

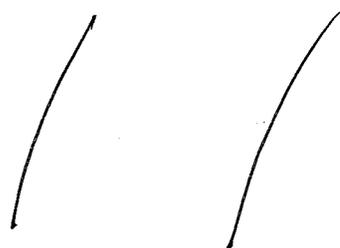
2.4.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

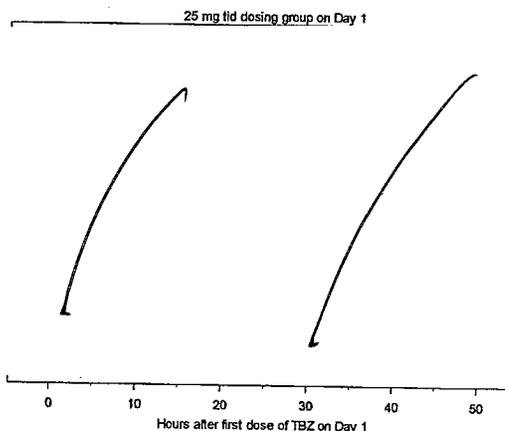
An exposure response relationship is not readily observed. In pivotal efficacy study TBZ 103,004 the subjects were titrated to “best dose” based on efficacy and tolerability, and therefore a dose-response relationship for efficacy cannot be determined. The range of doses at the end of treatment, with starting doses of 12.5 mg daily and titrated weekly by 12.5 mg increments, was 12.5 mg/day to 100 mg/day given in divided doses. (The maximum dose given at any administration time was 37.5 mg). The most common doses were 50 mg daily and 100 mg daily.

Efficacy Study TBZ 103,005 was a randomized, double-blind, placebo-controlled staggered withdrawal study in patients with Huntington’s Disease Treated with TBZ that was administered as the patients “best dose”. Daily doses at study entry ranged from 12.5 mg/day to 150 mg/day given while the patient was awake, with most doses between 25-75 mg/day. Ten subjects were to have a full PK profile. The plasma concentration time course for α -HTBZ in 3 subjects after 25 mg bid dosing and in 6 subjects on 25 mg tid dosing on Study Day 1 (before withdrawal) are shown in the figures below, and their PK parameters in the Table below. Subject 2 received a dose at approximately 34 hours, accounting for the high plasma concentrations at the later time points.

Plasma Concentration Time Course After 25 mg BID Dosing on Day 1



Hours after the first dose on Day 1



25 mg tid dosing group on Day 1

Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ (Study TBZ 103,005) on Day 1

	25 mg BID (% CV) n=3	25 mg TID (% CV) n=6
α-HTBZ		
C_{max} (ng/mL)	40.5 (66)	47.8 (65)
AUC_{0-t} (ng*h/mL) ^a	237.3 (63)	121.6 (39)
β-HTBZ		
C_{max} (ng/mL)	25.7 (47)	40.9 (69)
AUC_{0-t} (ng*h/mL) ^a	152.2 (85)	93.1 (55)

^a AUC, as calculated by Sponsor, based on a 12 hour dosing interval for bid regimens and a 4 hour dosing interval for tid regimens.

There was no apparent relationship between C_{max} and dose or AUC and dose. This may have been due to the lack of standard dosing intervals and the variability in the time from the last dose, as well as variability in drug metabolism. In fact, some individuals may not have received doses according to their regimens – for example subject 5 (bid regimen) has only 1 peak, although 2 would be expected.

The time to offset of response was evaluated in TBZ 103,005 within 12-18 hours after the last dose of TBZ at which time there was a statistically significant change in Total Maximal Chorea Scores compared to the Baseline on Study Drug.

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety? If relevant indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

The most common AEs during dose-optimization included somnolence, depression, insomnia, accidental injury, parkinsonism, and dysphagia. Since doses were titrated for tolerability, exposure response relationships for safety are difficult to determine in the Phase III studies.

Studies TBZ 203,008 and TBZ 202,001 evaluated the effect of single 12.5 mg and 25 mg doses and repeated (25 mg bid) doses of TBZ on various hormones and suggest a positive correlation between both α -HTBZ and β -HTBZ plasma concentration with serum prolactin. Increase in prolactin is consistent with an effect of drugs interfering with dopaminergic neurotransmission. However, since β -HTBZ is not thought to bind to hVMAT2, the increase in prolactin may be unrelated to this metabolite.

2.2.4.3 Does this drug prolong the QT or QTc interval?

The Sponsor has performed a HERG binding assay *in vitro* to assess displacement of ³H-astemizole by 10⁻⁷ M TBZ, α -HTBZ, and β -HTBZ and found no displacement of ³H-astemizole binding. However, it should be noted that clinically relevant concentrations range from approximately 40 ng/ml to more than 173 ng/ml for α -HTBZ or β -HTBZ (up to approximately 5.4 x 10⁻⁷ M) and concentrations of TBZ of up to 126 ng/ml (approximately 4 x 10⁻⁷ M) were measured in a subject with hepatic impairment. Thus, the concentration ranged examined *in vitro* is inadequate. The effects of any other circulating metabolites have not been evaluated *in vitro*.

The effect on QTc of TBZ given as a single 25 or 50 mg dose was evaluated in healthy male and female subjects in a placebo controlled study with moxifloxacin as a positive control. A slight positive correlation was seen between change from baseline QTcI or QTcF and β -HTBZ. TBZ caused an approximate 7 msec increase in QTc (F or I) and a 10 msec increase could not be excluded. There were no subjects with QTc > 500 msec and the 2 subjects characterized as CYP2D6 PMs were not QT outliers. A larger effect at higher exposure cannot be ruled out. In addition, excessive exposure to TBZ (as seen in hepatic impairment) has not been evaluated in terms of QT prolongation.

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In the ISS there were 3 subjects with change from baseline in QTc(F) > 30 msec, and one subject with a change in QTcF of 62.4 msec. No patient had on-treatment QTc exceeding 450 msec, according to the safety officer's review.

2.2.4.4. Is the dose and dosing regimen selected by the Sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues? (In some cases it may be possible to combine with 2.2.4.2 and 2.2.4.3)

The maximum daily dose (100 mg/day) is consistent with the doses used in the pivotal efficacy study as is the titration schedule. However, since the maximum single dose in that study was 37.5 mg, that should be the maximum single dose in labeling.

2.4.5 What are PK characteristics of the drug and its major metabolites?

2.2.5.1 What are the single dose and multiple dose PK parameters?

The following table shows the PK parameters after single doses (12.5-50 mg) and in 1 multiple dose study (25 mg) in healthy subjects. The PK parameters for a given dose are similar across studies.

Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ in Phase I Studies							
	Study 202,001 Single Dose Prolactin study 12.5 mg (% CV) n=6	Study 104012 Single dose BE study 25 mg (1x25 mg tablet) (% CV) n=23	Study 103,003 dose Food Effect Study 25 mg Single (% CV) n=25	Study 104,015 Single dose QT study		Study 203,008 Single and Repeat Dose Prolactin Study (% CV) n=12	
				25 mg n=50	50 mg	25 mg single dose	25 mg repeat dose (Day 4)
α-HTBZ							
t_{max} (h) ^a	1.25	1.50 (1.0-3.0)	1.0 (1.0-4.0)	1.5 (0.5-2.5)	1.0 (0.5-4.0)	1.0 (1.0-2.5)	1.0 (0.5-2.0)
C_{max} (ng/mL)	12.7 (16)	33.1 (44)	32.2 (40)	38.23 (47)	88.4 (48)	32.7 (34)	51.1 (63)
AUC _{0-t} (ng*h/mL)	91.8 (62)	187 (71)	175.9 (43)	185.84 (56)	425.64 (53)	154.3 (56)	244.4 (64)
AUC _{0-∞} (ng*h/mL)		219.9 (87)	199.3 (47)	214.16 (61)	458.66 (58)	159.6 (60)	0.153 (30)
λ_z (hr ⁻¹)		0.113 (48)	0.101 (38)			0.173 (31)	4.9 (31)
$t_{1/2}$ (h)		7.5 (47)	8.1 (51)	6.26 (36)	6.49 (33)	4.4 (32)	
β-HTBZ							
t_{max} (h) ^a	1.25	1.5 (1.0-2.5)	1.5 (1.0-4.0)	1.5 (0.5-3.0)	1.0 (0.5-3.0)	1.0 (1.0-1.5)	1.0(0.5-2.0)
C_{max} (ng/mL)	8.15 (65)	22.3 (73)	17.8 (79)	24.17 (72)	61.42 (59)	12.8 (62)	21.0 (96)
AUC _{0-t} (ng*h/mL)	66.8 (135)	136.8 (142)	87.6 (118)	127.1 (166)	274.1 (100)	43.4 (79)	74.2 (84)
AUC _{0-∞} (ng*h/mL)		189.9 (173)	95.8 (133)	161.1 (195)	327.7 (123)	43.9 (76)	
λ_z (hr ⁻¹)		0.238 (54)	0.259 (46)			0.313 (32)	0.258 (30)
$t_{1/2}$ (h)		5.2 (105)	3.6 (69)	4.23 (68)	4.9 (53)	2.4 (33)	3.0 (36)

^a median (range)

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

PK was not well characterized in patients. However, the available data suggest that it is similar to the PK described in healthy subjects with respect to exposure, variability in PK, and relationship of α - to β -HTBZ.

For data from Study 103,005, please refer to question 2.2.4.1. In that study, the AUC for the 25 mg BID dose was generally in agreement with results from Study 203,008 although the C_{max} was not. This discrepancy may have been due to lack of standard dosing intervals and variability in time from the last dose, as well as variability in drug metabolism. Variability in the PK was great, similar to that observed in the healthy volunteer studies. The ratio of α - to β -HTBZ ranged from 0.3 to 6.8 and this is consistent with the ratio reported in other studies.

Study 103,004 was a randomized, double-blind, placebo-controlled study in which 54 patients with Huntington's chorea were titrated to their best dose up to 100 mg/day. Sparse PK sampling was performed with approximately 2 samples per subject (1 at week 9 and 1 at week 12). Ratios of α - to β -HTBZ ranged from 0.43 to 6.4 consistent with the ratio reported in other studies.

2.2.5.3. What are the characteristics of drug absorption? (This may include discussion of transporter or pH effect).

In 6 healthy male volunteers receiving [¹⁴C]-tetrabenazine orally, 64.97-81.39% of the dose (mean 75.4%) was excreted in urine (Study RD 204/24124). Therefore, the extent of absorption can be considered to be at least 75.4% (mean) since that was the % of dose excreted in urine.

2.2.5.4. What are the characteristics of drug distribution? (Include protein binding)
Protein binding of TBZ, α -HTBZ, and β -HTBZ was not concentration dependent at concentrations of approximately 40-160 ng/ml for TBZ, 60-290 ng/ml for α -HTBZ, and 50-200 for β -HTBZ (Study AG-04001). The % protein binding was approximately 60-68% for α -HTBZ and approximately 59-63% β -HTBZ. The % bound for TBZ was approximately 81-84%, although this may have been overestimated as the assay was not performed under equilibrium conditions for TBZ.

In the mass balance study (Study RD 204/24124) exposure to radioactivity in whole blood was determined to be approximately 40% less than that in plasma.

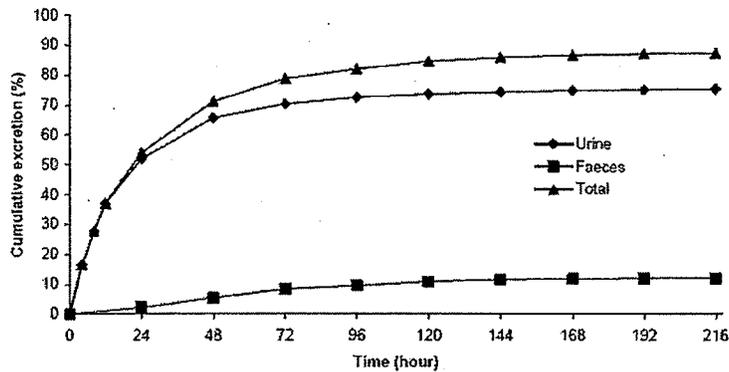
The Sponsor has provided literature that evaluates the use of ¹¹C- α -HTBZ, ¹¹C-HTBZ, and ¹¹C TBZ in positron emission tomography (PET) for VMAT2 imaging in humans. The literature shows rapid uptake of these compounds into the human brain after intravenous injections. The results also show binding predominately, although not exclusively, in the striatum.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination? (This may include table with results of mass balance study)

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The mass balance study suggests that TBZ is extensively metabolized, with metabolites being primarily renally eliminated.

Mass balance study 204/24124 evaluated the PK characteristics of total radioactivity, TBZ, and its metabolites following oral administration of 25 mg [¹⁴C-TBZ] in healthy male volunteers. The mean total recovery (urine + feces) was 87.49% (range 76.88-92.84%). 64.97-81.39% of the dose (mean 75.4%) was excreted in the urine in the 9 day collection period. More than 19 metabolites were reported to be found in the urine. TBZ was not reported to be found in the urine. Fecal recovery accounted for 7.07-16.05% of the dose. This is shown in the figure below, as provided by the Sponsor.



Mean excretion of total radioactivity following oral administration of 25 mg ¹⁴C-TBZ to 6 male human subjects

2.2.5.6 What are the characteristics of drug metabolism? (This may include data on extraction ratio; metabolic scheme; enzymes responsible for metabolism; fractional clearance of drug).

TBZ is extensively and rapidly metabolized so that TBZ is not generally detected in plasma or is seen at very low concentrations (10 ng/ml). In the mass balance study (RD 204/24124) at least 19 distinct metabolites have been identified in the urine, according to the Sponsor. α - and β -HTBZ have been considered to be the major circulating metabolites and have been measured in the clinical pharmacology studies. However, the mass balance study (conducted on June 30, 2005) has identified other circulating metabolites that may be circulating to a similar extent as the active metabolite α -HTBZ as shown in the table below (as provided by the Sponsor). The pharmacokinetics and pharmacologic activity of metabolites other than α - and β -HTBZ have not been characterized.

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Component	0.25 - 1.5 hours	2 - 3 hours	4 - 8 hours	Mean
P1	ND	ND	ND	NC
P2	ND	ND	ND	NC
P3	ND	ND	ND	NC
P4	ND	ND	ND	NC
P5	ND	ND	ND	NC
P6	ND	ND	4.16 (4.65)	NC
P7	ND	ND	ND	NC
P8	ND	ND	8.23 (9.14)	NC
P9	ND	ND	ND	NC
P10	ND	ND	ND	NC
P11	23.44 (16.67)	22.62 (20.41)	15.23 (16.90)	22.73 (17.99)
P12	ND	ND	ND	NC
P13	26.73 (19.01)	41.77 (25.88)	24.22 (26.88)	30.91 (24.92)
P14	ND	ND	ND	NC
P15	ND	ND	ND	NC
P16	49.54 (35.24)	50.11 (34.66)	19.77 (21.95)	39.81 (30.61)
P17	13.65 (9.71)	8.54 (4.52)	4.45 (4.97)	8.22 (6.40)
P18	25.73 (18.32)	18.70 (11.68)	14.05 (15.60)	18.83 (15.15)
Other	1.52 (1.08)	ND	ND	NC

Results expressed as ng equivalents/ml (Values in parentheses indicate % sample radioactivity)
 ND Component not detected NC Not calculable

These components represent:

P1-9: mono-hydroxy-HTBZ and glucuronides of O-dealkylated HTBZ

P10: monohydroxy HTBZ

P 11 and P13: sulfate conjugates of O-dealkylated HTBZ

P15 and P17: O-dealkylated HTBZ and/or β- HTBZ (tentative)

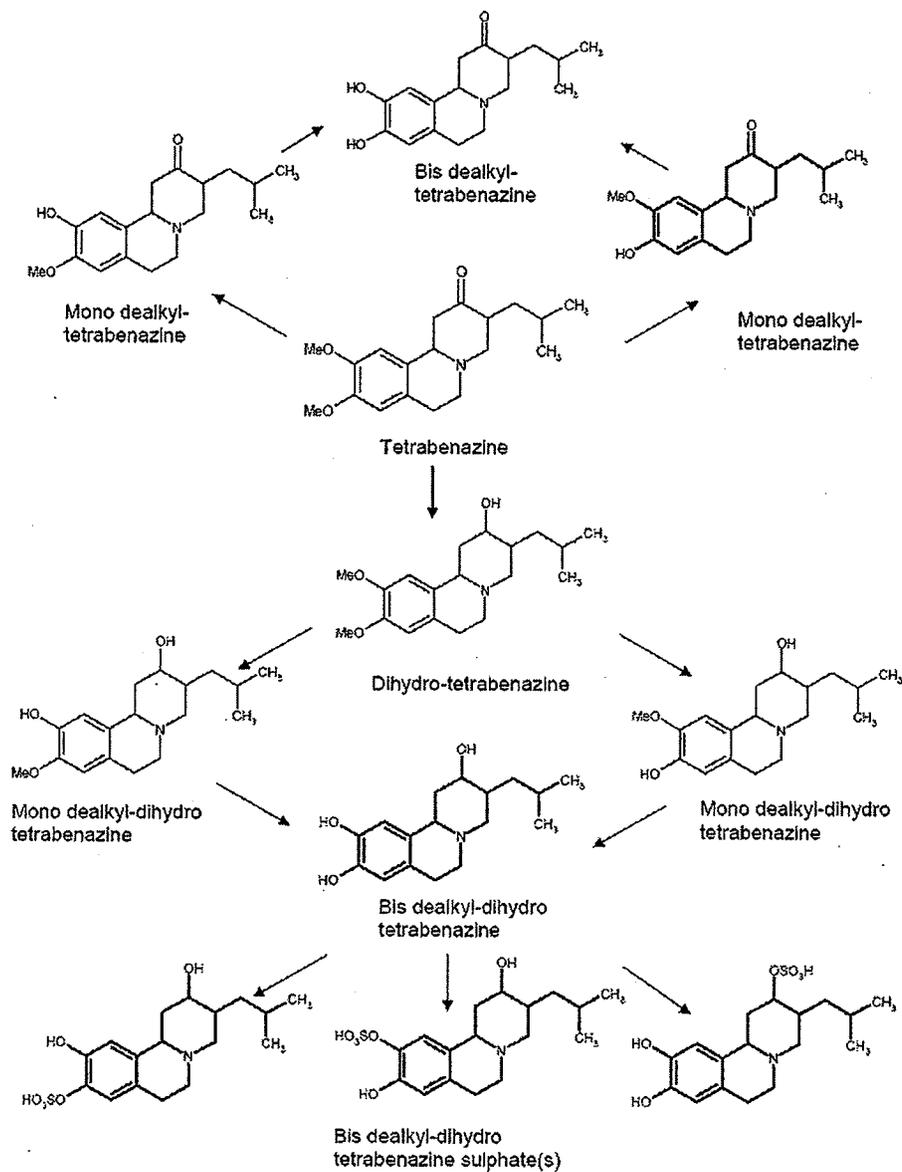
P18: α - HTBZ

P16, the largest component of the plasma, was not identified.

The results of this mass balance study are generally in agreement with the metabolites identified in the original submission that reported on metabolites in human plasma from Protocols TBZ 103,003 (food effect, 3 subjects) and from 2 subjects in Efficacy study TBZ 103004, targeting metabolites that had been identified by Schwartz et al (*Biochemical Pharmacology* 1966; 15: 645-655) based on detection in urine following a subcutaneous injection in humans. However, several oxygenated metabolites from that report are not reported in the most recent mass balance study.

The Sponsor has proposed the following metabolic scheme:

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Note: Dihydro-tetrabenazine is referred to throughout this review as HTBZ.

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Although the Sponsor shows mono- and bis-dealkyl TBZ, the extent of circulation of those metabolites is not described.

The Sponsor has evaluated the metabolism of TBZ and of α - and β -HTBZ using recombinant human CYP enzymes (Studies AG-A04002 and AG-A05001). The studies suggest a role for CYP2D6 in the metabolism of TBZ and α - and β -HTBZ. However, there were deficiencies in the approach to evaluating P450-mediated metabolism that does not allow for ruling out the role of other P450 pathways. These deficiencies include 1) the Sponsor has only used recombinant P450s, but has not used additional methods (such as use of inhibitors) as generally recommended, and 2) the probes used as controls in the present study are not classical, preferred probes so it is difficult to understand the acceptability of the reactions that showed negative results. The Sponsor has not evaluated the role of specific P450 isozymes in the formation or metabolism of other metabolites.

Stereoselectivity in exposure to metabolites is observed in clinical studies. On average, the exposure to α - HTBZ is approximately 1.5 x greater than exposure to β -HTBZ. The variability in the ratios of α : β is great and ranges from approximately 0.4 to 6 have been observed.

2.2.5.7 What are the characteristics of drug excretion?

As described above, TBZ is extensively metabolized, and the metabolites are primarily renally eliminated. Several circulating metabolites are substantially eliminated renally (11 and 13 are the predominant metabolites in the urine). Components identified in the urine in the mass balance study (RD 204/24124) are as follows:

Component	Assignment	Nominal HPLC retention time (minutes)*	% dose
U1 – U9	Monohydroxy-dihydro tetrabenazines and Glucuronides of O-dealkylated dihydro tetrabenazine(s)	4.2 – 13.9	1.01 – 4.02 (Total 17.93)
U10	Monohydroxy dihydro tetrabenazine	14.1 – 14.6	3.70
U11 and U13	Sulphate conjugates of O-dealkylated dihydro tetrabenazine(s)	15.4 – 15.7 17.3 – 17.5	12.68 – 17.09 (Total 29.77)
U15 and U17	O-dealkylated dihydro tetrabenazine and/or β -dihydro tetrabenazine	19.0 – 19.2 21.9 – 22.0	2.38 – 2.64
U18	α -dihydro tetrabenazine	24.9 – 25.1	0.43

* Retention times taken from HPLC analysis, slight shifts in retention times observed on MS analysis

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The elimination half-life for α -HTBZ has ranged from approximately 5-8 hours (mean) and the elimination half-life for β -HTBZ has ranged from approximately 2.5-5 hours (mean) in healthy volunteers.

