl Plasma Concentrations
Day 4



APPENDIX I
Study Schedule

Day of Dosing Cycle	Time of Day	DRUG ADMINISTRATION Dose Given, MG		Blood Sample	Hours Since Last Dose		Urine Collection Interval	Food Intake
		GITS & Dexatrim	Solution		GITS & Dexatrim	Sol'n		
1	07:30						Control Void Just Prior to 1st Dose Start	Breakfast*
1	08:00	75	37.5	X	0	0	↓ 4 hrs	
1	08:30			X	0.5	0.5		
1	09:00			X	1	1		
1	10:00			X	2	2		
1	11:00			X	3	3	↓ 4 hrs	Lunch*
1	12:00			X	4	4		
1	12:30						↓ 4 hrs	Dinner*
1	14:00			X	6	6		
1	16:00			X	8	8	↓ 4 hrs	
1	18:30							
1	20:00		37.5	X	12	12		
1	24:00			X	16	4		
2	07:30						↓ 8 hrs	Breakfast*
2	08:00	75	25	X	24	12		
2	12:00		25				↓ 24 hrs	Dinner
2	12:30		25					
2	16:00		25					
2	18:30							
3	07:30						↓ 24 hrs	Breakfast*
3	08:00	75	25	X	24	16		
3	12:00		25				↓ 24 hrs	Dinner
3	12:30		25					
3	16:00		25					
3	18:30							
4	07:30						↓ 4 hrs	Lunch*
4	08:00	75	25	X	24	16		
4	09:00			X	1	1	↓ 4 hrs	Dinner*
4	10:00			X	2	2		
4	12:00		25	X	4	4		
4	12:30							
4	13:00			X	5	1	↓ 4 hrs	
4	14:00			X	6	2		
4	16:00		25	X	8	4	↓ 4 hrs	Dinner*
4	17:00			X	9	1		
4	18:00			X	10	2		
4	18:30							
4	20:00			X	12	4	↓ 4 hrs	
4	24:00			X	16	8		
5	07:30						↓ 8 hrs	Breakfast*
5	08:00			X	24	16		
5	12:00			X	28	20	↓ 4 hrs	
5	16:00				32	24		
6	08:00				48	40	Finish	

* All subjects were served the same menu

The Determination of Phenylpropanolamine
in Plasma by Gas Liquid Chromatography
and Urine by High Pressure Liquid Chromatography

Analysis of the drug in plasma involved extraction of phenylpropanolamine from plasma into toluene, followed by derivatization with trifluoroacetic anhydride to yield an electron-capturing di-trifluoroacetyl derivative, prior to injection into the gas liquid chromatographic system. 2-amino-3-phenyl-1-propanol hydrochloride was used as an internal standard. Quantification was performed by peak area measurement and by use of a standard curve.

Analysis of the drug in urine involved injection of aliquots of urine into the high pressure liquid chromatographic system. Amphetamine sulfate was used as an internal standard. The compounds were chromatographed on a reverse phase column. The drug and internal standard were converted to fluorescent molecules⁽¹⁾ as they eluted from the column by post-column reaction with o-phthalaldehyde. The fluorophores were detected by a fluorescence detector with excitation at 340 nm and an emission cutoff at 418 nm. Quantification was performed by peak area measurement and by use of a standard curve. A detailed description of the high pressure liquid chromatographic system is contained in Exhibit I.

Reference:

- (1) Simons, S.S., Jr. and Johnson, D.F., J. Am. Chem. Soc. 98, pp. 7098-1099, 1976.

Appendix II
(continued)

I. REAGENTS AND EQUIPMENT

Plasma Assay

Varian Model 3700 gas chromatograph; equipped with a Ni⁶³ electron-capture detector and capillary pneumatics.

The gas chromatograph was fitted with a fused silica capillary column (Chrompack-Netherland B.V.) 25 meters x 0.25 mm i.d. coated with OV-101.

Carrier gas was dry, oxygen-free high purity helium at a linear velocity of 30 cm/sec.

Make-up gas was dry, oxygen-free high purity nitrogen, at 30 ml/min.

The temperatures used were: injector 220°C, detector 200°C, oven temperature at 120°C isothermal.