2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

In the Phase I studies shown in the table in Question 2.2.5.1, above, The C_{max} and AUC for both α -HTBZ and β -HTBZ appear to increase slightly more than dose proportionately (approximately 2.3-2.6 fold either across or within studies with a 2-fold increase in dose following single doses.

In addition to those studies in which α -HTBZ and β -HTBZ were characterized separately, Study 1700114 was a dose proportionality study that evaluated single 12.5 mg and 50 mg doses in healthy volunteers in a crossover design, using an achiral assay to determine HTBZ. Results from that study also show a greater than dose proportional increase in exposure with an increase in dose (please refer to individual study review in Appendix), such that a 4-fold increase in dose resulted in a 4.3-fold increase in C_{max} and an approximate 5-fold increase in AUC.

2.2.5.9 How do the PK parameters change with time following chronic dosing?

Based on a range of half-lives from approximately 4.4 to 8 hours for α -HTBZ, an accumulation factor of 1.2-1.5 would be predicted, for a dose given every 12 hours. Based on a range of half-lives from approximately 2.4-5 hours for β -HTBZ, an accumulation factor of 1-1.2 would be predicted. Following chronic dosing, a slightly greater than expected increase in exposure to α -HTBZ and β -HTBZ is observed as shown in the results for Study 203,008 in table in Question 2.2.5.1, above.

Study TBZ 203,008 evaluated the pharmacodynamic effect of single and repeated doses of oral TBZ on serum and plasma concentrations of various hormones in 12 healthy male volunteers and assessed the PK characteristics of TBZ and its metabolites α -HTBZ and β -HTBZ after single and multiple dose administration. TBZ was given as a single 25 mg dose on the mornings of Days 1 and 4, and morning and evening doses on Days 2 and 3. Blood samples were drawn on Day 1 and 4 to determine PK. There was an approximate 1.58 fold accumulation of α -HTBZ (based on AUC) and an approximate 1.7 fold increase for β -HTBZ (based on AUC, and was similar for C_{max}).

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The inter-subject variability, as shown by CV in the Table in Question 2.2.5.1 was approximately 40-60% for α -HTBZ for C_{max} and AUC and approximately 60-90% for C_{max} and even higher for AUC (80%-166%) for β -HTBZ in healthy volunteer studies. As shown in Question 2.2.5.2, there was similar extensive variability in patients receiving TBZ. Sources of variability could include variability in drug metabolism including formation and metabolism of α -HTBZ and β -HTBZ.

Data are not available to determine intra-subject variability.

2.5 Intrinsic Factors

2.5.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure and/or response and what is the impact of any differences in exposure on efficacy or safety responses?

Age – A population PK analysis (4 Phase I studies and 2 efficacy studies) explored age as a covariate. Although more rapid absorption and decreased clearance was predicted with increasing age, the other covariate that showed a statistical relationship with PK parameters was patients vs subjects. The 4 studies in healthy subjects used only younger subjects (maximum age 44 years) whereas the studies in patients included elderly subjects. Thus some of the effects attributed to age may result from status (patient vs healthy subject).

Gender – There is no evidence to support a gender effect.

Gender was evaluated in Study TBZ 104012. PK parameters were similar for men and women for T_{max} and C_{max}. Exposure (AUC) to β-HTBZ was slightly greater (1.5x) in women than in men, with slightly longer half-life, although variability was large. There does not appear to be a gender effect.

This was a BE study (TBZ 104,012) to determine BE of tetrabenazine 12.5 mg and 25 mg tablets using a single-dose, randomized, crossover design in 11 healthy males and 12 healthy females ages 18-44 y.o. Results in men and women are shown in the table below. As seen in all of the TBZ PK studies, variability was large.

	Male (n=11)		Female (n=12)	
	Test (2x12.5 mg tablet) TREATMENT B (% CV)	Reference (1x25mg tablet) TREATMENT A (% CV)	Test (2x12.5 mg tablet) TREATMENT B (% CV)	Reference (1x25mg tablet) TREATMENT A (% CV)
α-HTBZ				
t _{max} (h) ^a	1.5 (1.0-2.5)	2.0 (1.0-2.5)	1.5 (1.0-1.5)	1.25 (1.0-3.0)
C _{max} (ng/mL)	33.3 (27)	31.4 (34)	39.9 (53)	34.6 (51)
AUC _{0-∞} (ng*h/mL)	218.88 (62)	211.52 (61)	249.79 (112)	227.6 (107)
t _{1/2} (h)	6.3 (32)	7.5 (54)	6.9 (46)	7.6 (43)
β-HTBZ				
t _{max} (h) ^a	1.5 (1.0-2.5)	1.5 (1.0-2.5)	1.5 (1.0-2.5)	1.5 (1.0-2.5)
C _{max} (ng/mL)	21.7 (46)	21.6 (52)	27.0 (84)	22.9 (89)
AUC _{0-∞} (ng*h/mL)	177.59 (164)	153.3 (146)	270.58 (193)	223.5 (184)
t _{1/2} (h)	4.7 (100)	4.2 (102)	5.5 (112)	6.1 (106)

Population PK analysis using data from TBZ 103,003 (Food Effect), TBZ 104,012 (BE of 12.5 mg and 25 mg), TBZ 203,008 (Single and Repeat Dose in Healthy Male Volunteers), TBZ 203,009 (P-glycoprotein Study), TBZ 103,005 (Randomized Withdrawal in Patients), and TBZ 103,004 (Randomized, Double-Blind, Placebo Controlled Study in HD) also found no evidence of a gender effect.

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Race – This has not been evaluated. The racial composition of TBZ 104012 was Black, and the composition of 103,003 was 13 Black, 3 Caucasian. In the remainder of the Phase I studies, race was not provided as the studies were performed in France where race is not recorded. In efficacy study 103,004 the population was 93% Caucasian.

Weight – Not evaluated.

Height – Not evaluated.

Disease – Not evaluated.

Genetic Polymorphism – The effect of genetic polymorphisms on exposure to TBZ or its metabolites has not been carefully evaluated.

Pregnancy – This has not been evaluated.

Organ Dysfunction –

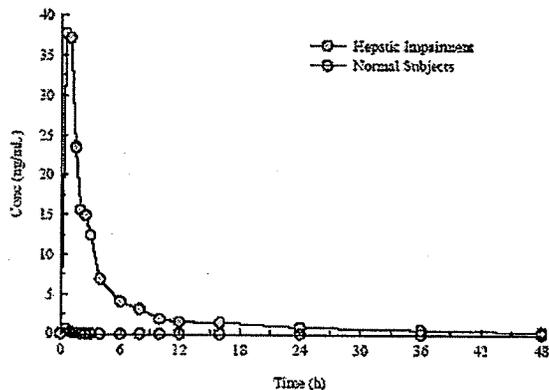
Renal Impairment - The PK population in the pivotal Phase III studies included 21 subjects with mild renal impairment (Cr Cl 50-80 ml/min). Patients with Cr > 3X ULN were excluded from the pivotal Phase III studies.

Tetrabenazine is a chronically administered drug that is extensively metabolized. Phase I and Phase II metabolites (including monohydroxy-dihydro tetrabenazines, glucuronides of O-dealkylated dihydrotetrabenazines, and sulfate conjugates of O-dealkylated dihydrotetrabenazines) are eliminated renally, and the activity of these metabolites with respect to safety and efficacy is unknown. However, TBZ does not appear in the urine, and the HTBZ metabolites accounted for less than 3% of the dose in the urine in the mass balance study.

Hepatic impairment reduces the metabolism of TBZ resulting in substantial exposures relative to normal subjects in which minimal exposure to TBZ occurs. The increase in TBZ exposure is not entirely accounted for by changes in exposure to α - and β -HTBZ.

The Sponsor is performing a study to compare PK characteristics of TBZ and α - and β -HTBZ in subjects with mild or moderate liver impairment to those of age-matched healthy subjects after administration of a single 12.5 mg dose of TBZ. An interim report was provided in the submission stating that the study is ongoing. Twelve male subjects have completed the study to date (6 healthy, 6 liver impaired). Five subjects were considered to be mildly hepatically impaired and 1 subject moderately hepatically impaired based on Child-Pugh classification. Plasma concentrations of TBZ are shown in the figure below and the PK parameters are shown in the table below.

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re 1. Mean plasma concentrations of tetrabenazine after oral administration of 25 mg of tetrabenazine (2x12.5 mg tablets) under fasting conditions to subjects with liver impairment and healthy subjects — linear axes - Protocol TBZ 203,010

Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ (Study TBZ 203,010)

	Normal (% CV) n=6	Hepatic Impairment (% CV) n=6
TBZ		
t_{max} (h) ^a	0.5 (0.5-0.5)	1.0 (0.5-1.5)
C_{max} (ng/mL)	0.67 (106)	47.0 (98)
AUC _{0-t} (ng*h/mL)	0.5 (112)	131.6 (112)
AUC _{0-∞} (ng*h/mL)	1.5	170.0 (97)
λz (hr ⁻¹)	1.67	0.201 (174)
$t_{1/2}$ (h)	.41	12.8 (54)
α-HTBZ		
t_{max} (h) ^a	1.0 (0.5-1.05)	1.75 (0.5-3.03)
C_{max} (ng/mL)	38.4 (21)	34.8 (44)
AUC _{0-t} (ng*h/mL)	211.0 (50)	262.2 (34)
AUC _{0-∞} (ng*h/mL)	215.1 (50)	282.7 (40)
λz (hr ⁻¹)	0.136 (44)	0.073 (44)
$t_{1/2}$ (h)	6.1 (45)	10.9 (39)
β-HTBZ		
t_{max} (h) ^a	1.0 (0.5-1.05)	1.75 (0.5-7.98)
C_{max} (ng/mL)	23.4 (44)	22.7 (66)
AUC _{0-t} (ng*h/mL)	101.3 (82)	125.2 (30)
AUC _{0-∞} (ng*h/mL)	103.1 (81) ^d	146.4 (52)
λz (hr ⁻¹)	0.201 (36) ^d	0.101 (58)
$t_{1/2}$ (h)	4.0 (51) ^d	10.7 (92)

^a median (range)

In hepatic impairment patients there were detectable concentrations of TBZ that were similar to or higher than exposures seen with α - or β -HTBZ. The mean TBZ concentration in hepatic subjects was 70-fold higher for C_{max} and approximately 100 to 200 fold higher for AUC than

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the mean in healthy subjects. There were also increases in exposure to α - and β -HTBZ, in general, although to a lesser extent than the increase in exposure to TBZ. Half-life was also prolonged.

The PK parameters in each hepatic impairment subject are shown in the tables below. The patient with moderate impairment can be distinguished from the mildly impaired subjects.

TBZ PK parameters by hepatic impairment subject

Subject Number	Child-Pugh Classification	C _{max} (ng/ml)	AUC _{0-t} (ng*hr/ml)	t _{1/2} (h)
13	5	No conc \geq LOQ	No conc \geq LOQ	No conc \geq LOQ
14	5	42.5	158.9	13.5
15	6	5.6	8.72	0.8
16	9	126.3	400.4	18.8
17	6	66.6	145.0	16.3
18	5	41.0	76.3	14.6

α -HTBZ PK parameters by hepatic impairment subject

Subject Number	Child-Pugh Classification	C _{max} (ng/ml)	AUC _{0-t} (ng*hr/ml)	t _{1/2} (h)
13	5	39.8	170.4	7.0
14	5	31.3	251.8	9.4
15	6	62.1	320.6	8.5
16	9	17	361.8	Could not be estimated
17	6	33.1	320.9	9.0
18	5	25.7	137	9.8

β -HTBZ PK parameters by hepatic impairment subject

Subject Number	Child-Pugh Classification	C _{max} (ng/ml)	AUC _{0-t} (ng*hr/ml)	t _{1/2} (h)
13	5	29.0	84.5	Could not be estimated
14	5	14.8	111.8	Could not be estimated
15	6	50.6	142.9	5.3
16	9	9.0	183.5	Could not be estimated
17	6	16.6	127.6	7.3
18	5	16.4	76.6	6.7

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The Sponsor reports that no safety or tolerability analysis was performed for the purposes of the interim pharmacokinetic analysis, but that no deaths or serious adverse events were reported for the first 12 participants who had completed the study.

2.5.2 *Based upon what is known about exposure-response relationships and their variability, and the groups studied, healthy volunteers vs patients vs. specific populations (examples shown below), what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.*

2.3.2.1 *Elderly* - None

2.3.2.2 *Pediatric patients. Also, what is the status of pediatric studies and/or any pediatric plan for study?*

The Sponsor has requested a waiver from the requirement of conducting pediatric studies, since TBZ has been granted orphan drug status.

2.3.2.3 *Gender* - None

2.3.2.4 *Race* - None.

2.3.2.5 *Renal Impairment* – None.

2.3.2.6 *Hepatic Impairment*

Without an understanding of the contribution of elevated levels of TBZ to safety, and without an understanding of the relationship between TBZ, α - and β -HTBZ, and other metabolites that have not been measure in the clinical pharmacology studies and their role in contributing to safety and efficacy, it is not possible to recommend a dosage adjustment in hepatic impairment. We recommend that TBZ be contraindicated in hepatic impairment.

2.3.2.7 *What pharmacogenetics information is there in the application and is it important or not?*

The QT study included 2 subjects characterized as poor metabolizers of CYP2D6. Their exposure was at the higher end of the range of exposures. Their ratios of α : β -HTBZ were lower than the ratios in extensive metabolizers, similar to what has been observed by the reviewer in patients taking CYP2D6 inhibitors (see 2.4.2.8 below). The relevance depends on the ratio of safety to efficacy in relation to the α : β -HTBZ ratio. This may not be as important in titrating the dose of the drug in a CYP2D6 poor metabolizer as would be adding a CYP2D6 inhibitor to a patient (extensive metabolizer) already taking a given dose of TBZ. The role of CYP2D6 polymorphisms should be confirmed.

2.3.2.8 *What pregnancy and lactation use information is there in the application?*

This application did not include human pregnancy or lactation use information.

2.3.2.9 *Other human factors that are important to understanding the drug's efficacy and safety*
None Identified.

2.6 *Extrinsic Factors*

2.6.1 *What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of any differences in exposure on response?*

The effect of smoking on exposure was not systematically evaluated. Study 1700114 that examined bioavailability after single and repeat doses of TBZ using an achiral assay included 6/12 subjects in the single dose group and 13/24 subjects in the repeat dose group. The reviewer has evaluated PK parameters for HTBZ by smoking status and did not see a consistent effect of smoking on exposure. Please refer to the Individual Study report for details.

The Sponsor has not evaluated the effect of herbal products, diet (other than food effect), or alcohol use on exposure and/or response. The effect of drugs on exposure/response has not been specifically evaluated, but some information can be extrapolated from the clinical studies and will be discussed in 2.4.2, below.

Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.

No recommendations.

2.6.2 *Drug-Drug Interactions*

2.4.2.1 *Is there an in vitro basis to suspect in vivo drug-drug- interactions?*

Yes, there is an *in vitro* basis to suspect *in vivo* drug-drug interactions. As discussed in 2.2.5.6 TBZ and α - and β -HTBZ appear to be substrates of CYP2D6 *in vitro*. A role for other P450s cannot be ruled out. In addition, the results of *in vitro* Study AG-03001 suggest (based on predicted I/Ki) the possibility of CYP2D6 inhibition following administration of TBZ due to the presence of β -HTBZ.

2.4.2.2 *Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?*

The drug appears to be a substrate for at least CYP2D6 (see above). The role of genetics has not been well characterized, although in the QT study, the 2 subjects characterized as poor metabolizers had α - and β -HTBZ concentrations that were among the highest in the study, and their ratios of α : β -HTBZ were at the low end of the range generally observed, suggesting a role for CYP2D6 in stereoselective metabolism. This is supported by similar low ratios of α : β -HTBZ that were observed by the reviewer in the Phase III studies in patients taking CYP2D6 inhibitors (see section 2.4.2.8).

2.4.2.3 Is the drug and inhibitor and/or an inducer of CYP enzymes?

As discussed above, it appears that there is a potential for *in vivo* inhibition of CYP2D6 at clinically relevant doses. The effect on induction has not been evaluated. The effect of TBZ on P450s has not been evaluated with an *in vivo* drug interaction study.

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

Whether TBZ is a PgP substrate has not been evaluated.

The Sponsor has provided a literature reference evaluating inhibition of PgP by 100 uM TBZ (Staal RGW et al. Brain Res 2001; 91):116-125). The authors' conclusion is that TBZ is a PgP inhibitor. The cell system was a mouse leukemia cell line P388 and assay methods involved assessment of vincristine resistance as determined by the MTT assay. This method is not in agreement with the current Agency thinking regarding performance of this type of study, and therefore cannot be used to conclude that TBZ is a PgP inhibitor.

The Sponsor has conducted an *in vivo* study to evaluate TBZ as a PgP inhibitor. TBZ given as 25 mg twice daily did not affect the PK parameters of digoxin, a P-gP substrate, when both drugs were co-administered to healthy volunteers (Study TBZ 203,009). The interaction at higher, but clinically relevant doses of TBZ has not been evaluated. In Study TBZ 203,009 the 90% CI for the ratio of geometric means for C_{max} and AUC were within the BE interval of 80-125%. In that study (TBZ 203,009), subjects received 6 days of oral digoxin (0.5 mg on the first day, 0.25 mg daily thereafter, Days 1-6), followed by 4 days of oral digoxin (0.25 mg) and TBZ 25 mg bid (Days 7-10).

2.4.2.5 Are there other metabolic/transporter pathways that may be important in the pharmacokinetics of tetrabenazine?

The role of sulfotransferases and UDP glucuronosyl transferase has not been evaluated. The formation of HTBZ may be mediated by reductases.

2.4.2.6 Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

The label does not specify co-administration of another drug,

_____ A potential for interaction with
antidepressants that are CYP2D6 inhibitors is discussed in 2.4.2.8 below.

2.4.2.7 What other co-medications are likely to be administered to the target patient population?

Antidepressants are likely to be co-administered, and 56% of patients enrolled in the pivotal study (TBZ 103,004) were taking antidepressants and 17% were taking benzodiazepines. Other medications in more than one subject included oral contraceptives, tizanidine, oxybutynin,

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sildenafil, acetaminophen/hydrocodone, risedronate, mirtazapine, estrogens, donepezil, CoQ₁₀, and creatine.

2.4.2.8 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

In the pivotal efficacy study 103,004, patients who were taking CYP2D6 inhibitors had a lower ratio of α : β HTBZ than did patients who were not taking CYP2D6 inhibitors. α : β ratios ranged from 0.43 to 6.4 in that study. For α : β ratios > 1.6, there was no concomitant medication identified by the Reviewer as being a CYP2D6 inhibitor. For ratios < 1.6, 26 of 47 samples were from subjects taking CYP2D6 inhibitors, and for ratios of < 1, twenty-one of 25 samples were from subjects taking CYP2D6 inhibitors.

2.4.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

CNS depression that occurs with TBZ would be a concern when taken with other CNS depressants.

2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions or protein binding?

Yes. With respect to *in vitro* metabolism studies, as discussed in Question 2.2.5.5 there were deficiencies in the approach to evaluating P450-mediated metabolism of TBZ that does not allow for ruling out the role of other P450 pathways. These deficiencies include 1) the Sponsor has only used recombinant P450s, but has not used additional methods (such as use of inhibitors) as generally recommended, and the Sponsor has not evaluated formation of metabolites other than HTBZ. In addition, there were deficiencies in the study evaluating inhibition of P450s by TBZ and HTBZ as the methodology did not conform to the recommended methodology, using substrates recognized as being acceptable. Therefore, the lack of inhibition of pathways other than CYP2D6 could not be confirmed. Finally, *in vitro* studies should also evaluate potential for induction of TBZ/HTBZ metabolism and potential for TBZ/HTBZ to induce P450s.

Since CYP2D6 is thought to participate in the metabolism of TBZ/HTBZ, the role for CYP2D6 should be better characterized *in vivo*, either with a drug interaction study, or with well characterized CYP2D6 poor metabolizers. The relevance of the altered ratio of α : β HTBZ should be evaluated. Since TBZ dose will be titrated for effect and for tolerability, the ratio should be examined in light of safety issues.

An *in vivo* interaction study should be conducted with a sensitive CYP2D6 substrate (such as desipramine) to determine clinical relevance of CYP2D6 inhibition after administration of TBZ.

The *in vitro* studies should guide the need for other *in vivo* studies.

The pharmacology of the relevant circulating metabolites should be characterized to help determine the clinical relevance of interactions.

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2.6.3 *What issues related to dose, dosing regimens, or administration are unresolved, and represent significant omissions?*

None

2.7 *General Biopharmaceutics*

2.7.1 *Based on the biopharmaceutics classification system (BCS principles), in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?*

TBZ is considered to be BCS Class 4 (low solubility, low permeability).

Based on the pH solubility profile provided by the Sponsor, TBZ does not meet the cutoff criteria for highly soluble (25 mg/250 ml) except at pH 2 and therefore cannot be considered highly soluble.

Permeability considerations for TBZ are based on the mass balance study. When the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination, a drug substance is considered to be highly permeable. For tetrabenazine, since 64.97-81.39% of the dose (mean 75.4%) was excreted in urine, the extent of absorption is considered to be 75.4 % (mean). Therefore tetrabenazine cannot be considered highly permeable.

2.7.2 *What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial formulation?*

2.5.2.1 *What data support or do not support a waiver of in vivo BE data?*

The Sponsor has not requested a biowaiver. The Sponsor has conducted a BE study (TBZ 104,012) to determine BE of tetrabenazine 12.5 mg and 25 mg tablets using a single-dose, randomized, crossover design in 11 healthy males and 12 healthy females ages 18-44 y.o. Bioequivalence was shown for 2x 12.5 mg vs 1x25 mg tablets on the basis of circulating metabolites α - and β -HTBZ.

TBZ concentrations were less than the LOQ for the majority of sampling times in most subjects. Three subjects had detectable TBZ, and the concentrations were less than 9 ng/ml. The pertinent PK parameters are shown in the table below.