Injections were made in the split mode, with a split ratio of 50:1.

Trifluoroacetic anhydride, Pierce Chemical Company

2-amino-3-phenyl-1-propanol hydrochloride, Aldrich Chemical Company,

4-Dimethylaminopyridine, Aldrich Chemical Company.

Appendix II
(continued)

Urine Assay

Water's Model 6000 A Pump (for mobile phase)

Water's Model 6000 A or Milton Roy Model 196
(for Fluoropa^o Solution)

Water's Model 710 A WISP Automatic Sample Processor

Coiled teflon tubing (15' x 0.027" i.d.) as a post-
column, in-line reactor

Thermonix 420 BKU water bath, room temperature

Schoeffel FS 970, L.C. Fluorometer

25 cm x 4.6 mm ODS-Hypersil 5 micron column, Shandon
Southern

Spectra-Physics 4100 Integrator-Calculator

Gelman Instrument Co., Glass Fiber Filter type A/E,
47 mm

Fluoropa^o crystals, Pierce Chemical Co.

Phenylpropanolamine HCl, Sigma

Amphetamine Sulfate

Appendix II
(continued)

II. PROCEDURES

A. Plasma Assay

Standard Solutions Preparation

Phenylpropanolamine HCl (Sigma) was used to prepare standard solutions. 2-amino-3-phenyl-1-propanol hydrochloride (Aldrich) was used to prepare internal standard solutions for the plasma assay. Phenylpropanolamine HCl (25 mg) was accurately weighed, transferred to a 25 ml volumetric flask, and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the phenylpropanolamine HCl stock standard solution (1 mg/ml).

2-amino-3-phenyl-1-propanol hydrochloride (10 mg) was accurately weighed, transferred to a 10 ml volumetric flask and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the 2-amino-3-phenyl-1-propanol hydrochloride stock internal standard solution (1 mg/ml).

Standard Curves, Spiked Plasma

Standard curves were generated by spiking control plasma samples (1 ml) with varying amounts of phenylpropanolamine HCl, and a constant amount of internal standard.

An aliquot (1.0 ml) of the phenylpropanolamine stock solution (1 mg/ml) was transferred to a 1L volumetric flask, and brought to mark with distilled water. Varying microliter aliquots of this dilution (1 µg/ml) were added to control plasma so that final concentrations were 5.23, 20.94, 104.70, 157.05 and 261.75 ng/ml. An aliquot (1.0 ml) of the 2-amino-3-phenyl-1-propanol hydrochloride stock internal standard solution (1 mg/ml) was transferred to a 1L volumetric flask, and brought to mark with distilled water. An aliquot (200 µl) of this dilution was added to each of the five standard plasma solutions for a final concentration of approximately 200 ng/ml. Standard concentrations of phenylpropanolamine HCl in plasma were converted to the equivalent base concentrations by multiplication by 0.8055.

Analysis of Plasma

Extraction and derivatization of phenylpropanolamine was carried out in silanized-glass 15 ml Teflon® stoppered centrifuge tubes. Plasma samples (1.0 ml) were spiked with internal standard 2-amino-3-phenyl-1-propanol hydrochloride (200 ng), followed by the addition of KH_2PO_4 buffer (0.2 ml of a 0.5 M solution, pH 11.0), saturated sodium chloride (0.2 ml), and toluene (1.5 ml). The centrifuge tubes were then sealed with teflon stoppers, and shaken by vortex mixing for 1.5 minutes. They were then centrifuged at 2000 RPM for 5 minutes, and the upper organic layer transferred to a second set of silanized 15-ml centrifuge tubes. The remaining aqueous phases were re-extracted with toluene (1.5 ml) by vortex mixing for 1.5 minutes. They were then centrifuged at 2000 RPM for 5 minutes, and the upper organic layer combined with the first toluene extracts.

The combined toluene extracts were then concentrated to approximately 0.5 ml under nitrogen, in a 40°C water bath. 4-dimethylaminopyridine (0.2 mg dissolved in 50 μl toluene) and trifluoroacetic anhydride (70 μl) were then added, and the tubes stoppered with teflon stoppers. The tubes were then heated in a 60°C water bath for 45 minutes. Na_2HPO_4 buffer (2 mls of a 0.5 M solution, pH 6.0) was then added to the tubes, followed by vortex mixing. The toluene layer was then transferred to a third set of silanized centrifuge tubes, followed by injection of microliter aliquots into the chromatographic system. Retention times of the di-trifluoroacetyl derivatives of phenylpropanolamine and 2-amino-3-phenyl-1-propanol were 2.5 and 3.5 minutes respectively.

B. Urine Assay

Standard Solution Preparation

Phenylpropanolamine HCl (Sigma) was used to prepare standard solutions. Amphetamine sulfate (Sigma) was used to prepare internal standard solutions for the urine assay. Phenylpropanolamine HCl (25 mg) was accurately weighed, transferred to a 25 ml volumetric flask, and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the phenylpropanolamine HCl stock standard (1 mg/ml).

Amphetamine sulfate (30 mg) was accurately weighed, transferred to a 500 ml volumetric flask and dissolved with distilled water. The solution was brought to mark with distilled water, and was used as the amphetamine sulfate stock standard solution (60 µg/ml).

The mobile phase was prepared by adding 6.8 gm KH_2PO_4 , 1.9 gm hexanesulfonate sodium (Regis Chemical Co.), and 1.0 ml triethylamine (Pierce Chemical Co.) to 950 ml distilled water. The pH was adjusted to 3.0 with H_3PO_4 . The solution was then transferred to a 1L volumetric flask and brought to mark with distilled water. Methanol (400 ml) was added to a 1L volumetric flask, which was then brought to mark with the above solution. The mobile phase was vacuum filtered through a 0.3 µ filter before use. The volumetric flow rate of the mobile phase was 1.5 ml/minute. Retention time of phenylpropanolamine and amphetamine were 5.8 minutes and 8.5 minutes respectively.

The eluent from the reverse phase column was mixed with a Fluoropa® solution which was introduced into the solvent stream via a T-fitting (Kel-F) at the rate of 1.5 ml/minute. The Fluoropa® solution was prepared by adding 25 gm boric acid to 950 ml distilled water in a 2L beaker. The pH was adjusted to 10.4 with 50% KOH in water. 2-mercaptoethanol (2.0 ml) was added to the solution, followed by the addition of Fluoropa® (800 mg o-phthalaldehyde) previously dissolved in approximately 10 ml methanol. This solution was vacuum filtered through a 0.3 µ filter before use.

Standard Curves, Spiked Urine

Standard curves were generated by spiking control urine samples (2.0 ml) with varying amounts of phenylpropanolamine HCl, and a constant amount of internal standard.

Varying microliter aliquots of the phenylpropanolamine HCl stock solution were added to control urine so that the final concentrations were 0.955, 3.82, 9.55, 38.2 and 95.5 µg/ml. An aliquot (1.0 ml) of the amphetamine sulfate stock solution was added to each of the five standard urine solutions for a final concentration of approximately 20 µg/ml. Standard concentrations of phenylpropanolamine HCl in urine were converted to the equivalent base concentrations by multiplication by 0.8055.

Analysis of Urine

Urine samples (2.0 ml) were transferred to 15-ml stoppered centrifuge tubes, followed by addition of internal standard amphetamine sulfate (approximately 60 µg). The solution was mixed on a vortex mixer, followed by centrifugation at 2000 RPM for five minutes. The supernatant was transferred to auto-sampler vials by pipet for injection into the chromatographic system.

III.

PLAN OF STUDY

The assay methods described in this report for measuring phenylpropanolamine levels in urine and plasma were evaluated for their precision, accuracy, reproducibility, specificity and linearity. In addition to this, the stability of phenylpropanolamine in urine and plasma was evaluated.

To evaluate the linearity of the methods, a five point standard curve over the expected concentration ranges was constructed. The precision of the method for measurement of drug in plasma and urine was determined as the coefficient of variation of the mean of five replicate assays at each level. Accuracy and reproducibility of the assays were determined by analysis of plasma and urine samples which had been spiked with phenylpropanolamine HCl, split into aliquots, frozen, and assayed on different days. Specificity of the methods for phenylpropanolamine and internal standards was determined.