Table 5. Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ (Study TBZ104,012)

	Test (2x12.5 mg tablet) TREATMENT B (% CV) n=23	Reference (1x25mg tablet) TREATMENT A (% CV) n=23
α-HTBZ		
t_{max} (h) ^a	1.5 (1.0-2.5)	1.50 (1.00-3.00)
C_{max} (ng/mL)	36.7 (45)	33.1 (44)
AUC _{0-t} (ng*h/mL)	202.8 (76)	187 (71)
AUC _{0-∞} (ng*h/mL)	235 (93)	219.9 (87)

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λ_z (hr ⁻¹)	0.120 (36)	0.113 (48)
$t_{1/2}$ (h)	6.5 (40)	7.5 (47)
β-HTBZ		
t_{max} (h) ^a	1.5 (1.0-2.5)	1.5 (1.0-2.5)
C_{max} (ng/mL)	24.4 (72)	22.3 (73)
AUC _{0-t} (ng*h/mL)	155.5 (151)	136.8 (142)
AUC _{0-∞} (ng*h/mL)	226.1 (186)	189.9 (173)
λ_z (hr ⁻¹)	0.232 (50)	0.238 (54)
$t_{1/2}$ (h)	5.1 (106)	5.2 (105)

^a median (range)

The 90% CIs for the ratio of geometric means for C_{max} and AUC were within the BE interval of 80-125%. Therefore bioequivalence was demonstrated between two 2x12.5 mg tablets and one 25 mg tablet.

2.5.2.2 What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%

Not applicable.

2.5.2.3 If the formulations do not meet the standard criteria for bioequivalence, what clinical pharmacology and/or clinical safety and efficacy data support the approval of the to-be-marketed product?

Not applicable.

2.7.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

There was no statistically significant effect of a high-fat, high calorie meal on C_{max} or AUC of tetrabenazine metabolites α - or β -HTBZ after administration of a single 25 mg dose of TBZ. TBZ can be taken without regard to meals.

Food effect was evaluated in an open-label, randomized, 2-period, 2-treatment (fasted and fed), 2 sequence crossover study using the highest strength tablet 25 mg in healthy volunteers, ages 19-43 years of age. TBZ concentrations were less than the LOQ for the majority of sampling times in most subjects, although concentration of up to 1.54 ng/ml were measured. The pertinent PK parameters for α - and β -HTBZ are shown in the Table below. A bioequivalence comparison for C_{max} and AUC with and without food showed the 90% CI for the ratio of geometric means within the BE interval of 80-125%.

Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ (Study TBZ 103,003)

	Fed TREATMENT A (% CV) n=25	Fasted TREATMENT B (% CV) n=25
α-HTBZ		
t_{max} (h) ^a	2.0 (1.0-5.0)	1.0 (1.0-4.0)
C_{max} (ng/mL)	30.6 (33)	32.2 (40)

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AUC _{0-t} (ng*h/mL)	196.2 (42)	175.9 (43)
AUC _{0-∞} (ng*h/mL)	214.5 (49)	199.3 (47)
λ _z (hr ⁻¹)	0.123 (30)	0.101 (38)
t _{1/2} (h)	6.1 (29)	8.1 (51)
<hr/>		
β-HTBZ		
t _{max} (h) ^a	2.0 (1.0-5.0)	1.5 (1.0-4.0)
C _{max} (ng/mL)	17.1 (71)	17.8 (79)
AUC _{0-t} (ng*h/mL)	95.5 (118)	87.6 (118)
AUC _{0-∞} (ng*h/mL)	105.4 (136)	95.8 (133)
λ _z (hr ⁻¹)	0.206 (34)	0.259 (46)
t _{1/2} (h)	3.9 (48)	3.6 (69)
<hr/>		
^a median (range)		

2.7.4 When would a fed BE study be appropriate and was one conducted?

Not required in this case. There is no significant effect of food on BA.

2.7.5 How do the dissolution conditions and specifications assure in vivo performance and quality of the product?

The Sponsor has not shown discriminatory ability of the proposed method, and has not provided complete information to determine the adequacy of the conditions (the rotation speed at which the dissolution method was generated is not clear). This clarification was requested of the Sponsor on 1/2/406 and as of 2/14/06 there is no response.

The Sponsor proposed the following dissolution method and specifications:

Apparatus: USP Apparatus 2 (Paddles)

Medium: 0.1 M HCl

Volume: 900 ml

Rotation Speed: 50 rpm

Specification: ≥ — at 20 minutes.

The Office of New Drug Chemistry has recommended changing the specification to ≥ — (Q) in 30 minutes (with the specification of — based on ICH Q6 and the 30 minute time point to account for possible accumulation of the drug at time points beyond 20 minutes).

Provided that the Sponsor can document the development of the dissolution method as above, we agree concur with the Office of New Drug Chemistry's specification and recommend this as an interim method and specification until the discriminatory ability of the method can be shown.

2.7.6 If different-strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to-be-marketed product?

Not applicable.

2.7.7 *If the NDA is for a modified release formulation of an approved immediate product without supportive safety and efficacy studies, what dosing regimen changes are necessary, if any, in the presence or absence of PK-PD relationship?*

Not applicable.

2.7.8 *If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either in vitro or in vivo data to evaluate BE?*

Not applicable.

2.7.9 *What other significant, unresolved issues related to in vitro dissolution or in vivo BA and BE need to be addressed?*

The Sponsor should show discriminatory ability of the proposed dissolution method and should clarify the rotation speed used in method development.

In addition, since the 25 mg tablet is scored, the sponsor should show similar dissolution (using the approved interim methodology) for 2 half-tablets vs 1 whole tablet.

2.8 *Analytical Section*

2.8.1 *How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?*

In all of the clinical pharmacology and efficacy studies in which PK is determined, a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay has been used. In the initial dose proportionality study (1700114), the assay did not distinguish between α - and β -HTBZ are identified and measured. However subsequent studies used Method — 1266/1 that could distinguish between the 2 enantiomers. TBZ could be determined by either method.

2.8.2 *Which metabolites have been selected for analysis and why?*

The α - and β -HTBZ metabolites have been selected for analysis since they were thought to be the result of first pass metabolism and to be the major circulating components. (These studies were conducted prior to the mass balance study conducted in June 2005).

2.8.3 *For all moieties measured, is free, bound or total measured? What is the basis for that decision, if any, and is it appropriate?*

The assays measure total TBZ or metabolite. This is acceptable since the metabolites are < 70% protein bound and the TBZ is at least less than 85% protein bound, as discussed in Question 2.2.5.4

2.8.4 *What bioanalytical methods are used to assess concentrations?*

2.8.4.1 *What is the range of the standard curve and how does it relate to the requirements for the clinical studies? What curve fitting techniques are used?*

For the chiral method — 1266/1 the range of the standard curve for TBZ is 0.2 ng/ml to 200 ng/ml, and for α - and β -HTBZ the range is 0.5 ng/ml to 200 ng/ml. The plasma concentrations observed in the clinical pharmacology and efficacy studies were within these ranges. Linearity was established using $1/x^2$ linear regression analysis.

For method — 1133/1 (achiral method), the range of the calibration curve was 0.2 ng/ml to 50 ng/ml for TBZ, and for HTBZ was 1 ng/ml to 150 ng/ml for both urine and plasma. Linearity was established using $1/x^2$ linear regression analysis. However, although the assay itself would be acceptable, it cannot distinguish between α - and β -HTBZ, and therefore is not a useful assay for characterizing the clinical pharmacology of TBZ.

2.8.4.2 *What are the lower and upper limits of quantification (LLOQ/ULOQ)?*

For TBZ the LLOQ is 0.2 ng/ml and for α - and β -HTBZ the LLOQ is 0.5 ng/ml (for HTBZ in method 1133 the LLOQ was 1 ng/ml). The ULOQ is beyond the range of the calibration curve, with dilution integrity having been shown for method 1266 (25 fold) and for method 1133 (10-fold for plasma and 50-fold for urine).

2.8.4.3 *What are the accuracy, precision, and selectivity at these limits?*

Selectivity was determined in both assays. For method 1266/1 inter-assay accuracy and precision ranged from -8.6% to 8.2% and from 3.3% to 10%, respectively. Intra-assay accuracy and precision were similarly acceptable. For method 1133/1 (plasma) the inter-assay accuracy and precision ranged from -8.23% to 11.76% and from 3.54% to 11.61% for TBZ and for HTBZ. The intra-assay accuracy and precision were similarly acceptable.

2.8.4.4 *What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?*

	Method 1266/1	1133/1 (plasma)	1133/3 (urine)
Freeze-thaw In process	3 cycles 4 hrs (benchtop)	3 cycles 4 hours	3 cycles 4 hours (benchtop) 4 days at 4° C
Autosampler	24 hours at room temperature	72 hours at room temperature 72 hours at 4° C	3 days at room temperature 5 days at 4° C
Long-term stability	321 days at -80° C	2.5 months at -20° C, 5 months at - 70° C	6 months at - 20° C 6 months at - 70° C

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2.8.4.5 What is the QC sample plan?

Duplicate QC samples were analyzed with each batch of study samples analyzed.

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Deliberative Process

4.2 Clinical Pharmacology and Biopharmaceutics Individual Study Reviews

4.2.1 BIOANALYTICAL METHOD (TBZ AND HTBZ IN HUMAN PLASMA)

Bioanalytical Method — 1266/1 for Tetrabenazine (TBZ) and Hydroxytetrabenazine (HTBZ) in Human Plasma

A liquid chromatography tandem mass spectrometry (LC-MS/MS) assay was developed and validated for analysis of TBZ and its metabolites α -HTBZ and β -HTBZ in human plasma (Study — 1266/1). The standards were tetrabenazine from _____ and α -HTBZ and β -HTBZ, both from _____. Internal standard was _____. Aliquots of plasma are combined with internal standard along with 0.2 M ammonium hydroxide solution. After addition of ethyl acetate, mixing, and centrifugation, the supernatant was removed and samples were evaporated to dryness under nitrogen at 40° C. Samples were reconstituted in mobile phase prior to injection into a LC-MS/MS system. Chromatography was carried out using an _____ analytical column (for chiral separations), with a mobile phase comprised of _____. The method was developed and performed at _____.

Standard operating procedures (SOPs) were in place for sample preparation, the analytical procedure, and for acceptance of the bioanalytical run (system suitability and acceptance of calibration standards and quality control (QC) samples).

Selectivity, Accuracy, Precision, and Recovery

Selectivity was determined by analysis of blank samples from 6 independent sources of blank human plasma for presence of interfering endogenous peaks with respect to tetrabenazine, HTBZ, and _____. Ranges of the calibrations curves, LOQ for each analyte, and nominal values for the QC samples are shown in Table 1 below.

Table 1. Summary of standard curves and QC samples

Analyte	Range of Calibration Curve	LOQ	QC Samples
TBZ	0.2 ng/ml to 200 ng/ml	0.2 ng/ml	0.2 ng/ml 0.5 ng/ml 25 ng/ml 100 ng/ml
α -HTBZ	0.5 ng/ml to 200 ng/ml	0.5 ng/ml	0.5 ng/ml 1 ng/ml 25 ng/ml 100 ng/ml
β -HTBZ	0.5 ng/ml to 200 ng/ml	0.5 ng/ml	0.5 ng/ml 1 ng/ml 25 ng/ml

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			100 ng/ml
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A calibration curve for each analyte consisted of a minimum of 75% of the calibration points (out of 11 standards for TBZ and 9 standards for the metabolites) being acceptable. In addition, the SOP for the method specified inclusion of a blank and a zero sample (internal standard) for each analytical run. Three sets of calibration curves (in duplicate) were performed. Linearity was established for each analyte ($r > 0.993$, $1/x^2$ linear regression analysis). The accuracy for each nonzero standard for each analyte ranged from -14.89 to 14.42 % and is therefore acceptable.

Accuracy and precision were analyzed for 6 replicates of each of 4 quality control (QC) concentrations for each analyte, with three separate sets of analysis performed on different occasions. The intra-assay accuracy and precision ranged from -13.0 % to 14.1 % and from 1.3% to 10.8%, respectively. Inter-assay accuracy and precision ranged from -8.6 % to 8.2% and from 3.3% to 10.0% respectively. These values are acceptable.

Stability

Stability of TBZ, α -HTBZ, and β -HTBZ was demonstrated as follows. Freeze-thaw stability in plasma was demonstrated for six aliquots of low and high concentrations after three freeze/thaw cycles (although for the 100 ng/ml concentration of α -HTBZ, and β -HTBZ the % stability had dropped to 90 and 87%, respectively). In-process stability was demonstrated in human plasma for 4 hours on benchtop prior to extraction. Autosampler stability of extracted samples was demonstrated for approximately 24 hours at room temperature. Extracts were also stored for 4 days in a refrigerator but some samples had completely evaporated and the batch could not be quantified. Long term stability in plasma was demonstrated for 2 months at -80° C. Dilution integrity was demonstrated for dilution with plasma by a factor of 25-fold.

Stability of stock solutions of internal standard of standard stock solutions was not evaluated. According to the SOP these were to be stored in the refrigerator for up to 1 month.

Partial Validation Using Robotic Arm for Sample Extraction

The intra-batch accuracy and precision (for 6 replicates at 4 QC concentrations) were acceptable for all three analytes.

Conclusion

The bioanalytical method — 1266/1 used for analysis of human plasma samples with respect to TBZ and its metabolites α -HTBZ and β -HTBZ is considered adequately documented and validated (although there is no data on storage of extracts for 4 days in the refrigerator).

4.2.2 BIOANALYTICAL METHOD (LONG TERM STABILITY IN PLASMA)

BIOANALYTICAL METHOD

Assessment of Long Term Stability of Tetrabenazine (TBZ) and α - and β -Hydroxytetrabenazine (HTBZ) in Human Plasma (STUDY 1266/2)

This study assessed long-term stability of TBZ and its metabolites α -HTBZ and β -HTBZ in human plasma after being stored frozen (nominally -80° C) for approximately 321 days. A liquid chromatography tandem mass spectrometry (LC-MS/MS) assay (previously validated in study 1266/1) was used. The standards were tetrabenazine from [redacted] and α -HTBZ and β -HTBZ, both from [redacted]. Internal standard was [redacted].

Table 1. Summary of standard curves and QC samples

Analyte	Range of Calibration Curve	LOQ	QC Samples
TBZ	0.2 ng/ml to 200 ng/ml	0.2 ng/ml	0.5 ng/ml 100 ng/ml
α -HTBZ	0.5 ng/ml to 200 ng/ml	0.5 ng/ml	1 ng/ml 100 ng/ml
β -HTBZ	0.5 ng/ml to 200 ng/ml	0.5 ng/ml	1 ng/ml 100 ng/ml

A calibration curve for each analyte consisted of a minimum of 75% of the calibration points (out of 11 standards for TBZ and 9 standards for the metabolites). Points falling outside of the acceptance criteria were not used in the linear regression. Calibration curves were performed in duplicate for each analyte. Linearity was established for each analyte ($r > 0.994$, $1/x^2$ linear regression analysis). The accuracy for each nonzero standard included in the curve for each analyte ranged from -13.7 to 14.4% and is therefore acceptable.

Long term stability in plasma was demonstrated for 321 days at -80° C for each analyte.

4.2.3 BIOANALYTICAL METHOD FOR TBZ AND HTBZ IN SUTDY 1700114
BIOANALYTICAL METHOD

Bioanalytical Method for Tetrabenazine (TBZ) and Hydroxytetrabenazine (HTBZ) in Human Plasma for Use in Study 1700114 (Method — 1133/1)

A liquid chromatography tandem mass spectrometry (LC-MS/MS) assay (achiral) was developed and validated for analysis of TBZ and its metabolite, HTBZ in human plasma (Study — 1133/1). The standards were tetrabenazine and α -HTBZ, both from Cambridge Laboratories. Aliquots of plasma are combined with internal standard (—) along with 0.2 M ammonium hydroxide solution. After addition of ethyl acetate, mixing, and centrifugation, the supernatant was removed and samples were evaporated to dryness under nitrogen at 40 C. Samples were reconstituted in 10 mM ammonium hydroxide pH 8.5:acetonitrile (70:30 v/v) prior to injection into a LC-MS/MS system. Chromatography was carried out using a — analytical column, with a mobile phase gradient comprised of — r. This method was used for plasma samples in Study 1700114. The method was developed and performed at —

Standard operating procedures (SOPs) were in place for sample preparation, the analytical procedure, and for acceptance of the bioanalytical run (system suitability and acceptance of calibration standards and quality control (QC) samples).

Selectivity, Accuracy, Precision, and Recovery

Selectivity was determined by analysis of blank samples from 6 independent sources of blank human plasma for presence of interfering endogenous peaks. A matrix effect was observed when spiked extracted plasma samples were compared with aqueous standard. Ranges of the calibrations curves, LOQ for each analyte, and nominal values for the QC samples are shown in Table 1 below.

Table 1. Summary of standard curves and QC samples

Analyte	Range of Calibration Curve	LOQ	QC Samples
TBZ	0.2 ng/ml to 50 ng/ml	0.2 ng/ml	0.2 ng/ml 0.4 ng/ml 10 ng/ml 30 ng/ml 50 ng/ml
HTBZ	1 ng/ml to 150 ng/ml	1 ng/ml	1 ng/ml 3 ng/ml 40 ng/ml 100 ng/ml 150 ng/ml

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A calibration curve for each analyte consisted of a minimum of 5 calibration points (out of 8 standards). In addition, the SOP for the method specified inclusion of a blank and a zero sample (internal standard) for each analytical run. Three sets of calibration curves (in singlicate) were performed. Linearity was established for each analyte ($r > 0.997$, $1/x^2$ least-squares linear regression analysis). The accuracy for each nonzero standard for each analyte ranged from -12.0 to 13.39 % and is therefore acceptable.

Accuracy and precision were analyzed for 6 replicates of each of 5 quality control (QC) concentrations for each analyte, with three separate sets of analysis performed on different occasions. The intra-assay accuracy and precision ranged from -8.23% to 11.76% and from 1.88% to 18.9%, respectively. The value of 18.9% was for TBZ at the LLOQ; all other values were less than 15%. Inter-assay accuracy and precision ranged from -8.23% to 11.76% and from 3.54% to 11.61% respectively. These values are acceptable.

Stability

Stability of TBZ and HTBZ was demonstrated as follows. Freeze-thaw stability in plasma was demonstrated for six aliquots (except where indicated) of low and high concentrations after three freeze/thaw cycles. In-process stability was demonstrated in human plasma for 4 hours on benchtop prior to extraction and for 4 days at 4° C. Autosampler stability of extracted samples was demonstrated for approximately 72 hours at room temperature and samples were stable for 72 hours at 4° C (for 3 replicates each). Long term stability (3 aliquots for each condition) in plasma was demonstrated for 2.5 months at -20° C and for 5 months at -70° C. Dilution integrity was demonstrated for dilution (with either water or plasma) by a factor of 10-fold.

Stability of stock solutions of internal standard of standard stock solutions was not evaluated. According to the SOP these were to be stored at 4° C with an expiry date of 1 month.

In conclusion, the bioanalytical method used for analysis of plasma samples in the clinical studies in NDA 21-894 is considered adequately documented and validated.

**APPEARS THIS WAY
ON ORIGINAL**

4.2.4 BIOANALYTICAL METHOD FOR TBZ AND HTBZ IN HUMAN URINE
BIOANALYTICAL METHOD

Bioanalytical Method for Tetrabenazine (TBZ) and Hydroxytetrabenazine (HTBZ) in Human Urine (Method 1133/3)

A liquid chromatography tandem mass spectrometry (LC-MS/MS) assay (achiral) was developed and validated for analysis of TBZ and its metabolite, HTBZ in human urine (Study 1133/3). The standards were tetrabenazine and α -HTBZ, both from Cambridge Laboratories. Aliquots of urine are combined with internal standard along with 0.2 M ammonium hydroxide solution. After addition of ethyl acetate, mixing, and centrifugation, the supernatant was removed and samples were evaporated to dryness under nitrogen at 40° C. Samples were reconstituted in 10 mM ammonium hydroxide pH 8.5:acetonitrile (70:30 v/v) prior to injection into a LC-MS/MS system. Chromatography was carried out using a analytical column, with a mobile phase gradient comprised of . This method was used for urine samples in Study 1700114. The method was developed and performed at

Standard operating procedures (SOPs) were in place for sample preparation, the analytical procedure, and for acceptance of the bioanalytical run (system suitability and acceptance of calibration standards and quality control (QC) samples).

Selectivity, Accuracy, Precision, and Recovery

Selectivity was determined by analysis of blank samples from 6 independent sources of blank human plasma for presence of interfering endogenous peaks. A matrix effect was observed when spiked extracted plasma samples were compared with aqueous standard. Ranges of the calibrations curves, LOQ for each analyte, and nominal values for the QC samples are shown in Table 1 below.

Table 1. Summary of standard curves and QC samples

Analyte	Range of Calibration Curve	LOQ	QC Samples
TBZ	0.2 ng/ml to 50 ng/ml	0.2 ng/ml	0.2 ng/ml 0.4 ng/ml 10 ng/ml 30 ng/ml 50 ng/ml
α -HTBZ	1 ng/ml to 150 ng/ml	1 ng/ml	1 ng/ml 3 ng/ml 40 ng/ml 100 ng/ml 150 ng/ml

A calibration curve for each analyte consisted of a minimum of 5 calibration points (out of 8 standards). In addition, the SOP for the method specified inclusion of a blank and a zero sample

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(internal standard). Three sets of calibration curves (in singlicate) were performed. Linearity was established for each analyte ($r > 0.997$, $1/x^2$ least-squares linear regression analysis). The accuracy for each nonzero standard for each analyte ranged from -8.95 to 10.02 % and is therefore acceptable.

Accuracy and precision were analyzed for 6 replicates of each of 5 quality control (QC) concentrations for each analyte, with three separate sets of analysis performed on different occasions. Inter-assay accuracy and precision for TBZ ranged from -13.75 % to 5.32 % and from 3.96 % to 6.61 %, respectively. For α -HTBZ, the intra-assay accuracy and precision ranged from -10.00 % to 1.76 % and from 6.52 % to 9.35 %, respectively. These values are acceptable.

Stability

Stability of TBZ and HTBZ in human urine was demonstrated for six aliquots of low and high concentrations as follows. Freeze-thaw stability in urine was demonstrated after three freeze/thaw cycles. In-process stability was demonstrated in human urine for 4 hours on benchtop prior to extraction and for 4 days at 4° C. Autosampler stability of extracted samples was demonstrated for 3 days at room temperature and samples were stable for 5 days at 4° C. Long term stability in urine was demonstrated for 6 months at -20° C and for 6 months at -70° C. Dilution integrity was demonstrated for dilution with urine by a factor of 50-fold.

Extraction recovery was approximately 107% and 104% for the low and high QC samples, respectively for tetrabenazine, but only 64% and 54% for the low and high QC samples, respectively for HTBZ.

Stability of stock solutions of internal standard of standard stock solutions was not evaluated. According to the SOP these were to be stored at 4° C with an expiry date of 1 month.

β -HTBZ

Determination of β -HTBZ was performed using a set of calibration standards made up from β -HTBZ. The samples were extracted, analyzed, and back-calculated from an α -HTBZ calibration curve. For 8 theoretical concentrations (performed in singlicate), the back-calculated concentration of β -HTBZ was approximately 2.5-fold greater than the nominal concentration. Thus this assay is not adequate to determine β -HTBZ.

Conclusion

In conclusion, the bioanalytical method used for analysis of urine samples (in the clinical study 170014) in NDA 21-894 is considered adequately documented and validated for determining TBZ and α -HTBZ, but not for β -HTBZ .

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Tetrabenazine

4.2.5 IN VITRO TBZ METABOLISM STUDY

ASSESSMENT OF THE METABOLISM OF TETRABENAZINE BY NINE HUMAN RECOMBINANT CYTOCHROME P-450 ENZYME SUBTYPES

Study Investigators and Site:

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Protocol Number: AG-A04002

OBJECTIVES:

The purpose of the study was to determine the potential for metabolism of tetrabenazine (TBZ) by each of nine specific human P450 enzyme subtypes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4).

DRUGS STUDIED:

Tetrabenazine, _____ Batch # 100730.

METHODS:

Recombinant human P450 enzymes

Recombinant human CYP enzymes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) expressed in microsomes were obtained from _____ Reactions were initiated by the addition of 75 μ L of pre-warmed NADPH regenerating system and buffer solution (a 4-fold concentrated solution). The test (TBZ) and probe substrates were prepared in acetonitrile (final acetonitrile concentration in the assay was 0.5%). Reactions were performed (in triplicate) in a 0.3 ml total incubation volume containing buffer, microsomes, and probe substrate (positive control) or test compound at 37° C for 15 and 30 minutes in the presence of an NADPH regenerating system and MgCl₂. The concentration of TBZ was 0.05 μ M. For time zero samples of test compound, an aliquot of the reaction mixture was added to ice cold methanol and NADPH regenerating system. For time zero samples of probe substrates, an aliquot was added to STOP solution and NADPH regenerating system. After 15 and 30 minutes of incubation, an aliquot of the reaction mixture containing test compound was added to an equal volume of ice cold methanol to stop the reaction. After centrifugation, an aliquot of the supernatant was stored a -70° C until analysis. For the probe substrates, the reaction was stopped using a CYP subtype specific STOP solution. Specific reaction conditions are shown in the table below.

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	Amount of microsomes per incubation	Probe Substrate	Metabolic Product	STOP buffer
CYP1A2	0.5 pmol	3-cyano-7-ethoxycoumarin (CEC) (5 uM)	3-Cyano-7-hydroxycoumarin (CHC)	80% acetonitrile/20% 0.5 M Tris base
CYP2A6	1.0 pmol	Coumarin (3 uM)	7-Hydroxycoumarin	80% acetonitrile/20% 0.5 M Tris base
CYP2B6	1.0 pmol	7-ethoxy-4-trifluoromethylcoumarin (EFC) (2.5 uM)	7-hydroxy-4-trifluoromethylcoumarin (HFC)	80% acetonitrile/20% 0.5 M Tris base
CYP2C8	4.0 pmol	Dibenzylfluorescein (DBF) (1 uM)	Fluorescein	2 mM NaOH
CYP2C9*1	1.0 pmol	7-methoxy-4-trifluoromethylcoumarin (MFC) (75 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base
CYP2C19	0.5 pmol	CEC (25 uM)	CHC	80% acetonitrile/20% 0.5 M Tris base
CYP2D6*1	1.5 pmol	3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4methylcoumarin (AMMC) (1.5 uM)	AHMC	80% acetonitrile/20% 0.5 M Tris base
CYP2E1	2.0 pmol	MFC (100 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base
CYP3A4	1.0 pmol	7-Benzoyloxy-4-trifluoromethylcoumarin (BFC) (50 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base

With the exception of 7-hydroxycoumarin for CYP2A6, none of the probe substrates are generally preferred or acceptable alternate substrates.

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ON ORIGINAL

ASSAY:

Formation of the fluorescent metabolic product from the probe substrates was detected in a fluorescence plate reader. Triplicate fluorescent signals were corrected for background (mean fluorescence of the zero time samples) and mean fluorescence signals (for 15 and 30 minutes) were calculated.

Concentrations of TBZ, α -HTBZ, and β -HTBZ were determined using LC-MS/MS at — using method 1494/1. The method is based on method 1266/1 that was validated in plasma. Method 1491/1 is partially validated in the following buffer solutions (these were the buffers used in the in vitro metabolism method):

- Phosphate buffered saline (1x) (Dulbecco's buffer)
- Potassium phosphate buffer (200 mM, pH 7.4) containing 0.5% acetonitrile:methanol (50:50, v/v)
- Tris buffer (100 mM, pH 7.5) containing 0.5% acetonitrile:methanol (50:50 v/v).

All analytes were found to be stable in all buffer samples stored at room temperature for 4 hours, frozen (-80° C) for 27 weeks (Dulbecco's buffer), 29 weeks (phosphate buffer), or 15 weeks (Tris buffer), and following 3 freeze-thaw cycles. Extracted samples were stable for 24 hours at 4° C.

Performance of Analytical Method for AG-04002

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.989	0.5	1.0	7.4	0.1
					25.0	5.0	-1.0
					100.0	5.6	1.9
α -HTBZ	LC/MS/MS	1-200 ng/ml	r > 0.992	1.0	2.0	4.1	6.4
					25.0	7.3	2.7
					100.0	5.7	0.7
β -HTBZ	LC/MS/MS	1-200 ng/ml	r > 0.986	1.0	2.0	6.9	3.8
					25.0	7.8	2.4
					100.0	7.7	1.9

Two calibration curves and duplicate QC samples were analyzed with each batch of samples. Study samples were stored at -70° C. Samples were analyzed within the period for which the samples are stable. The performance of the assays for all analytes is considered acceptable.

RESULTS:

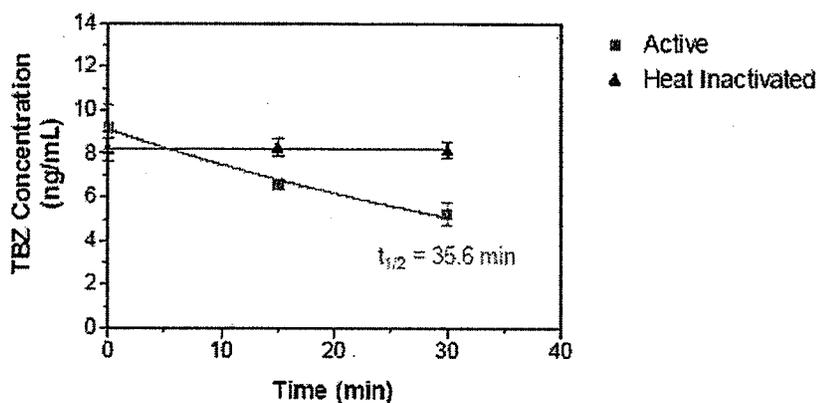
Fluorescence indicating formation of probe metabolites increased relatively linearly over time for CYP1A2, CYP2A6, CYP2B6, CYP2D6, CYP2E1, CYP2C9 began to plateau somewhat at 30 minutes for CYP2C19 and more noticeably for CYP3A4 and CYP2C8.

The results of incubations with tetrabenazine are shown in the Table below, and for CYP2D6 in the figure below, as provided by the Sponsor.

Table 1. Mean tetrabenazine concentrations (\pm S.D. of 3 replicates) as a function of incubation time in active and heat inactivated microsomes each expressing a human CYP subtype.

CYP Subtype	Condition of Microsomes	TBZ Concentration		
		0 min	15 min	30 min
		(ng/mL)		
CYP1A2	Active	7.689 \pm 0.409	7.039 \pm 1.021	6.897 \pm 1.495
	Heat inactivated	7.632 \pm 0.916	7.853 \pm 1.984	7.276 \pm 2.464
CYP2A6	Active	7.874 \pm 0.797	7.043 \pm 0.976	7.381 \pm 0.950
	Heat inactivated	8.095 \pm 1.133	7.948 \pm 1.236	7.529 \pm 0.527
CYP2B6	Active	8.789 \pm 1.477	8.188 \pm 1.768	8.319 \pm 2.178
	Heat inactivated	8.807 \pm 1.227	8.557 \pm 1.606	8.734 \pm 1.078
CYP2C8	Active	8.061 \pm 1.106	8.208 \pm 0.678	8.596 \pm 1.947
	Heat inactivated	8.210 \pm 0.363	8.411 \pm 0.289	7.838 \pm 0.546
CYP2C9	Active	8.275 \pm 0.578	8.182 \pm 0.490	7.594 \pm 0.872
	Heat inactivated	8.421 \pm 0.704	8.253 \pm 1.548	8.745 \pm 1.099
CYP2C19	Active	7.702 \pm 0.136	7.356 \pm 0.411	7.503 \pm 0.528
	Heat inactivated	7.682 \pm 0.687	8.486 \pm 0.150	7.698 \pm 0.307
CYP2D6	Active	9.176 \pm 1.094	8.579 \pm 0.279	5.217 \pm 0.543
	Heat inactivated	8.185 \pm 0.926	8.263 \pm 0.711	8.172 \pm 0.635
CYP2E1	Active	8.943 \pm 0.852	7.942 \pm 1.360	8.514 \pm 0.937
	Heat inactivated	8.310 \pm 1.501	8.212 \pm 1.919	8.853 \pm 1.783
CYP3A4	Active	10.230 \pm 0.290	8.973 \pm 0.393	9.230 \pm 3.125
	Heat inactivated	9.586 \pm 1.004	9.849 \pm 1.703	9.764 \pm 0.320

CYP2D6



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Tetrabenazine (0.05 uM) appeared to be stable in all assays over time, except for CYP2D6 in which approximately 43% disappeared, suggesting that CYP2D6 is in part responsible for metabolism of tetrabenazine. The metabolites α -HTBZ and β -HTBZ were not detectable in the CP2D6 incubation samples.

CONCLUSIONS and COMMENTS:

This study suggests a role for CYP2D6 in the metabolism of TBZ. The role of other P450 isozymes or other non P450 pathways cannot be ruled out.

The following points should be considered and included as comments to the Sponsor.

1. The Sponsor has not taken a step-wise approach to understanding the metabolism of TBZ. The preferred first approach would be to directly identify metabolites after incubation with hepatocytes or liver slices. Subsequent studies can also eliminate non CYP oxidative pathways.
2. This study to evaluate CYP pathways is methodologically deficient. It is recommended that recombinant enzymes not be used alone, but in combination with other methods (such as use of inhibitors) for identifying drug metabolizing P450 isozymes. In addition, the probes used as controls in the present study are not classical, preferred probes, and the Sponsor has not provided justification, so it is difficult to understand the acceptability of the reactions.
3. Studies characterizing the metabolism of TBZ and HTBZ *in vitro* should measure the formation of metabolites (including the oxidative metabolites of TBZ and oxidative metabolites of HTBZ) to identify the pathways by which they are formed.

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ON ORIGINAL**

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Tetrabenazine

4.2.6 IN VITRO METABOLISM OF HTBZ

ASSESSMENT OF THE METABOLISM OF ALPHA-DIHYDROTETRABENAZINE AND BETA-DIHYDROTETRABENAZINE BY NINE HUMAN RECOMBINANT CYTOCHROME P-450 ENZYME SUBTYPES

Study Investigators and Site:

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Protocol Number: AG-A05001

OBJECTIVES:

The purpose of the study was to determine the potential for metabolism of α -dihydrotetrabenazine (α -HTBZ) and β -dihydrotetrabenazine (β -HTBZ) by each of nine specific human P450 enzyme subtypes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4).

TEST COMPOUNDS:

α -HTBZ (MW 319.43 g/mole, batch RUS0406, _____)

β -HTBZ (MW 319.43 g/mole, batch RUS0407, _____)

METHODS:

Recombinant human P450 enzymes

Recombinant human CYP enzymes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) expressed in microsomes were obtained from _____ Reactions were initiated by the addition of 75 μ L of pre-warmed NADPH regenerating system and buffer solution (a 4-fold concentrated solution). The test and probe substrates were prepared in acetonitrile (final acetonitrile concentration in the assay was 0.5%). Reactions were performed (in triplicate) in a 0.3 ml total incubation volume containing buffer, microsomes, and probe substrate (positive control) or test compound at 37° C in the presence of an NADPH regenerating system and MgCl₂. Formation of a fluorescent metabolite from a non-fluorescent probe substrate was determined for each isozyme as the positive control. The concentration of either α -HTBZ or β -HTBZ was 0.1 μ M. (Note: from the Food effect study 103,003, C_{max} of the metabolites following a single 25 mg dose of TBZ was approximately 30 ng/ml or 0.94 μ M). Time zero samples of the test compound were stopped by the addition of ice cold methanol and NADPH regenerating system. After 15 and 30 minutes of incubation, an aliquot of the reaction mixture containing test compound was added to an equal volume of ice cold methanol to stop the

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Tetrabenazine

reaction. After centrifugation, an aliquot of the supernatant was stored a -70° C until analysis. For the probe substrates, the reaction was stopped using a CYP subtype specific STOP solution. Specific reaction conditions are shown in the table below.

	Amount of microsomes per incubation	Probe Substrate	Metabolic Product	STOP buffer
CYP1A2	5 pmol/ml	3-cyano-7-ethoxycoumarin (CEC) (10 uM)	3-Cyano-7-hydroxycoumarin (CHC)	80% acetonitrile/20% 0.5 M Tris base
CYP2A6	10 pmol/ml	Coumarin (6 uM)	7-Hydroxycoumarin	80% acetonitrile/20% 0.5 M Tris base
CYP2B6	10 pmol/ml	7-ethoxy-4-trifluoromethylcoumarin (EFC) (5 uM)	7-hydroxy-4-trifluoromethylcoumarin (HFC)	80% acetonitrile/20% 0.5 M Tris base
CYP2C8	40 pmol/ml	Dibenzylfluorescein (DBF) (2 uM)	Fluorescein	2 mM NaOH
CYP2C9*1	10 pmol/ml	7-methoxy-4-trifluoromethylcoumarin (MFC) (150 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base
CYP2C19	5 pmol/ml	CEC (50 uM)	CHC	80% acetonitrile/20% 0.5 M Tris base
CYP2D6*1	15 pmol/ml	3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4methylcoumarin (AMMC) (3 uM)	AHMC	80% acetonitrile/20% 0.5 M Tris base
CYP2E1	20 pmol/ml	MFC (200 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base
CYP3A4	10 pmol/ml	7-Benzoyloxy-4-trifluoromethylcoumarin (BFC) (100 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base

With the exception of CYP2A6, none of the probe substrates are generally preferred or acceptable alternate substrates and the Sponsor has not provided justification for their use.

ASSAY:

Formation of the fluorescent metabolic product from the probe substrates was detected in a fluorescence plate reader. Triplicate fluorescent signals were corrected for background (mean fluorescence of the zero time samples) and mean fluorescence signals (for 15 and 30 minutes) were calculated.

Concentrations α -HTBZ and β -HTBZ were determined using LC-MS/MS at \curvearrowright using method 1494/1 as described for Study AG-A04002.

Performance of Analytical Method for AG-05001

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
α - HTBZ	LC/MS/MS	1-200 ng/ml	r > 0.988	1.0	2.0	5.7	3.8
					25.0	6.2	-0.8
					100.0	6.8	-6.5
β -HTBZ	LC/MS/MS	1-200 ng/ml	r > 0.988	1.0	2.0	8.8	3.3
					25.0	9.0	1.6
					100.0	8.8	-1.3

Two calibration curves and duplicate QC samples were analyzed with each batch of samples. Study samples were stored at -80° C. Samples were analyzed within the period for which the samples are stable. The performance of the assays for all analytes is considered acceptable.

RESULTS:

Fluorescence indicating formation of probe metabolites increased relatively linearly over time for CYP2A6, CYP2C8, CYP2C9, CYP2B6, CYP2C19, CYP2D6, CYP2C9 began to plateau somewhat at 30 minutes for CYP2D6 and more noticeably for CYP1A2, CYP3A4, CYP2E1, and CYP2C8.

The results of incubations with α -HTBZ and β -HTBZ are shown in the Tables below, and for CYP2D6 in the figures below, as provided by the Sponsor.

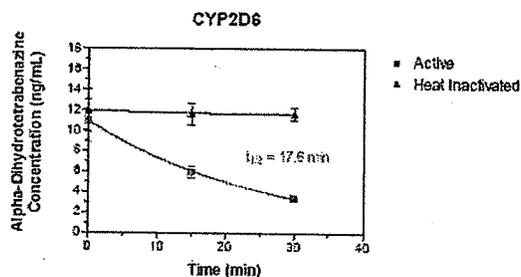
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Tetrabenazine

Table 1. Mean α -dihydratetrabenazine concentrations (\pm S.D. of 3 replicates) as a function of incubation time in active and heat inactivated microsomes each expressing a human CYP enzyme subtype.

CYP Subtype	Condition of Microsomes	Mean α -Dihydratetrabenazine Concentration (ng/mL)		
		0 min	15 min	30 min
CYP1A2	Active	10.8 \pm 0.567	10.6 \pm 0.299	10.6 \pm 0.566
	Heat inactivated	11.6 \pm 0.894	11.7 \pm 1.02	12.5 \pm 0.759
CYP2A6	Active	14.0 \pm 0.695	13.7 \pm 1.43	14.2 \pm 0.987
	Heat inactivated	13.5 \pm 1.96	13.7 \pm 0.616	14.3 \pm 0.338
CYP2B6	Active	12.0 \pm 1.80	12.1 \pm 1.46	10.9 \pm 1.24
	Heat inactivated	9.81 \pm 0.964	11.0 \pm 0.539	11.4 \pm 0.898
CYP2C8	Active	14.9 \pm 2.23	15.0 \pm 1.92	14.2 \pm 0.489
	Heat inactivated	12.7 \pm 1.35	12.5 \pm 1.51	12.6 \pm 1.25
CYP2C9	Active	12.7 \pm 1.29	12.6 \pm 0.922	12.2 \pm 1.27
	Heat inactivated	13.8 \pm 1.17	13.6 \pm 0.586	13.7 \pm 0.240
CYP2C19	Active	14.2 \pm 0.754	13.7 \pm 1.21	14.3 \pm 0.866
	Heat inactivated	13.5 \pm 0.242	14.3 \pm 0.463	14.4 \pm 0.165
CYP2D6	Active	10.9 \pm 2.10	5.97 \pm 0.571	3.44 \pm 0.251
	Heat inactivated	11.9 \pm 3.54	11.6 \pm 1.80	11.7 \pm 1.04
CYP2E1	Active	12.4 \pm 1.65	13.9 \pm 1.12	12.5 \pm 2.04
	Heat inactivated	12.5 \pm 2.92	12.2 \pm 0.831	11.3 \pm 0.555
CYP3A4	Active	13.1 \pm 2.10	11.2 \pm 1.46	12.7 \pm 0.982
	Heat inactivated	13.2 \pm 2.65	11.6 \pm 0.603	14.1 \pm 2.21

Figure 6. Stability of α -dihydratetrabenazine (0.10 μ M) following incubation with microsomes, expressing either the human CYP2D6, CYP2E1 or CYP3A4 enzyme subtypes (active) and with the corresponding heat inactivated microsomes as controls.



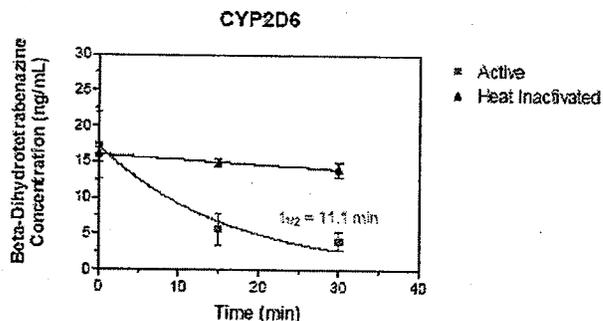
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Table 2. Mean β -dihydrotetrabenazine concentrations (\pm S.D. of 3 replicates) as a function of incubation time in active and heat inactivated microsomes each expressing a human CYP enzyme subtype.

CYP Subtype	Condition of Microsomes	Mean β -Dihydrotetrabenazine Concentration (ng/mL)		
		0 min	15 min	30 min
CYP1A2	Active	20.5 \pm 4.01	21.6 \pm 4.91	19.6 \pm 3.83
	Heat inactivated	21.8 \pm 2.95	23.0 \pm 5.10	23.2 \pm 2.28
CYP2A6	Active	17.2 \pm 1.30	15.7 \pm 2.43	18.1 \pm 3.69
	Heat inactivated	16.9 \pm 0.713	15.0 \pm 1.17	17.2 \pm 1.13
CYP2B6	Active	18.4 \pm 0.890	18.4 \pm 1.61	18.7 \pm 0.893
	Heat inactivated	20.9 \pm 1.44	19.4 \pm 2.87	18.7 \pm 3.32
CYP2C8	Active	16.1 \pm 1.36	17.2 \pm 0.926	17.4 \pm 0.293
	Heat inactivated	17.8 \pm 2.48	16.8 \pm 1.72	18.0 \pm 1.26
CYP2C9	Active	18.9 \pm 1.27	19.6 \pm 1.13	19.9 \pm 0.117
	Heat inactivated	20.1 \pm 3.52	19.4 \pm 1.92	19.6 \pm 1.68
CYP2C19	Active	17.7 \pm 0.625	15.9 \pm 0.629	16.4 \pm 1.54
	Heat inactivated	17.2 \pm 0.922	16.8 \pm 1.20	17.6 \pm 0.727
CYP2D6	Active	17.3 \pm 4.61	5.66 \pm 2.19	3.95 \pm 1.30
	Heat inactivated	16.1 \pm 1.91	14.9 \pm 0.885	14.0 \pm 1.86
CYP2E1	Active	17.7 \pm 1.98	22.3 \pm 4.99	19.3 \pm 2.11
	Heat inactivated	19.4 \pm 2.09	18.5 \pm 1.35	17.9 \pm 1.78
CYP3A4	Active	20.2 \pm 1.15	22.5 \pm 0.989	20.9 \pm 2.82
	Heat inactivated	19.1 \pm 3.17	19.6 \pm 2.66	20.0 \pm 3.17

Figure 9. Stability of β -dihydrotetrabenazine (0.10 μ M) following incubation with microsomes, expressing either the human CYP2D6, CYP2E1 or CYP3A4 enzyme subtypes (active) and with the corresponding heat inactivated microsomes as controls.