Stability of the drug in urine was evaluated by analysis of samples which had been spiked with phenylpropanolamine HCl, split into aliquots, and stored at room temperature, 4°C and -20°C. Stability was evaluated over a one month period of time. Stability of the drug in plasma was evaluated in the same manner, except that spiked plasma samples were only stored at -20°C.

IV.

RESULTS AND CONCLUSIONS

Concentrations of phenylpropanolamine in the biological fluids were determined from calibration graphs constructed by plotting the ratio of peak-area measurements of the

drug to the internal standard (2-amino-3-phenyl-1-propanol in plasma and amphetamine in urine) against the concentrations of phenylpropanolamine in the standards. The concentration ranges for the standards in plasma and urine were 5.23-261.75 ng/ml and 0.955-95.5 µg/ml, respectively. Plots of peak area ratios (Y) against phenylpropanolamine concentration (X) were linear.

Typical standard curves for phenylpropanolamine in plasma and urine had correlation coefficients of 0.9997 and 0.9999 respectively. Sample standard curves for plasma and urine are shown in Figures 1 and 2 respectively. Sample chromatograms of plasma and urine extracts are shown in Figures 3 and 4 respectively. The methods were specific for drug in that control samples of plasma and urine contained no responses which interfered with either phenylpropanolamine or internal standards.

The precision of the method for measurement of plasma concentrations of phenylpropanolamine, determined as the coefficient of variation of the mean of five replicate assays, was ± 5.63 , ± 1.80 , ± 6.48 , ± 1.64 , and $\pm 1.63\%$ at 5.23, 20.94, 104.70, 157.05 and 261.75 ng/ml respectively. Precision for measurement of urine concentrations of phenylpropanolamine was ± 2.23 , ± 0.55 , ± 0.41 , ± 0.64 , and $\pm 0.19\%$ at 0.955, 3.82, 9.55, 38.2, and 95.5 µg/ml respectively. The above data are shown in Table I and Ia.

Reproducibility and accuracy of the assays was determined by analysis of blank plasma and urine samples which had been spiked with known amounts of phenylpropanolamine HCl. They were each then divided into aliquots and frozen, followed by analysis on consecutive days. The results of these analyses are shown in Table II and III for plasma and urine respectively. A given extract was injected into the chromatographic systems five successive times to determine the variation of detector response. The results are shown in Table IV. Reproducibility of the plasma assay was $\pm 7.03\%$ (Table II) while that of the urine assay was $\pm 2.69\%$ (Table III). Accuracy of the plasma assay measured as the percent difference between actual amount spiked into the plasma and the average of five assay values was 6.37%. Accuracy of the urine assay measured as the percent difference between the actual amount spiked into urine and the average of eight assay values was 1.58%. The variation of detector response measured as the coefficient of variation of area ratios between standard and internal standard for five successive injections of the same extract was $\pm 0.53\%$ and $\pm 0.75\%$ for plasma and urine respectively (Table IV).

Appendix II,
(continued)

Stability of the drug in plasma was determined by assay of control plasma samples which had been spiked at three levels (approximately 20, 100 and 190 ng/ml), separated into separate aliquots, and stored in silicone coated blood collection tubes at -20°C . Aliquots were assayed periodically over a 33 day period. The results are shown in Table V. The drug in plasma at levels of approximately 100 and 190 ng/ml are stable after 33 days of storage. At approximately 20 ng/ml, however, the level of drug fell off at day 33. It was therefore concluded that at this lower level, samples are stable for at least 28 days, but not 33 days. Samples from clinical studies must therefore be assayed within 28 days of collection.

Stability of the drug in urine was determined by assay of control urine samples which had been spiked at three levels (approximately 2, 25 and 50 $\mu\text{g}/\text{ml}$), separated into separate aliquots, and stored in brown one quart polyethylene bottles at three different temperatures (room temperature, 4°C and -20°C). Aliquots were assayed over a 28 day period. The results are shown in Table VI. The drug in urine at all three levels is only stable for 7 days when stored at room temperature. When stored at 4°C , the drug is stable for 11, 15 and 15 days at levels of approximately 2, 25 and 50 $\mu\text{g}/\text{ml}$ respectively. The drug at all levels in urine was stable for the full 28 days when stored at -20°C .