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Both α -HTBZ and β -HTBZ appeared to be stable in all assays over time, except for CYP2D6. For CYP2D6 approximately 68% of α -HTBZ and 77% of β -HTBZ disappeared, suggesting that CYP2D6 is in part responsible for metabolism of these metabolites.

CONCLUSIONS and COMMENTS:

This study suggests a role for CYP2D6 in the metabolism of α -HTBZ and β -HTBZ.

The following points should be considered and conveyed to the Sponsor.

1. The Sponsor has not taken a step-wise approach to understanding the metabolism of TBZ or its metabolites. The preferred first approach would be to directly identify metabolites after incubation with hepatocytes or liver slices. Subsequent studies can also eliminate non CYP oxidative pathways.
2. This study to evaluate CYP pathways is methodologically deficient. It is recommended that recombinant enzymes not be used alone, but in combination with other methods (such as use of inhibitors) for identifying drug metabolizing P450 isozymes. In addition, the probes used as controls in the present study are not classical, preferred probes, so it is difficult to understand the acceptability of the reactions.
3. Because of these deficiencies, the role of other isozymes cannot be ruled out.

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4.2.7 *IN VITRO* INHIBITION OF P450

ASSESSMENT OF THE POTENTIAL FOR TETRABENAZINE, ALPHA-DIHYDROTETRABENAZINE AND BETA-DIHYDROTETRABENAZINE TO INHIBIT THE CATALYTIC ACTIVITY OF NINE HUMAN CYTOCHROME P450 ENZYME SUBSTRATES

Study Investigators and Site:

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Protocol Number: AG-A03001

OBJECTIVES:

The purpose of the study was to determine the potential for TBZ and the metabolites α - and β -HTBZ to inhibit the catalytic activity of nine human CYP subtypes: 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4.

DRUGS STUDIED:

Test compounds were received from _____

Tetrabenazine, Lot D9645A
 β -HTBZ, Lot MK110102
 α -HTBZ, Lot MK060201

METHODS:

Recombinant human P450 enzymes

Recombinant human CYP enzymes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) expressed in microsomes were obtained from _____ The assays monitor via fluorescence detection the formation of a fluorescent metabolite following incubation of the microsomes with a specific CYP substrate. (Note: these are not the substrates that are generally recommended for use and the Sponsor has not justified their use). Reactions were performed at 37° C in the presence of an NADPH regenerating system. Inhibition of metabolic product formation was tested in the presence and absence of 0.457 to 1000 μ M TBZ, α - , or β -HTBZ (9 concentrations). Solutions of test compounds included acetonitrile, such that the final concentration of acetonitrile in the assay was 2%. An enzyme-selective inhibitor was also tested at 8 concentrations in each assay as a positive control. All determinations were performed in duplicate. Reactions were initiated by addition of microsome/substrate solution to wells of a microtitre plate containing pre-warmed NADPH regenerating system, buffer, and inhibitor

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solutions. Following specified incubation times, the reactions were stopped by the addition of a STOP solution. Blank samples were assayed by adding the STOP solution prior to addition of microsome/substrate mix to the NADPH regenerating system. The amount of metabolic product formed was quantified by fluorescence detection.

Assay conditions for Study AG-A03001

	Amount of microsomes per incubation	Probe Substrate	Metabolic Product	STOP buffer	Positive Control Inhibitor
CYP1A2	0.5 pmol	3-cyano-7-ethoxycoumarin (CEC) (5 uM)	3-Cyano-7-hydroxycoumarin (CHC)	80% acetonitrile/20% 0.5 M Tris base	Furafylline (0.0457-100 uM)
CYP2A6	1.0 pmol	Coumarin (3 uM)	7-Hydroxycoumarin	80% acetonitrile/20% 0.5 M Tris base	Tranlycypromine (0.0457 to 100 uM)
CYP2B6	1.0 pmol	7-ethoxy-4-trifluoromethylcoumarin (EFC) (2.5 uM)	7-hydroxy-4-trifluoromethylcoumarin (HFC)	80% acetonitrile/20% 0.5 M Tris base	Tranlycypromine (0.057-125 uM)
CYP2C8	4.0 pmol	Dibenzylfluorescein (DBF) (1 uM)	Fluorescein	2 mM NaOH	Quercetin (0.009 – 20 uM)
CYP2C9*1	1.0 pmol	7-methoxy-4-trifluoromethylcoumarin (MFC) (75 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base	Sulfaphenazole (0.00457-10 uM)
CYP2C19	0.5 pmol	CEC (25 uM)	CHC	80% acetonitrile/20% 0.5 M Tris base	Tranlycypromine (0.229 to 500 uM)
CYP2D6*1	1.5 pmol	3-[2-(N,N-diethyl-N-methylamin)ethyl]-7-methoxy-4methylcoumarin (AMMC) (1.5 uM)	AHMC	80% acetonitrile/20% 0.5 M Tris base	Quinidine (0.00023-0.5 uM)
CYP2E1	2.0 pmol	MFC (100 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base	Diethyldithiocarbamic acid (0.0457-100 uM)
CYP3A4	1.0 pmol	7-Benzyloxy-4-trifluoromethylcoumarin (BFC) (50 uM)	HFC	80% acetonitrile/20% 0.5 M Tris base	Ketoconazole (0.00229 to 5 uM)
CYP3A4	3.0 pmol	7-Benzyloxyquinoline (BQ) (40 uM)	Quinolinol	80% acetonitrile/20% 0.5 M Tris	Ketoconazole (0.00229 to 5 uM)

With the exception of coumarin for CYP2A6, none of the probe substrates are generally preferred or acceptable alternate substrates. (It should be noted, however, that the concentration of coumarin used in the CYP2A6 assay is greater than the Km reported for coumarin for CYP2A6. It is recommended that when IC₅₀ values are determined for inhibitors, as in the present study, that the concentration of the substrate should be less than the Km). In addition, in several cases (CYP2B6 and CYP2C19) the inhibitor used as a positive control is not considered to be a preferred or acceptable inhibitor and the Sponsor has not justified their use.

RESULTS:

The Sponsor states that the observed IC₅₀ values for the various inhibitors (shown in the table below, as provided by the Sponsor) are similar to those obtained previously in their laboratory during assay validation, indicating that enzyme activity was not compromised in any of the assays. However, as noted above, for CYP2B6 and CYP2C19 the inhibitor used as a positive control is not considered to be a preferred or acceptable inhibitor.

Table 1. Summary of the IC₅₀ values estimated for the known inhibitors of each CYP subtype.

CYP Subtype	Substrate	Inhibitor (positive control)	Expected ^a Inhibitor IC ₅₀	Observed Inhibitor IC ₅₀
			(µM)	
CYP1A2	CEC	Furafylline	2.34 ± 0.423 (3)	1.97
CYP2A6	Coumarin	Tranlycypromine	0.642 ± 0.175 (4)	1.08
CYP2B6	EFC	Tranlycypromine	16.7 ± 0.785 (4)	13.6
CYP2C8	DBF	Quercetin	0.886 ± 0.079 (4)	1.83
CYP2C9	MFC	Sulfaphenazole	0.275 ± 0.111 (4)	0.256
CYP2C19	CEC	Tranlycypromine	4.37 ± 0.871 (4)	4.03
CYP2D6 ^b	AMMC	Quinidine	0.00519 ± 0.00343 (4)	0.00292
				0.00327
CYP2E1	MFC	DDTC	8.49 ± 3.37 (5)	7.82
CYP3A4	BFC	Ketoconazole	0.0242 ± 0.0208 (3)	0.0464
	BQ		0.200 ± 0.0687 (4)	0.194

^a value expected from assay validation (mean ± S.D. (n)).

^b assay was performed twice.

The following table, as calculated by the reviewer, predicts the Ki values for the positive control inhibitors based on the reported IC₅₀ values, assuming competitive inhibition, for the case when substrate concentration is substantially lower than the substrate Km (Ki=IC₅₀), or when the substrate concentration (S) is the same as substrate Km (Ki=IC₅₀/2). For the inhibitors that are generally accepted as inhibitors that are useful in *in vitro* studies, the predicted Ki values are generally similar to those cited in the *draft preliminary concept paper*, for CYP2C8, CYP2C9, and CYP3A4. The assay for quinidine as an inhibitor of CYP2D6 and diethyldithiocarbamic acid as an inhibitor of CYP2E1 have lower predicted Ki values than generally cited, such that the assays would appear more sensitive to inhibition than the generally recommended assays. The

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assays for CYP1A2 and CYP2A6 would appear to be slightly less sensitive to inhibition by standard inhibitors than generally reported. For CYP2B6 and CYP2C19 the control inhibitors are not among the generally acceptable inhibitors making results with these isozymes difficult to interpret.

Calculation of Ki for Control Inhibitors and Comparison to Generally Cited Ki Values.

	Positive Control Inhibitor	Predicted Ki=IC ₅₀ (uM)	Predicted Ki=IC ₅₀ /2 (uM)	Ki (uM) cited in Draft Preliminary Concept Paper
CYP1A2	Furafylline	1.97	.985	0.6-0.73
CYP2A6	Tranlycypromine	1.08	0.54	0.02-0.2
CYP2B6	Tranlycypromine ^a			
CYP2C8	Quercetin	1.83	0.915	1.1
CYP2C9*1	Sulfaphenazole	0.256	0.128	0.3
CYP2C19	Tranlycypromine ^a			
CYP2D6	Quinidine	~0.003	0.0015	0.027-0.4
CYP2E1	Diethyldiothiocarbamic Acid	7.82	3.91	9.8-34
CYP3A4	Ketoconazole	.0464	0.0232	.0037-0.18
CYP3A4	Ketoconazole	0.194	0.097	.0037-0.18

^a Not a preferred inhibitor, not reviewed in the Concept Paper.

The results for the inhibition studies (inhibition of P450s by TBZ and its metabolites) are summarized in the Table below, as provided by the Sponsor. For CYP2D6 the experiment was repeated and the results of that experiment are shown.

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Table 2. Summary of the IC₅₀ values estimated for TBZ, α-HTBZ and β-HTBZ for each CYP subtype.

CYP Subtype	Substrate	TBZ IC ₅₀	α-HTBZ IC ₅₀	β-HTBZ IC ₅₀
			(μM)	
CYP1A2	CEC	83.1	≈ 1000	> 1000
CYP2A6	Coumarin	n/a ^a	n/a	n/a
CYP2B6	EFC	334	475	n/a
CYP2C8	DBF	≈ 1000	n/a	n/a
CYP2C9	MFC	> 1000	≈ 540	n/a
CYP2C19	CEC	≈ 569	> 1000	n/a
CYP2D6 ^b	AMMC	72.3	27.3	not reliable ^c
		55.1	16.0	2.63
CYP2E1	MFC	n/a	n/a	n/a
CYP3A4	BFC	150	≈ 484	>1000
	BQ	≈ 521	> 1000	n/a

^a n/a denotes not applicable (i.e. no inhibition was observed over concentration range tested)

^b assay was performed twice.

^c an IC₅₀ value could not be reliably estimated.

CYP2D6: Inhibition of the metabolism of AMMC to AHMC (repeat experiment)

Quinidine		Test Compound Concentration (μM)	TBZ	α-HTBZ	β-HTBZ
Concentration (μM)	Inhibition (%)		Inhibition	Inhibition (%)	Inhibition
0.500	84.2	1000	36.80	25.8	0.000
0.167	78.6	333	52.10	65.7	0.000
0.0556	76.5	111	44.40	71.0	54.1
0.0185	60.1	37.0	15.40	53.7	67.9
0.00617	26.4	12.3	3.67	35.2	60.9
0.00206	0.880	4.12	0.00	14.7	41.8
0.000686	2.35	1.37	0.00	0.000	17.5
0.000229	0.000	0.457	0.00	2.64	1.03

Data represent the mean of 2 replicate determinations.

TBZ and both metabolites were most potent at inhibiting CYP2D6. Percent inhibition at each concentration is shown in the table above for the repeat experiment. The effect of both metabolites as well as TBZ appeared to decrease at higher concentrations, particularly for β-HTBZ where no inhibition was observed at the highest concentrations. In the hepatic impairment study (203,010) TBZ was detectable, with mean C_{max} of approximately 56 ng/ml. Concentrations of α- and β-HTBZ in sparse PK sampling from Clinical Study 103,004 in which patients received doses of up to 100 mg/day were 105 ng/ml and 163 ng/ml, respectively. These concentrations are higher than in the clinical pharmacokinetic studies that used lower doses. The range of concentrations in Study 103,004 is unknown. These concentrations, expressed as μM concentrations, as well as the reviewer's predicted K_i values, and 1/K_i are shown in the table below for inhibition of CYP2D6. The predicted K_i values assume competitive inhibition and that S=K_m, and use the IC₅₀ values above that are most likely to predict inhibition.

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	MW	Conc (uM)	Predicted Ki=IC ₅₀ /2	Predicted I/Ki
TBZ	314.72	0.177 uM	27.55 uM	0.006
α-HTBZ	319.43	0.33 uM	8 uM	0.04
β-HTBZ	319.43	0.51 uM	1.3 uM	0.39

These results, suggest that there is a possibility of CYP2D6 inhibition following administration of TBZ due to inhibition of CYP2D6 by β-HTBZ.

CONCLUSIONS and COMMENTS:

1. The results of this study suggest *in vitro* inhibition of CYP2D6 by β-HTBZ. Based on these results it is possible that there may be an *in vivo* interaction mediated by CYP2D6 when TBZ is given clinically.
2. The results of this study, in general, are difficult to interpret since the methodology does not conform to the methodology such as substrates that are well recognized as being acceptable.
3. The Sponsor should perform an *in vivo* interaction study with TBZ and a sensitive CYP2D6 substrate (such as desipramine).
4. The Sponsor should follow-up with the results of the present study with *in vitro* inhibition studies that use well accepted methodology to confirm lack of involvement in inhibition of other P450s.

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4.2.8 MASS BALANCE STUDY

A PHASE I, OPEN-LABEL STUDY TO ASSESS THE ABSORPTION, METABOLISM AND ELIMINATION OF [¹⁴C]-LABELLED TETRABENAZINE

Study Investigators and Site:

/ /

Protocol Number: RD 204/24124

OBJECTIVES:

To define the PK characteristics (absorption, metabolism and elimination) of total radioactivity, TBZ, and its metabolites α - and β -HTBZ following oral administration of [¹⁴C]-tetrabenazine.

To further investigate the safety and tolerability of TBZ.

FORMULATION:

The study medication consisted of 25 mg ¹⁴C-TBZ (50 uCi, 1.85 MBq). The study was conducted on June 30, 2005.

STUDY DESIGN:

This was a Phase I, open-label study. The dose of 25 mg TBZ was chosen as it was well-tolerated in Phase I studies. Six healthy male subjects were enrolled and received a single oral dose of 25 mg ¹⁴C-TBZ (50 uCi, 1.85 MBq), after a 10 hour overnight fast. Following administration of study drug, each bottle was rinsed twice with 50 ml water which the subjects were asked to swallow to ensure that the total dose was administered. Subjects were allowed to eat a light lunch 4 hours after dosing. Water was not allowed from 1 hour pre-dose until 2 hours post-dose. Subjects were admitted to the clinical Center on the evening before each dose and remained in the Center for at least 168 h (until the morning of Day 8) post-dose. Sampling of blood, urine and feces was scheduled to cease at 168 hours provided that at least 90% of the radioactive dose was recovered. Five of the 6 subjects had not met this criterion and were asked to continue their collections up to the morning of day 10 (216 h post-dose). Adverse events were monitored, and vital signs, 12-lead ECGs and laboratory safety tests were performed during the study for tolerability assessments.

Blood samples were collected for determination whole blood and for plasma ¹⁴C total radioactivity and plasma TBZ at pre-dose and at 0.25, 0.5, 1, 1.25, 1.5, 2, 2.25, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, and 48 and 60, 72, 96, 120, 144, and 168 hours after drug administration. Urine sample were collected for determination of ¹⁴C total radioactivity and TBZ at pre-dose and 0-4,

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4-8, 8-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours post dose. Each subject collected a ¹⁴C free fecal sample before dosing. After study drug administration, all fecal samples were collected for a minimum period of 168 hours and up to 216 hours post dose in some subjects.

Inclusion criteria included healthy males, between the ages of 18 and 55 years of age. Exclusion criteria included a history of gastrointestinal disorder likely to influence drug absorption, receipt of regular medication within 14 days of the first study day, and evidence of renal, hepatic, central nervous system, respiratory, cardiovascular, or metabolic dysfunction. Over the counter drugs taken during the 2 weeks before the study start were to be recorded and should have been avoided throughout the duration of the study dosing period with the exception of acetaminophen and _____ which was taken as a bulking agent (to aid regular bowel movement) for 3 days prior to dosing and on a daily basis for the duration of the study period. Consumption of alcohol was limited to a maximum of 2 units per day from 7 days prior to the administration of the first dose and avoided completely for a period of not less than 48 hours prior to the first dose and throughout the study period.

Safety monitoring included adverse event assessments, vital signs (including supine and standing systolic and diastolic blood pressure, pulse, and oral temperature), laboratory tests including hematology, serum chemistry, and urinalysis, 12-lead ECG, and physical exam.

ASSAY:

Plasma concentrations of TBZ, α -dihydro-TBZ (α -HTBZ), and β -dihydroTBZ (β -HTBZ) were measured using a validated LC/MS/MS method (1266/1).

Table 1. Performance of Analytical Method for TBZ 204/24124

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.2-200 ng/ml	r > 0.990	0.2	0.5	4.8	3.4
					25.0	5.1	-3.2
					100.0	6.0	-0.6
α -HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.992	0.5	1.0	6.7	-1.1
					25.0	4.2	-5.9
					100.0	6.9	3.0
β -HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.992	0.5	1.0	9.6	-0.2
					25.0	7.6	-3.2
					100.0	8.4	1.9

Two calibration curves and duplicate QC samples were analyzed with each batch of study samples. The performance of the assays for all analytes is considered acceptable.

RESULTS:

Demographics

Table 2. Demographics of Subjects Completing Study 204-24124

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Mean Age (Range)	Gender	Weight (mean \pm SD)	Race
36.3 (21-46)	6 males	73.8 \pm 11.4 kg	Not provided

Pharmacokinetics

Recovery of Radioactivity

After administration of TBZ, 64.97-81.39% of the dose (mean 75.4%) was excreted in urine in the 9 day collection period. The majority was recovered within 72 hours after dosing. Fecal recovery in the 9 days after dose accounted for 7.07-16.05% of the dose. The mean total recovery (urine + feces) was 87.49% (range 76.88-92.84%). The mean percent recovery is shown in the figure below, as provided by the Sponsor.

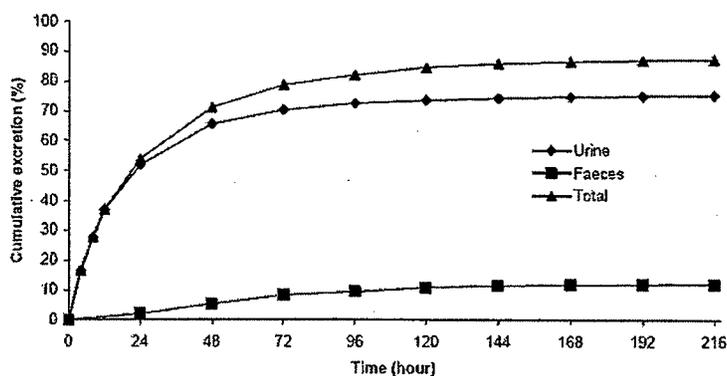


Figure 1 Mean excretion of total radioactivity following oral administration of 25 mg ¹⁴C-TBZ to 6 male human subjects

PK analysis of total radioactivity in plasma and in whole blood showed the following results, as provided by the Sponsor. Exposure to radioactivity in whole blood was approximately 40% less than that seen in plasma, based on AUC_{0-t}.

Table 3. Pharmacokinetic parameters (arithmetic mean, %CV) for total radioactivity in plasma and in whole blood in Study RD 204 2124)

	Plasma	Whole Blood
t _{max} (h) ^a	1.0 (0.5-1.5)	1.25 (0.5-1.25)
C _{max} (ng equiv/mL)	191.5 (10)	149.9 (13)
AUC _{0-t} (ng*h/mL)	1490.41 (12)	852.76 (24)
AUC _{0-inf} (ng*h/mL)	1833.69 (13)	1188.73 (25)
t _{1/2} (hrs)	11.38 (19)	6.72 (29)

^a Median (range)

PK Analysis

Pharmacokinetic analysis of TBZ and α - and β - HTBZ in plasma was performed using noncompartmental analysis. The mean results are shown in the table below, calculated from

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individual parameters as provided by the Sponsor (in Appendix 3, Table 6 of the Study Report). (Note: although these data were from PK parameters, provided by the Sponsor, the mean data provided by the Sponsor did not agree with the data provided below). For TBZ, all subjects had detectable concentrations up to approximately 2.5 hours post-dose, at which point all time points had TBZ concentrations below the limit of quantification.

Table 4. Pharmacokinetic parameters (arithmetic mean, %CV) for TBZ, α - and β -HTBZ in Plasma Study RD 204 2124)

	TBZ	α -HTBZ	β -HTBZ
t_{max} (h) ^a	0.25 (0.25-0.5)	1.0 (0.5-1.5)	1.0 (0.5-1.5)
C_{max} (ng equiv/mL)	1.27 (46)	43.59 (43)	18.08 (71)
AUC _{0-t} (ng*h/mL)	1.12 (67)	161.80 (68)	46.33 (93)
AUC _{0-inf} (ng*h/mL)	1.36 (58)	170.63 (65)	50.46 (87)
$t_{1/2}$ (hrs)	0.72 (23)	5.24 (35)	3.00 (26)

^a Median (range)

Identification of Metabolites

Chromatographic analysis of urine showed up to 19 distinct metabolites (U1-U19). None of these components exhibited co-chromatography with ¹⁴C-TBZ. Analysis of urine using LC-MS/MS indicated the presence of the following compounds (as summarized by the Sponsor):

Component	Assignment	Nominal HPLC retention time (minutes) [†]	% dose
U1 – U9	Monohydroxy-dihydro tetrabenazines and Glucuronides of O-dealkylated dihydro tetrabenazine(s)	4.2 – 13.9	1.01 – 4.02 (Total 17.93)
U10	Monohydroxy dihydrotetrabenazine	14.1 – 14.6	3.70
U11 and U13	Sulphate conjugates of O-dealkylated dihydro tetrabenazine(s)	15.4 – 15.7 17.3 – 17.5	12.68 – 17.09 (Total 29.77)
U15 and U17	O-dealkylated dihydro tetrabenazine and/or β -dihydro tetrabenazine	19.0 – 19.2 21.9 – 22.0	2.38 – 2.64
U18	α -dihydro tetrabenazine	24.9 – 25.1	0.43

[†] Retention times taken from HPLC analysis, slight shifts in retention times observed on MS analysis

The following components and their proportions were found circulating in the plasma in pooled plasma samples. It can be seen that there were several metabolites (P11, P13, P 16) that were circulating to a greater extent than α – HTBZ (P 18). In addition, although P17 was not circulating to a greater extent than P18, it represented more than 10% of components circulating in the plasma up to 1.5 hours after the dose.

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Component	0.25 – 1.5 hours	2 – 3 hours	4 – 8 hours	Mean
P1	ND	ND	ND	NC
P2	ND	ND	ND	NC
P3	ND	ND	ND	NC
P4	ND	ND	ND	NC
P5	ND	ND	ND	NC
P6	ND	ND	4.10 (4.65)	NC
P7	ND	ND	ND	NC
P8	ND	ND	8.23 (2.14)	NC
P9	ND	ND	ND	NC
P10	ND	ND	ND	NC
P11	23.44 (18.67)	29.52 (20.41)	15.23 (18.90)	22.73 (17.09)
P12	ND	ND	ND	NC
P13	28.73 (19.01)	41.77 (28.88)	24.22 (28.88)	32.91 (24.02)
P14	ND	ND	ND	NC
P15	ND	ND	ND	NC
P16	49.54 (35.24)	59.11 (34.65)	19.77 (21.95)	59.81 (30.61)
P17	13.85 (9.71)	6.54 (4.52)	4.48 (4.97)	8.22 (6.40)
P18	25.73 (18.32)	18.70 (11.65)	14.05 (16.80)	18.83 (15.15)
Other	1.32 (1.08)	NC	NC	NC

Results expressed as ng equivalents/ml. Values in parentheses indicate % sample radioactivity.
 ND Component not detected NC Not calculable

These components represent:

P1-9: mono-hydroxy-HTBZ and glucuronides of O-dealkylated HTBZ

P10: monohydroxy HTBZ

P11 and P13: sulfate conjugates of O-dealkylated HTBZ

P15 and P17: O-dealkylated HTBZ and/or β -HTBZ (tentative)

P18: α -HTBZ

P16, the largest component of the plasma, was not identified.

Major metabolites in feces (from subject 2, taken as representative samples) were qualitatively similar as those found in plasma. Proportions are shown in the table below, as provided by the Sponsor.

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Component	0 - 24 hours	24 - 48 hours	Total
F1	0.42 (4.83)	0.97 (17.62)	1.39
F2	0.35 (4.01)	ND	0.35
F3	0.95 (10.84)	ND	0.95
F4	ND	ND	ND
F5	ND	ND	ND
F6	ND	ND	ND
F7	ND	ND	ND
F8	ND	ND	ND
F9	ND	ND	ND
F10	0.37 (4.20)	0.32 (5.80)	0.69
F11	0.65 (7.40)	ND	0.65
F12	0.84 (9.61)	0.62 (11.25)	1.46
F13	2.92 (33.53)	1.50 (27.07)	4.42
F14	ND	ND	ND
F15	0.46 (5.20)	1.43 (25.84)	1.89
F16	ND	0.17 (3.10)	0.17
F17	ND	ND	ND
F18	0.14 (1.59)	ND	0.14
F20	0.05 (0.54)	0.13 (2.27)	0.18
Other	1.59 (18.25)	0.39 (7.05)	1.98

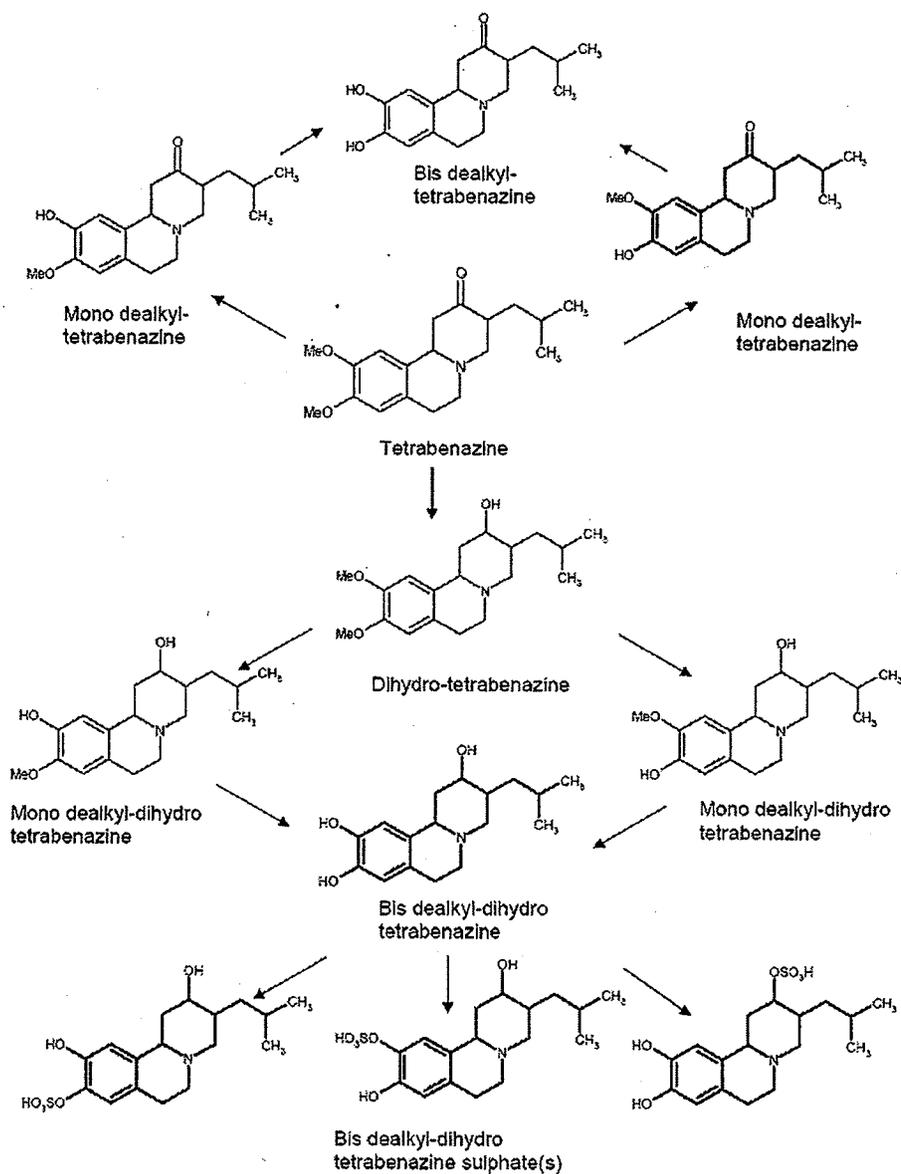
Results expressed as % dose administered. Values in parentheses indicate % sample radioactivity.
 ND Component not detected. NC Not calculable.
 Components 1 - 9 were identified as mono-hydroxy-dihydro tetrabenazine(s) and glucuronides of O-desalkylated dihydro tetrabenazine(s).
 Component 10 was identified as monohydroxy dihydro tetrabenazine.
 Components 11 and 13 were identified as sulphate conjugates of O-desalkylated dihydro tetrabenazine(s).
 Components 15 and 17 were tentatively identified as O-desalkylated dihydro tetrabenazine and/or 2-dihydro tetrabenazine.
 Component 16 was identified as o-dihydro tetrabenazine.

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Proposed Metabolic Scheme

The Sponsor has proposed the following metabolic scheme.



Note: Dihydro-tetrabenazine is referred to as HTBZ throughout the review.

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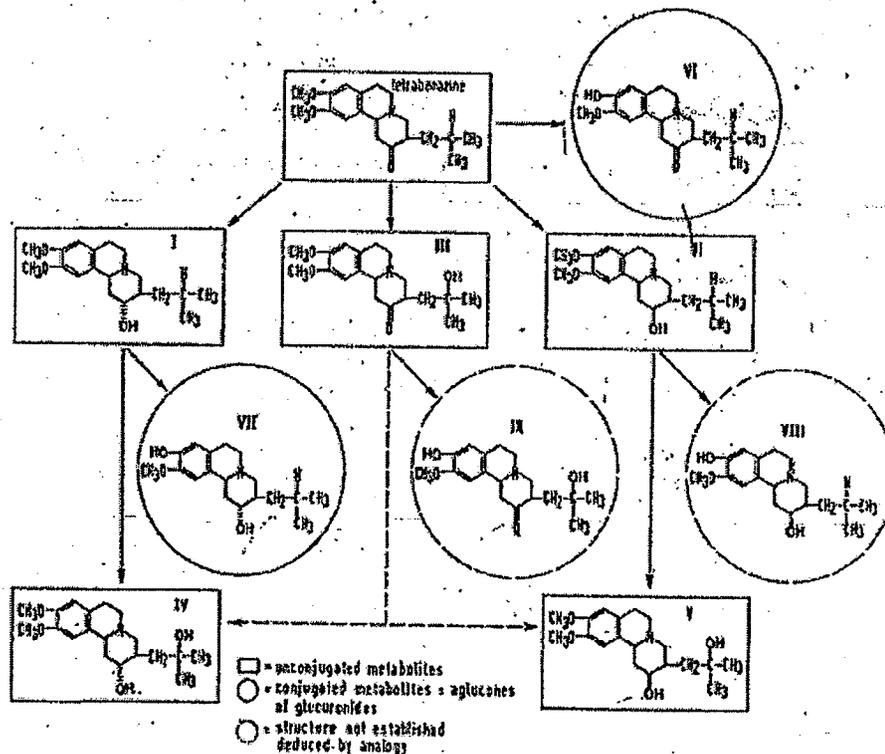
Comparison to original metabolite assignments

In the original submission, Report — 1627 reported on the metabolites in human, dog, rabbit, and mouse plasma from studies — 14711 and — 1546 from human Protocols TBZ 103,003 (food effect) from 3 subjects and TBZ 103004 (efficacy study) from 2 subjects using precursor scanning for metabolite detection targeting metabolites that had been identified by Schwartz et al (*Biochemical Pharmacology* 1966; 15: 645-655). The Sponsor had the following putative assignment of metabolites, identified along with the Schwartz Assignment. The Schwartz assignments (based on detection in urine following a subcutaneous dose in humans) are shown in the figure below.

Putative assignment of metabolites found in plasma samples in humans and animals.

Schwartz Assignment*	Molecular Formula	Metabolite	Theoretical Transition	Transition Detected			
				HUMAN	DOG	RABBIT	MOUSE
I & II	C19H29NO3	HYDROGENATION	320 → 165/228	✓	✓	✓	
III	C19H27NO4	OXIDATION	334 → 165/228	✓	✓	✓	
IV & V	C19H29NO4	OXIDATION & HYDROGENATION	336 → 165/220	✓	✓		
VI	C18H25NO3	DEMETHYLATION	304 → 151/206	✓	✓		✓
VII & VIII	C18H27NO3	DEMETHYLATION & HYDROGENATION	306 → 151/206	✓	✓	✓	
IX	C18H25NO4	DEMETHYLATION & OXIDATION	320 → 151/206	✓	✓		✓

Scheme of tetrabenazine metabolism in human as described by Schwartz et al.



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In Study RD 204/24124, the metabolites are in agreement with those reported in the initial submission (metabolites I and II are HTBZ, metabolite VI is mono-dealkyl TBZ, and VII and VIII are mono-dealkyl HTBZ, IV and V are mono-hydroxy HTBZ), although metabolites III and IX from the Schwarz scheme are oxidative metabolites that are not reported in Study RD 204/24124. The bis-dealkyl HTBZ and their sulfate metabolites as well as the bis-dealkyl TBZ (in Study RD 204/24124) were not reported in the original report. A comparison is shown in the table below.

Current Mass Balance Study		Schwarz
		Mono-hydroxy TBZ (III)
		mono hydroxy-O-dealkylated TBZ (IX) – <i>structure was deduced</i>
P6	Mono-hydroxy-HTBZ and glucuronides of O-dealkylated HTBZ	IV and V are mono-hydroxy HTBZ
P8	Mono-hydroxy-HTBZ and glucuronides of O-dealkylated HTBZ	VII is glucuronides of O-dealkylated HTBZ
P11	Sulfate conjugates of O-dealkylated HTBZ	
P13	Sulfate conjugates of O-dealkylated HTBZ	
P16	Not identified	
P17	O-dealkylated HTBZ and/or β -HTBZ	VII and VIII
P18	α -HTBZ	I and II (α and β HTBZ)
Other	Mono dealkyl TBZ	VI (glucuronide conjugated)
	Bis-dealkyl TBZ	
	Bisdealkyl HTBZ	

Safety

There were no serious or severe adverse events reported in Study RD 204/24124. A total of four adverse events were reported by 2 subjects, although in 1 of those subjects the event occurred prior to dosing. The other subject reported nausea (8 hours post-dose that lasted 1.8 hours), headache (9 hours post dose that lasted for 2.5 hours and was treated with acetaminophen), and vomiting (9.8 hours post-dose).

CONCLUSIONS:

After administration of TBZ, the majority of a radioactive dose (mean 75.4%) was excreted in the urine, with fecal recovery accounted for approximately 7-16% of the dose. The mean total recovery (urine + feces) was 87.49%. TBZ did not appear in the urine and α - and β -HTBZ accounted for less than 10% of the radioactive dose in the urine.

TBZ was only detectable in plasma up to 2.5 hours after the dose, beyond which all TBZ time points were below the limit of quantification for TBZ. The mean exposure for α -HTBZ was approximately 2.4 fold and 3.5 fold greater for C_{max} and for AUC, respectively than that for β -HTBZ. The mean elimination half-lives of α - and β -HTBZ were 5.24 and 3.0 hours, respectively.

The Sponsor has identified several Phase I and Phase II metabolites (glucuronidation and sulfation) circulating with significant exposures in the plasma. P16, the largest component circulating in the plasma, has not been identified. The Sponsor has not accounted for enantiomers of each metabolite. The extent of circulating mono- and bis-dealkyl TBZ is not provided.

Several circulating metabolites are also substantially eliminated renally (11 and 13 are the predominant metabolites in the urine).

The results are generally in agreement with the metabolites identified in the original submission although metabolites III, and IX from the Schwartz scheme are oxidative metabolites that are not reported in Study RD 204/24124. The bis-dealkyl HTBZ and their sulfate metabolites as well as the bis-dealkyl TBZ (in Study RD 204/24124) were not reported in the original report.

4.2.9 IN VITRO PROTEIN BINDING STUDY

EQUILIBRIUM DIALYSIS OF 50, 100, AND 200 NG/ML TETRABENAZINE, ALPHA-DIHYDROTETRABENAZINE AND BETA-DIHYDROTETRABENAZINE IN HUMAN, DOG RABBIT, RAT, AND MOUSE PLASMA FOR THE DETERMINATION OF PLASMA PROTEIN BINDING

Study Investigators and Site:

/ /

Protocol Number: AG-A04001

Note: This study will be reviewed with respect to human protein binding only.

OBJECTIVES:

The objective of the study was to assess the binding of tetrabenazine (TBZ), α -dihydrotetrabenazine (α -HTBZ) and β -dihydrotetrabenazine (β -HTBZ) to pooled mixed gender human, dog, rabbit, rat, and mouse plasma proteins.

TEST COMPOUNDS:

α -HTBZ (Batch RUS0406, _____)

β -HTBZ (Batch RUS0407, _____)

TBZ (Batch 100730, _____)

METHODS:

Plasma from each species was spiked with each compound at 50, 100, and 200 ng/ml. Buffer (Dulbecco's phosphate buffered saline) was also spiked with each test compound at a concentration of 200 ng/ml. Six replicate aliquots of each spike plasma and buffer solution were collected prior to dialysis. The standards _____

_____ were prepared in plasma from each species at a concentration of 1 μ Ci/ml.

Equilibrium dialysis was performed in a 96-well _____ dialysis unit. One chamber of each well was filled with 150 μ L of blank buffer. Aliquots (150 μ L) of spiked plasma or spiked buffer were then added to the opposing chamber in each well. The top of the plate was sealed to prevent evaporation and maintain constant pH during incubation. All experiments were assessed using six replicate samples, with the exception of the standards in human and dog plasma for which 3 replicate samples were used. An aliquot (120 μ L) from each plasma and buffer compartment was collected following 24 hours of incubation. The samples containing the test

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compounds were stored frozen at -80° C until analysis by — The samples containing the radiolabeled standards were counted using scintillation spectrometry.

The fraction of each standard and test compound unbound (f_u) to human serum proteins was calculated according to the following equation: $f_u = C_{\text{buffer}}/C_{\text{plasma}}$ and fraction bound (f_b) to plasma proteins was determined as $1-f_u$.

ASSAY:

Concentrations of TBZ, α -HTBZ and β -HTBZ were determined using LC-MS/MS at — using validated methods — 494/1 and — 1266/1.

Performance of Analytical Method for AG-04001

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.5-200 ng/ml	$r > 0.998$	0.5	1.0	9.5	-4.84
					25.0	0.56	-5.39
					100.0	4.46	-5.05
α -HTBZ	LC/MS/MS	1-200 ng/ml	$r > 0.988$	1.0	2.0	6.6	6.62
					25.0	7.34	-2.2
					100.0	2.91	-7.82
β -HTBZ	LC/MS/MS	1-200 ng/ml	$r > 0.987$	1.0	2.0	7.38	-4.68
					25.0	5.54	6.25
					100.0	7.08	2.7

Two calibration curves and duplicate QC samples were analyzed with each batch of samples. Samples were analyzed within the period for which the samples are stable. The performance of the assays for all analytes is considered acceptable.

RESULTS:

The following table summarizes the results for propranolol and for warfarin binding in human plasma, representing the mean \pm SD for 3 replicate determinations. The % bound agrees with what has previously been reported in the literature.

	Plasma concentration at 24 h (pM)	Unbound (%)	Bound (%)
(S)-propranolol	108 \pm 4.39	9.40 \pm 0.862	90.6 \pm 0.862
(R,S)-warfarin	76.6 \pm 1.90	0.87 \pm 0.066	99.1 \pm 0.066

To determine whether equilibrium binding had been reached for the test compounds, buffer spiked with each compound was dialyzed against blank buffer, in parallel with the dialysis of the plasma samples. If equilibrium were reached, the concentrations of the compounds should be equal in the opposing chambers and the calculated % unbound should be equal to 100%. The data indicated that α -HTBZ and β -HTBZ were at equilibrium but that tetrabenzazine was not, and

may result in an overestimation of the % bound, since the concentration of TBZ in the chamber to which spiked buffer was added was approximately 25-27% higher than the opposing chamber to which blank buffer was added.

The results for the equilibrium dialysis in human plasma are summarized in the table below. The results represent the means \pm of 3 determinations.

	Plasma Concentration at 0 h (ng/ml)	Plasma Concentration at 24 h (ng/ml)	Unbound (%)	Bound (%)
TBZ	159 \pm 7.67	123 \pm 0.26	15.5 \pm 0.669	84.5 \pm 0.669
	80.9 \pm 4.16	59.5 \pm 2.52	16.4 \pm 1.93	83.6 \pm 1.93
	41.9 \pm 1.12	29.2 \pm 2.22	18.1 \pm 2.86	81.7 \pm 2.86
α -HTBZ	293 \pm 30.5	177 \pm 4.92	35.7 \pm 3.42	64.3 \pm 3.42
	127 \pm 11.3	78.5 \pm 3.19	39.8 \pm 8.55	60.2 \pm 8.55
	64.1 \pm 3.92	40.9 \pm 1.6	32.1 \pm 2.37	67.9 \pm 2.37
β -HTBZ	207 \pm 12.0	145 \pm 2.04	37.2 \pm 2.35	62.8 \pm 2.35
	99.9 \pm 6.49	70.1 \pm 6.42	41.3 \pm 9.1	58.7 \pm 9.1
	51.7 \pm 6.18	36.1 \pm 1.53	38.8 \pm 10.8	61.2 \pm 10.8

CONCLUSIONS and COMMENTS:

1. Protein binding of TBZ, α -HTBZ, and β -HTBZ was not concentration dependent at concentrations of approximately 40-160 ng/ml for TBZ, 60-290 ng/ml for α -HTBZ, and 50-200 for β -HTBZ.
2. The % protein binding was approximately 60-68% for α -HTBZ and approximately 59-63% β -HTBZ. The % bound for TBZ was approximately 81-84%, although this may have been overestimated as the assay was not performed under equilibrium conditions for TBZ.

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4.2.10 BCS CLASSIFICATION OF TETRABENAZINE

Tetrabenazine Tablets: 12.5 and 25 mg tablets

Physical Properties: (as reported in the NDA, section 2.3S)

Molecular Weight: 317.4

Partition Coefficient: 3.187

pKa: 6.51

Solubility in water:

The Sponsor states that the drug is sparingly soluble in water, soluble in ethanol, and that 10 mg/ml aqueous solutions have a pH of

Solubility in other media:

The Sponsor has provided the following information (in the 1/18/06 submission) about the pH solubility profile:

pH of Solution	Concentration of Tetrabenazine (tetrabenazine/pH adjusted water)	USP Solubility Classification
2		
3		
4		
6		
8		
10		
12		

Absolute Bioavailability: Not determined.

Relative Bioavailability: Not determined.

Dissolution specification: To be determined.

Mass balance studies: In 6 healthy male volunteers receiving [¹⁴C]-tetrabenazine orally), 64.97-81.39% of the dose (mean 75.4%) was excreted in urine and fecal excretion of radioactivity represented 7.07-16.05% of the dose. Up to 19 distinct metabolites have been identified representing Phase I and Phase II metabolism.

In Vitro Intestinal Permeability Studies: Not performed.

Summary:

Highly soluble: According to the BCS, a drug substance is classified as *highly soluble* if the highest strength is soluble in less than 250 ml of aqueous media over the pH range of 1-7.5. (The cutoff for solubility for TBZ would be 25/250=0.1 mg/ml. It only meets this cutoff for pH

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2, based on the solubility profile provided above. Therefore, TBZ does not meet criteria for highly soluble).

Highly permeable: When the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination, a drug substance is considered to be highly permeable. For tetrabenazine, since 64.97-81.39% of the dose (mean 75.4%) was excreted in urine, the extent of absorption is considered to be 75.4 % (mean). Therefore tetrabenazine cannot be considered highly permeable.

Rapidly dissolving: An IR drug product is considered to be rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 30 minutes. (TBZ tablets are rapidly dissolving using USP Apparatus II (paddle) in pH 4.5 media, and 0.1 M HCl (although the rotating speed was increased to 100 rpm in this case), but not in pH 6.8 Simulated Intestinal Fluid. Therefore it is not rapidly dissolving.

Conclusion:

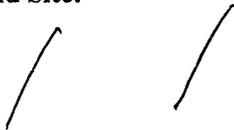
Tetrabenazine is classified as BCS Class 4 (low solubility, low permeability).

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4.2.11 DOSE PROPORTIONALITY AND REPEAT DOSE STUDY (1700114)

AN OPEN LABEL STUDY TO ESTABLISH THE BIOAVAILABILITY OF TETRABENAZINE AND DIHYDROTETRABENAZINE COMPRISING (A) A SINGLE DOSE TWO-WAY CROSS-OVER STUDY COMPARING 12.5 MG AND 50 MG DOSES AND (B) A REPEAT DOSE STUDY (25 MG) IN HEALTHY MALE AND FEMALE SUBJECTS

Study Investigators and Site:



Protocol Number: 1700114

OBJECTIVES:

To evaluate the single dose proportionality of 12.5, 25, and 50 mg doses of tetrabenazine in healthy male and female subjects.

To evaluate the steady-state pharmacokinetics of repeat 25 mg doses of tetrabenazine in healthy male and female subjects.

FORMULATIONS:

Table 1. Products used in 1700114

	Description	Batch Number	Exp. Date (Dates of Study)
Tetrabenazine 25 mg tablets	Round, yellowish-buff in color, with a single break bar on one face	L027/2CC	April 2005 (August 6, 2001- September 7, 2001)

The 12.5 mg doses were prepared by cutting the 25 mg tablets in half along the break bar using a scalpel. Each half tablet was weighed and the weight of the tablet administered was recorded to two decimal places to determine the actual dose administered.

STUDY DESIGN:

This study was an open-label, single and repeat dose study. The single dose study was a randomized, two-way cross-over study evaluating PK after administration of 12.5 mg and 50 mg tetrabenazine. There was a washout period of at least 7 days between doses. The repeat dose

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study was conducted in a different group of subjects. In that study, tetrabenazine 25 mg was given for 5 days, with pharmacokinetics evaluated on Days 1 and 5, with pre-dose samples on days 2, 3, and 4 to monitor the occurrence of steady state. The Sponsor has combined data from the 2 parts of this study to evaluate dose proportionality.

Inclusion criteria included healthy males or females, 18 to 45 years of age (inclusive). Exclusion criteria included use of medication (including OTC drugs) within 2 weeks of dosing except for "simple analgesics" such as acetaminophen. Oral contraceptives were permitted as were vitamins.

In each study period of the single dose state, subjects received a single oral dose of tetrabenazine (12.5 mg or 50 mg) on the morning of Day 1 after an overnight fast. The dose was given with 150 ml water. The subjects fasted until 1 hour post-dose. In the repeat dose stage, subjects received a single oral 25 mg dose of tetrabenazine every morning for 5 days. Subjects were fasted overnight (at least 10 hours) and remained fasted until 1 hour post-dose on Days 1, 2, and 5 when they were resident in the Clinic. Doses on Days 3 and 4 were administered during visits to the Clinic, and subjects were asked to fast overnight prior to those visits. Standardized lunch (4 hours after dosing) and an evening meal and snack were provided when subjects were resident in the Clinic. Subjects were requested not to consume alcohol, grapefruit-, caffeine-, or xanthine-containing products for 48 hours before admission until final discharge from the Clinic.

Blood samples were collected for determination of plasma concentrations of TBZ and its metabolite HTBZ. In the single dose state, samples were collected pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, and 24 hours post-dose. In the repeat dose stage samples were collected on Day 1 pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 12 hours post-dose. On Days 2-4 samples were collected pre-dose (24, 48, and 72 hours post-Day 1 dose). On Day 5 samples were collected pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, and 24 hours post-dose. Plasma was stored at -20° C.

Urine samples were collected pre-dose and then all urine was collected from 0-6, 6-12, and 12-24 hours post-dose on Day 1 of the single dose stage and on Days 1 and 5 of the repeat dose stage. Urine was stored at 4° C during the collection interval. Urine volumes were calculated by measurement of specific gravity and weight. After collection, the sample was mixed and two 10ml aliquots were removed and stored at approximately -20° C.

Safety assessments included vital signs at pre-dose and at 1 and 3 hours post-dose on Day 1 of the single dose stage, and on Days 1 and 5 of the repeat dose stage, and at 1 hour post-dose on Days 2 to 4 of the repeat dose stage.

ASSAY:

Table 3. Performance of Analytical Method for Plasma and Urine Samples in 1700114

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ plasma	LC/MS/MS (Method 1133/1)	0.2-50 ng/ml	$r > 0.997$	0.2	0.4	9.38	-10.52
					10	6.61	3.09
					30	5.60	1.22
HTBZ plasma	LC/MS/MS (Method 1133/1)	1-150 ng/ml	$r > 0.997$	1.0	3	8.2	3.9
					40	4.7	2.7
					100	3.9	1.3
TBZ urine	LC/MS/MS (Method 1133/1)	0.2-50 ng/ml	$r > 0.997$	0.2	0.4	8.15	-0.04
					10	9.13	0.00
					30	8.34	-0.02
HTBZ urine	LC/MS/MS (Method 1133/1)	1-150 ng/ml	$r > 0.995$	1.0	3	8.07	-0.11
					40	12.71	-0.09
					100	12.11	-0.11

The method was validated and performed at [redacted]. In addition to the items outlined in the review of the SOP for the analytical method, the SOP included criteria for acceptance of assay batches and for repeat analysis. During the analysis of human plasma samples it was found that the HTBZ samples showed 2 unresolved peaks. It was assumed that one peak was the α -form and one peak was the β -form. (It is stated in the analytical study report that during validation it was shown that the response factor for the β -form is 2.5x greater than that for the α -form. This data is found in the validation for the bioanalytical method in urine samples). The peaks were integrated together to give a combined total of HTBZ present.

A single calibration curve and duplicate QC samples were analyzed with each batch of study samples. Study samples were initially stored at -20°C and then transferred to storage at -70°C on October 19, 2001. (Stability in plasma was demonstrated for 2.5 months at -20°C and for 5 months at -70°C in [redacted] 1133/1. Study [redacted] 1266/2 showed long-term stability for 321 days at -80°C . Thus, samples were analyzed within the period for which they have been demonstrated to be stable).

In 2 runs for HTBZ (runs 4 and 5) and for run 5 for TBZ in plasma both replicates of the low QC samples were outside of 15% of their nominal values. It does not appear that the samples in these runs were re-assayed, although according to the SOP, this QC problem would not be acceptable. It is stated that only data from accepted batches are reported.

The assays are acceptable in terms of performance. However, the interpretation of the results must be done with caution due to the integration of the 2 peaks representing "total HTBZ".

RESULTS:

Demographics

Twelve subjects were enrolled in the single dose stage of the study and 25 subjects were enrolled in the repeat dose stage of the study. One subject withdrew from the repeat dose stage of the study (reason was listed as consent withdrawn). Demographics of the subjects completing the study are shown in the table below.

Table 4. Demographics of Subjects Completing the Study

	Mean Age (Range)	Gender	Weight (mean \pm SD)	Race
Single dose study	33.7 (22-45)	3 males (25%)	69.2 \pm 12.11 kg (n=12)	11 Caucasian
		9 females (75%)	74.6 \pm 16.5 kg (male)	1 Mixed
			67.4 \pm 10.9 kg (female)	
Repeat dose study	33.9 (21-45)	10 males (42%)	71.7 \pm 12.2 kg (n=24)	23 Caucasian
		14 females (58%)	81.4 \pm 10.0 kg (male)	1 Mixed
			64.7 \pm 8.5 kg (female)	

Two subjects in the single dose study and 5 subjects on the repeat dose study took oral contraceptives. 6 subjects in the single dose group and 13 subjects in the repeat dose group were current cigarette smokers. Two subjects in the repeat dose study took evening primrose, a component of which interacts *in vitro* with CYP2C9.

Pharmacokinetics

Although TBZ was undetectable in most subjects following a single dose, TBZ was detectable in several subjects with the highest C_{max} of 0.48 ng/ml and 3.9 ng/ml after 12.5 mg and 50 mg, respectively. In the repeat dose portion of the study, the highest detectable TBZ C_{max} was 2.9 on Day 1 and 1.8 on Day 5.

Pharmacokinetic parameters for HTBZ were determined using noncompartmental analysis. The parameters shown in the table below, as calculated by the reviewer, are in general agreement with the results provided by the Sponsor. The plasma concentration time course and the pertinent pharmacokinetic parameters for HTBZ in plasma are shown in Figures 1 (single dose) and 2 (repeat dose) and Table 5, below. Trough concentration values for the last 3 days of dosing (Days 11, 12, and 13) in the repeat dose study suggest that steady state was achieved.

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Figure 1. Mean Plasma Concentration Time Course for HTBZ after administration of single doses of 12.5 mg and 50 mg TBZ

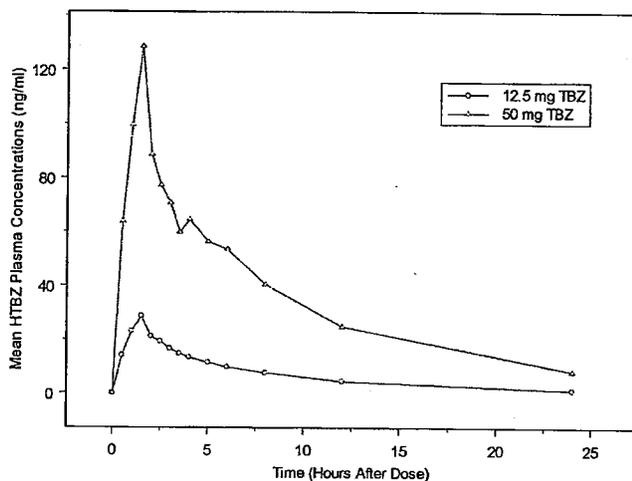
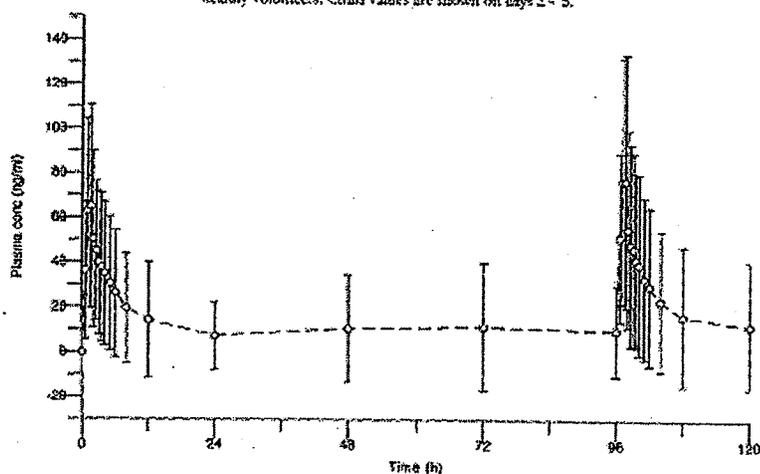


Figure 2. Mean Plasma Concentration Time Course for HTBZ after administration of single and multiple (once daily) doses of 25 mg TBZ (as provided by Sponsor)

Figure 4. Mean plasma concentration time profiles \pm SD of dihydro-tetrabenazine following repeated oral doses of 25 mg *q.d.* tetrabenazine to healthy volunteers. C_{trough} values are shown on days 2 - 5.



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Table 5. Pharmacokinetic parameters (arithmetic mean; %CV) for HTBZ (Study 1700411)

12.5 mg single dose (n=12)	t_{max} (h) ^a	1.25 (0.5-1.5)
	C_{max} (ng/mL)	33.13 (32)
	AUC _{0-t} (ng*h/mL)	170.6 (62)
	AUC _{0-∞} (ng*h/ml)	186.57 (71)
	λ_z (h ⁻¹)	0.1528 (36)
	t1/2 (h)	5.27 (44)
50 mg single dose (n=12)	t_{max} (h) ^a	1.5 (0.5-4)
	C_{max} (ng/mL)	143.00 (45)
	AUC _{0-t} (ng*h/mL)	848.06 (69)
	AUC _{0-∞} (ng*h/ml)	941.53 (77)
	λ_z (h ⁻¹)	0.1205 (24)
	t1/2 (h)	6.09 (27)
25 mg single dose (Day 1) (n=24)	t_{max} (h) ^a	1.00 (0.5-4)
	C_{max} (ng/mL)	78.94 (57)
	AUC _{0-t} (ng*h/mL)	348.6 (92)
	AUC _{0-∞} (ng*h/ml)	594.81 (196)
	λ_z (h ⁻¹)	0.1681 (33)
	t1/2 (h)	5.14 (82)
25 mg repeat dose (Day 5)	t_{max} (h) ^a	1.00 (0.5-1.5)
	C_{max} (ng/mL)	91.15 (63)
	AUC _{0-t} (ng*h/mL)	412.5 (102)
	λ_z (h ⁻¹)	0.1615 (36)
	t1/2 (h)	5.4 (69)
	C_{min} (ng/ml)	6.7 (255)
	C_{av} (ng/ml)	22.39 (131)
	Degree of Fluctuation (%)	562 (42)
Swing (%) ^b	32 (72)	

^a median (range)

^b calculated for 16 subjects in whom C_{min} was measurable.

Extensive variability was observed in the PK data, both in appearance of TBZ and exposure to HTBZ. The elimination half-life for HTBZ, although approximately 5-6 hours on average, was as long as approximately 34 hours in 1 subject.

Urine concentrations of TBZ were below the limit of quantification. For HTBZ the urinary excretion rate constant was used to calculate rate of excretion at the time of collection of the last urine sample and then to extrapolate the mass time curves to infinity (A_{einf}). The renal clearance was calculated from the ratio of the amount excreted in urine to infinity and the plasma AUC_{inf} . The results (averages based on results provided by Sponsor) are shown in the table below. The proportion of dose excreted was calculated from the amount of HTBZ excreted in moles compared to the molar dose of TBZ and was less than 15% for the maximum reported (mean was 2.7%, % CV was 99%).

NDA 21,894
Tetrabenzazine

	Cl _R (ml/min) Mean (%CV)
Single 12.5 mg dose	27.67 (45)
Single 50 mg dose	18.50 (31)
Single 25 mg dose	30.77 (64)
Repeat 25 mg dose	43.24 (89)

Dose Proportionality and Accumulation

C_{max} for HTBZ increased approximately 2.4-fold and 4.3-fold with a 2 fold- and a 4-fold increase in TBZ dose, respectively. AUC_{inf} increases more than dose proportionally such that there is an approximate 3-fold increase with a 2-fold increase in dose and a 5-fold increase with a 4-fold increase in dose.

With repeat dosing every 24 hours, there was an accumulation of approximately 1.15 fold for C_{max} and approximately 1.18 fold for AUC. Based on the half-life after a single dose, accumulation of 1.04-fold would have been expected.

Effect of Smoking on PK

Since 6/12 subjects in the single dose group and 13/24 subjects in the repeat dose group were smokers, the reviewer has evaluated PK parameters for HTBZ by smoking status. There is not a consistent effect of smoking on exposure to HTBZ.

		Smoker	Non-Smoker
		Arithmetic mean (CV)	Arithmetic mean (CV)
12.5 mg	C _{max} (ng/ml)	31.6 (38)	34.6 (28)
	AUC _{inf}	235 (70)	138 (54)
50 mg	C _{max} (ng/ml)	141 (59)	144 (32)
	AUC _{inf}	1247 (76)	635 (32)
25 mg single dose	C _{max} (ng/ml)	65.4 (49)	94.9(57)
	AUC _{inf}	257.8	993.06
25 mg repeat dose	C _{max} (ng/ml)	61.9 (43)	125.7 (52)
	AUC _t	236.7 (57.6)	620.2 (87.8)

Safety

There were no SAEs reported. There were 72 treatment-emergent AEs reported in 28 subjects. Nineteen AEs were considered to be possibly related and 28 AEs were considered to be probably related to the test product. The most commonly reported AEs (occurring in more than 1 subject) are shown in the table below according to dose. Asthenia and headache also occurred in 1 subject each in the 12.5 mg dose group, and headache also occurred in 1 patient in the 50 mg dose group.

NDA 21,894
Tetrabenazine

Dose	AE	# of subjects	Severity		Relationship To Test Product			
			Mild	Moderate	Probable	Possible	Unlikely	Not Related
12.5 mg (single)	Rhinitis	2	2				2	
50 mg (single)	Asthenia	3	1	2	2	1		
25 mg (repeat)	Asthenia	4		9	6	3		
	Headache	10	7	3	1	4	5	
	Nausea	3	3		2	1		
	Somnolence	13	7	10	14	3		

CONCLUSIONS:

TBZ was not detectable in plasma or urine in most subjects when TBZ was given at single doses of 12.5, 25, or 50 mg or in repeat doses of 25 mg given once daily. When present, concentrations of TBZ in plasma were less than 3 ng/ml.

The pharmacokinetics of HTBZ (determined using an achiral method) showed extensive variability with CV ranging from 32-63% for C_{max} and >62% for AUC. Elimination half-life of HTBZ was a mean of 5-6 hours.

C_{max} and AUC increased more than dose proportionately, and with repeated dosing, HTBZ appeared to accumulate to a slightly greater extent than would have been predicted.

There is not a consistent effect of smoking on exposure to HTBZ.

Headache and somnolence were the most commonly reported adverse events.

These results can generally be applied to an understanding of TBZ PK. However, since the enantiomers of HTBZ cannot be distinguished, it does not allow for characterizing the PK of TBZ.

NDA 21,894
Tetrabenazine

4.2.12 BIOEQUIVALENCE STUDY

AN OPEN-LABEL, RANDOMIZED, TWO-TREATMENT, TWO-SEQUENCE, TWO-PERIOD STUDY OF THE BIOEQUIVALENCE OF 12.5 AND 25 MG TETRABENAZINE TABLETS IN HEALTHY ADULT VOLUNTEERS UNDER FASTED CONDITIONS

Study Investigators and Site:

/ / / /

Protocol Number: TBZ 104,012

OBJECTIVES:

The primary objective was to determine the bioequivalence of tetrabenazine 12.5 mg and 25 mg tablets using a single-dose, randomized, two-treatment, two-period, two-sequence crossover design.

The secondary objective was to compare the pharmacokinetic characteristics of tetrabenazine (TBZ) and its two isomeric metabolites, α - and β -dihyrotetrabenazine (HTBZ), in men and women.

FORMULATIONS:

Table 1. Products used in TBZ 104,012

	Tablet Description	Batch No.	Exp. Date (Dates of Study)
Tetrabenazine 12.5 mg tablet	Flat, white tablets	6573804	12/31/04 (5/19/04-6/18/04)
Tetrabenazine 25 mg tablet	Flat, yellowish-buff tablets	1MM	5/07 (5/19/04-6/18/04)

The batch size for each tablet was _____ tablets weighing 125 mg each).

STUDY DESIGN:

This study was an open-label, randomized, 2-period, 2-treatment, 2-sequence crossover study of the bioequivalence of 12.5 mg and 25 mg tetrabenazine tables when administered in fasting state to healthy volunteers. Subjects randomized to Sequence 1 were to receive one 25 mg tablet of tetrabenazine orally on Day 1 of the first dosing period and two 12.5 mg tables of tetrabenazine orally on Day 8 of the second dosing period. Subjects randomized to sequence 2 were to receive treatments in the opposite order, as shown in the table below. All subjects were to be fasted for a minimum of 10 hours prior to study drug dosing on Days 1 and 8. There was a minimum 7 day washout period between Periods 1 and 2.

Table 2. Treatment Sequence in TBZ 104,012

Sequence Number	Dosing Period	
	1	2
1	A	B
2	B	A

A=1x25 mg tetrabenazine tablet
B=2x12.5 mg tetrabenazine tables

Inclusion criteria included healthy males or females, between the ages of 18 and 45 years of age (inclusive). Females had to be on adequate contraception that could include oral contraceptives. Exclusion criteria included "abnormal diet" during the 4 weeks preceding the study treatment with any known enzyme altering drugs within 30 days prior to or during the study, smoking or use of tobacco products within 6 months prior to or during the study. Subjects could not take OTC products including vitamins for the 7 days preceding the study or any prescription medications (except hormonal contraceptives) for the 14 days preceding the study. Acetaminophen and OTC antacids could be used during the washout period between doses (Days 2 through 7). Consumption of alcohol or xanthine-containing beverages and foods was prohibited for 48 hours before dosing and throughout the period of sample collection.

A standardized meal was served on the evening of check-in (Days 0 and 7) at 9PM which had to be consumed by 10 PM. Subjects then fasted overnight for at least 10 hours prior to dosing. At 8AM (\pm 30 minutes) on the morning of Days 1 and 8, all subjects were given their assigned dose of tetrabenazine with 240 ml of room temperature water. Subjects were not permitted to lie down for the first 4 hours after study drug. Standardized, caffeine-free meals were provided during the course of confinement and were identical for each dosing period. On Days 1 and 8, meals were provided approximately 4 and 9 hours after dosing. Ad lib consumption of water was allowed except for 1 hour prior to dosing and 1 hour immediately following study drug administration. Blood samples were collected at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 16, and 24 hours after study drug administration to determine plasma concentrations of TBZ, α -HTBZ, and β -HTBZ. Plasma was stored at -20° C or lower prior to shipment to --- for analysis.

Safety monitoring included vital signs at 1.75 hours before and 4, 8, and 24 hours post-dosing on Days 1 and 8. Twelve-lead ECGs were performed at screening and at 1.75 hours before and 1.75 hours after study drug administration (the estimated time to C_{max} for TBZ, according to the Sponsor) on Days 1 and 8.

ASSAY:

Plasma concentrations were measured using a validated LC/MS/MS method (1266/1).

Table 3. Performance of Analytical Method for TBZ 104,012

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.2-200 ng/ml	r > 0.990	0.2	0.5	7.4	0.5
					25.0	5.0	2.7
					100.0	5.2	-3.8
α-HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.988	0.5	1.0	7.6	0.9
					25.0	7.5	1.8
					100.0	6.7	-2.2
β-HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.985	0.5	1.0	8.1	1.0
					25.0	7.4	3.0
					100.0	6.9	-3.1

Two calibration curves and duplicate QC samples were analyzed with each batch of study samples. Study samples were stored at -70 to -90° C. Samples were analyzed within the 321 day period for which the samples are stable at -70° C. The performance of the assays for all analytes is considered acceptable.

RESULTS:

Demographics

Twenty-eight healthy volunteers (14 males and 14 females) were enrolled in the study. Twenty-three subjects completed the study. Five subjects were withdrawn: 3 had positive urine drug screens prior to Period 2 dosing, one withdrew for personal reasons during Period 2 check-in, and one withdrew because of headache prior to Period 2 dosing. Demographics of subjects completing the study are shown in the table below.

Table 4. Demographics of Subjects Completing the Study

Mean Age (Range)	Gender	Weight (mean ± SD)	Race
27 (18-44)	11 males	70 ± 12 kg (n=23)	Asian 1
	12 females	73 ± 13 kg (male)	Black 15
		68 ± 10 kg (female)	Hispanic 7

One female subject (#1247127) used the contraceptive medroxyprogesterone acetate (150 mg IM q 13 weeks). That was the only concomitant medication in subjects completing the study.

Pharmacokinetics

Pharmacokinetic parameters were determined using noncompartmental analysis.

TBZ concentrations were less than the LOQ for the majority of sampling times in most subjects. However, TBZ plasma concentrations were detectable in both study periods in several subjects including subjects 1247111 (Asian), 1247129 (Black), and 1247130 (Hispanic) in whom C_{max} stood out from the remainder of the study subjects. In these subjects C_{max} and T_{max} for each study period are shown below. The mean AUC_{inf} in those subjects was calculated to be approximately 5 ng*hr/ml. In the other 20 subjects, C_{max} when detectable was less than 1.2 ng/ml.

Table 5. Pharmacokinetics of TBZ in selected subjects from Study 104012

25 mg tablet				2x12.5 mg tablet		
Subject	C _{max} (ng/ml)	T _{max} (hours)	# Detectable Time points between 0 and 6 hours	C _{max} (ng/ml)	T _{max} (hours)	# Detectable Time points between 0 and 6 hours
111	3.16	0.5	10	2.91	0.5	9
129	5.94	1.5	9	2.44	1	6
130	5.19	0.5	8	8.11	0.5	9

The plasma concentration time course for α-HTBZ and β-HTBZ are shown in Figures 1 and 2 below, as provided by the Sponsor. The pertinent pharmacokinetic parameters and the bioequivalence assessment for the 1x25 mg and 2x12.5 mg tablets are shown in Tables 6 and 7, respectively, as calculated by the reviewer. The values are generally in agreement with those reported by the Sponsor, with the exception of AUC_{inf} for which the Sponsor reported values that were lower than the AUC_{0-t} values.

Figure 1. Mean Plasma Concentration Time Course for α-HTBZ after oral administration of 25 mg TBZ in study 104012

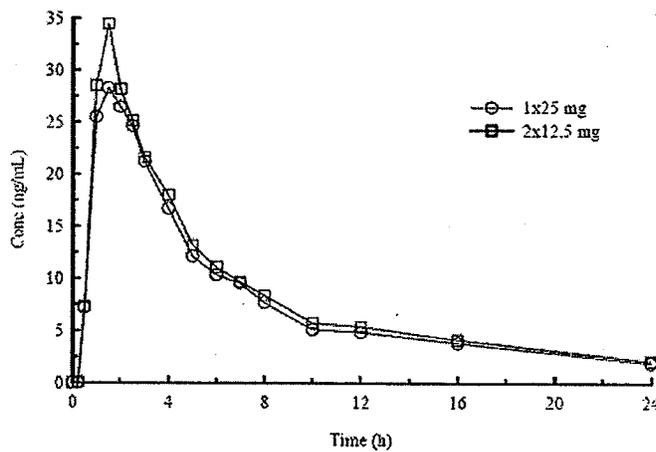


Figure 2. Mean Plasma Concentration Time Course for β -HTBZ after oral administration of 25 mg TBZ in study 104012

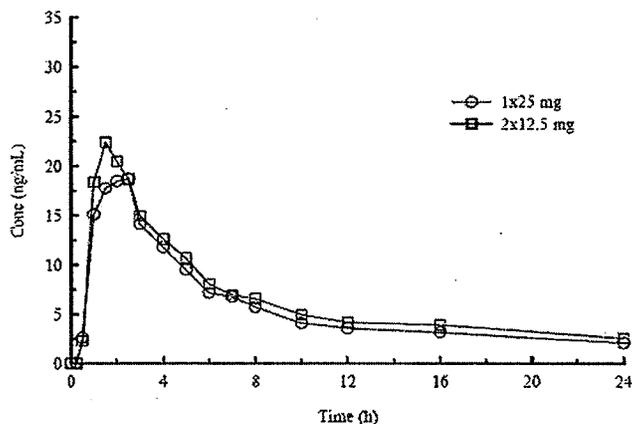


Figure 4. Mean plasma concentrations of β -HTBZ after oral administration of a 25 mg dose of tetrabenazine as either a 1 \times 25 mg tablet or 2 \times 12.5 mg tablets under fasting conditions to healthy volunteers — linear axes — Protocol TBZ 104,012

Table 5. Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ (Study TBZ104,012)

	Test (2x12.5 mg tablet) TREATMENT B (% CV) n=23	Reference (1x25mg tablet) TREATMENT A (% CV) n=23
α-HTBZ		
t_{max} (h) ^a	1.5 (1.0-2.5)	1.50 (1.00-3.00)
C_{max} (ng/mL)	36.7 (45)	33.1 (44)
AUC_{0-t} (ng*h/mL)	202.8 (76)	187 (71)
$AUC_{0-\infty}$ (ng*h/mL)	235 (93)	219.9 (87)
λ_z (hr ⁻¹)	0.120 (36)	0.113 (48)
$t_{1/2}$ (h)	6.5 (40)	7.5 (47)
β-HTBZ		
t_{max} (h) ^a	1.5 (1.0-2.5)	1.5 (1.0-2.5)
C_{max} (ng/mL)	24.4 (72)	22.3 (73)
AUC_{0-t} (ng*h/mL)	155.5 (151)	136.8 (142)
$AUC_{0-\infty}$ (ng*h/mL)	226.1 (186)	189.9 (173)
λ_z (hr ⁻¹)	0.232 (50)	0.238 (54)
$t_{1/2}$ (h)	5.1 (106)	5.2 (105)

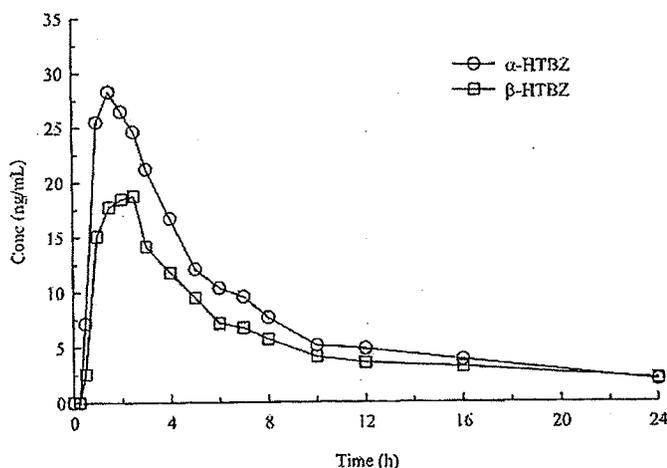
^a median (range)

Table 6. Bioequivalence Assessment for Study TBZ104,012

	Geometric Mean		Ratio of Geometric Means	90% CI for the Ratio of Geometric Means
	Treatment A	Treatment B		
α-HTBZ				
C_{max} (ng/ml)	30.1	33.9	112.32	(1.02, 1.24)
AUC_{0-t} (ng*h/ml)	155.4	166.9	107.32	(1.03, 1.12)
$AUC_{0-\infty}$ (ng*h/mL) ^b	171.1	180.3	105.28	(1.01, 1.10)
β-HTBZ				
C_{max} (ng/ml)	17.4	19.3	110.84	(1.00, 1.23)
AUC_{0-t} (ng*h/ml)	74.5	80.8	108.65	(1.02, 1.16)
$AUC_{0-\infty}$ (ng*h/mL) ^b	83.4	89.3	107.09	(1.00, 1.14)

Bioequivalence was demonstrated between 2x12.5mg tablets and one 25mg tablet.

Stereoselectivity in the pharmacokinetics of the HTBZ enantiomers was observed, with exposure to α -HTBZ greater than exposure to β -HTBZ. As an example, the mean plasma concentration time course comparing the 2 enantiomers following administration of a single 1x25 mg tablet is shown below, as provided by the Sponsor.



The mean C_{max} for α -HTBZ was approximately 1.5x greater than mean C_{max} for β -HTBZ. The mean AUC_{0-t} was approximately 1.3 x greater for α -HTBZ than for β -HTBZ, although AUC_{inf} was similar for both enantiomers. The elimination rate constant was approximately 2-fold greater for β -HTBZ than for α -HTBZ. The elimination half-life for α -HTBZ was approximately 1.3-1.4x longer than for β -HTBZ. Variability in the stereoselectivity was observed with a ratio for α -HTBZ to β -HTBZ of up to approximately 6 in C_{max} as well as AUC , although in several subjects the ratios were close to 1 or in some cases, exposure to β -HTBZ was greater.

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Tetrabenazine

Gender

The effect of gender on pharmacokinetics was evaluated. The means by gender for selected PK parameters are shown in the table below. PK parameters were similar for men and women for T_{max} and C_{max}. The AUC for β-HTBZ was approximately 1.5 fold greater in women than in men, and the half-life was slightly longer for β-HTBZ in women, although the variability was large for both parameters.

	Male (n=11)		Female (n=12)	
	Test (2x12.5 mg tablet) TREATMENT B (% CV)	Reference (1x25mg tablet) TREATMENT A (% CV)	Test (2x12.5 mg tablet) TREATMENT B (% CV)	Reference (1x25mg tablet) TREATMENT A (% CV)
α-HTBZ				
t _{max} (h) ^a	1.5 (1.0-2.5)	2.0 (1.0-2.5)	1.5 (1.0-1.5)	1.25 (1.0-3.0)
C _{max} (ng/mL)	33.3 (27)	31.4 (34)	39.9 (53)	34.6 (51)
AUC _{0-∞} (ng*h/mL)	218.88 (62)	211.52 (61)	249.79 (112)	227.6 (107)
t _{1/2} (h)	6.3 (32)	7.5 (54)	6.9 (46)	7.6 (43)
β-HTBZ				
t _{max} (h) ^a	1.5 (1.0-2.5)	1.5 (1.0-2.5)	1.5 (1.0-2.5)	1.5 (1.0-2.5)
C _{max} (ng/mL)	21.7 (46)	21.6 (52)	27.0 (84)	22.9 (89)
AUC _{0-∞} (ng*h/mL)	177.59 (164)	153.3 (146)	270.58 (193)	223.5 (184)
t _{1/2} (h)	4.7 (100)	4.2 (102)	5.5 (112)	6.1 (106)

Safety

Seventeen adverse events (AEs) occurred in 10 subjects. None was considered to be serious or clinically significant and all were assessed as mild in severity. AEs occurring in more than 1 subject were comparable across treatment groups. Six AEs were judged to have a probably relationship to TBZ (1 dizziness, drowsiness/somnolence) and 2 were judged to have a possible relationship to study drug (headache, nausea). Somnolence (29.4%) and headache (17.7%) were the most common adverse events and the only AEs reported in more than 1 subject. The distribution of AEs by gender was comparable.

There were 5 episodes of transient orthostatic hypotension (four at 4 hours after dosing and 1 at 8 hours after dosing; supine to standing). Subjects were not reported to be symptomatic.

Twelve-lead ECGs at pre-dose and at 1.7 hours post-dose were evaluated with respect to QT interval. The analysis was performed blinded by a consultant cardiologist : _____ MD). The maximum pre-dose QTcF was 446 msec and the maximum post-dose QTcF was 439 msec. The maximum change in QTcF was 28 msec. The mean change in QTcF was -0.78 msec in Period 1 and -1.15 msec in period 2.

CONCLUSIONS:

1. Dosage strength equivalence was shown for the 2x12.5 mg vs 1x25 mg TBZ tablets on the basis of circulating metabolites α -and β -HTBZ.
2. TBZ was detectable in the plasma of several individuals in concentrations up to approximately 8 ng/ml, with AUC_{inf} calculated from those individuals being approximately 5 ng*hr/ml.
3. Exposure (AUC) to β -HTBZ was slightly greater (1.5x) in women than in men, with slightly longer half-life, although variability was large.
4. This population may not be representative demographically of the population that will use TBZ in that there were no Caucasians (in efficacy study 103,004 the population was 93% Caucasian).

**APPEARS THIS WAY
ON ORIGINAL**

NDA 21,894
Tetrabenazine

4.2.13 FOOD EFFECT STUDY

AN OPEN-LABEL, RANDOMIZED, TWO-TREATMENT, TWO-SEQUENCE, TWO-PERIOD STUDY OF THE EFFECT OF FOOD ON THE ABSORPTION OF TETRABENAZINE IN HEALTHY ADULT VOLUNTEERS

Study Investigators and Site:

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Protocol Number: TBZ 103,003

OBJECTIVES:

The primary objective was to determine the effect of a high-fat, high calorie meal on the relative bioavailability of a 25 mg orally tablet of tetrabenazine in healthy, adult volunteers.

The secondary objectives were 1) to compare the pharmacokinetic characteristics of tetrabenazine in men and women and 2) to evaluate the tolerability and safety of two, single 25 mg oral doses of tetrabenazine administered to healthy adult volunteers.

FORMULATION:

Table 1. Product used in TBZ,103,003

	Tablet Description	Batch No.	Exp. Date (Dates of Study)
Tetrabenazine 25 mg tablet	Flat, yellowish-buff tablets	1MM	5/07 (6/13/03-6/22/03)

STUDY DESIGN:

This study was an open-label, randomized, 2-period, 2-treatment (fasted and fed), 2-sequence crossover study of the effect of a high-fat, high-calorie meal on the relative bioavailability of a 25 mg tablet oral dose of tetrabenazine administered to healthy volunteers. Subjects randomized to Sequence 1 were to receive one 25 mg tablet of tetrabenazine orally at the start of each dosing period and would be evaluated under fed conditions during Period 1 and under fasted conditions during Period 2. Subjects randomized to Sequence 2 would have the fed and fasted conditions in the opposite order, as shown in the table below. There was a minimum 7 day washout period between Periods 1 and 2.

Table 2. Treatment Sequence in TBZ 103,003

Sequence Number	Dosing Period	
	1	2
1	A	B
2	B	A

A=fed

B=fasted

Inclusion criteria included healthy males or females, between the ages of 18 and 45 years of age (inclusive). Females had to be on adequate contraception that could include oral contraceptives. Exclusion criteria included "abnormal diet" during the 4 weeks preceding the study, treatment with any known enzyme altering drugs within 30 days prior to or during the study, smoking or use of tobacco products within 6 months prior to or during the study. Subjects could not take OTC products including vitamins for the 7 days preceding the study or any prescription medications (except hormonal contraceptives) for the 14 days preceding the study. Acetaminophen and OTC antacids could be used during the washout period between doses (Days 2 through 7). Consumption of alcohol or xanthine-containing beverages and foods was prohibited for 48 hours before dosing and throughout the period of sample collection.

A meal was served on the evening of check-in (Day 0 and Day 7). All subjects were then required to fast overnight for at least 10 hours prior to dosing. On the morning of Day 1 and Day 8, subjects scheduled to be given study drug under fed conditions were given a standard high-fat (50% of total caloric content of the meal), high-calorie (approximately 1000 calories) breakfast consisting of 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 4 ounces of hash brown potatoes, and 8 ounces of whole milk. The meal was to be consumed no later than ½ hour prior to the 8AM dosing time. Subjects who were to receive study drug under fasted conditions did not receive a meal prior to study drug dosing.

Subjects received study drug on the mornings of Day 1 and Day 8 with 240 ml of water at room temperature. Standard caffeine-free meals were provided to all subjects approximately 4 and 9 hours after study drug administration. Ad lib water was allowed except for 1 hour immediately prior to dosing and for 1 hour immediately following dosing. Blood samples were collected pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 16, and 24 hours after study drug administration to determine plasma concentrations of TBZ, α -HTBZ, and β -HTBZ. Plasma was stored at -20° C or lower until shipped to — for analysis.

Safety monitoring included vital signs in the seated position, 12-lead ECG, laboratory values, and physical exam pre-dose and at the end of the study.

ASSAY:

Plasma concentrations were measured using a validated LC/MS/MS method (1266/1).

Table 3. Performance of Analytical Method for TBZ 103,003

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.2-200 ng/ml	r > 0.995	0.2	0.5	9.9	0.1
					25.0	4.9	-1.1
					100.0	5.8	-0.8
α-HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.995	0.5	1.0	6.3	3.6
					25.0	6.0	1.1
					100.0	4.8	0.0
β-HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.993	0.5	1.0	5.9	-1.9
					25.0	6.7	0.1
					100.0	5.6	-3.3

Two calibration curves and duplicate QC samples were analyzed with each batch of study samples (except for 1 run that had analysis of only 1 sample (re-injection)). Study samples were stored at -70° C. Samples were analyzed within approximately 1 month, within the period for which the samples are stable at -70° C. The performance of the assays for all analytes is considered acceptable.

RESULTS:

Demographics

Twenty-eight healthy volunteers (14 males and 14 females) were enrolled in the study. Twenty-five subjects completed the study. Three female subjects who completed Period 1 were withdrawn prior to Period 2: 1 had a presumed positive drug screen, one withdrew for personal reasons, and 1 had a positive alcohol screen. These subjects were not included in the pharmacokinetic analysis. Demographics of subjects completing the study are shown in the table below.

Table 4. Demographics of Subjects Completing the Study

Mean Age (Range)	Gender	Weight (mean ± SD)	Race
25 (19-43)	14 males	71 ± 9 kg (n=25)	Caucasian 3
	11 females	76 ± 9 kg (male)	Black 13
		65 ± 5 kg (female)	Hispanic 9

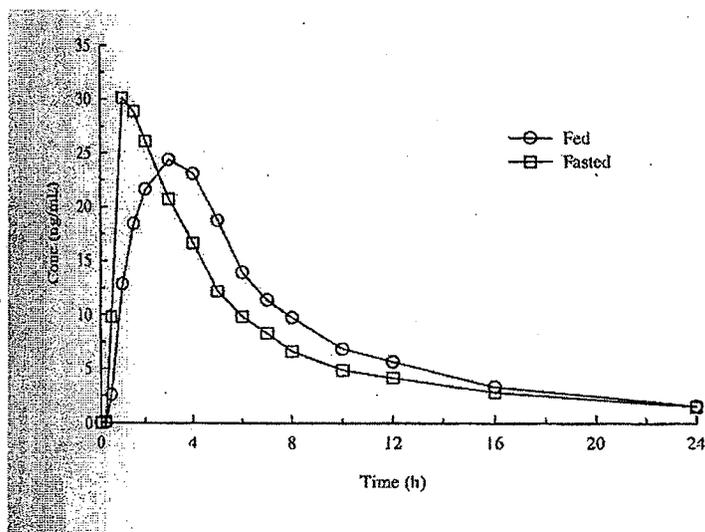
Three female subjects used hormonal contraceptives. There was no other use of concomitant medications.

Pharmacokinetics

TBZ concentrations were less than the LOQ for the majority of sampling times in most subjects. However, TBZ plasma concentrations were detectable in both study periods in several subjects including subjects 34704 (Black) who had C_{max} values of 1.08 ng/ml and 1.54 ng/ml in the fed and fasted periods, respectively and subject 34718 (Caucasian) who had a C_{max} of 1.23 ng/ml in the fasted period. In the remainder of the subjects, C_{max} when detectable was less than 1.0 ng/ml.

The plasma concentration time course and the pertinent pharmacokinetic parameters for for α -HTBZ and β -HTBZ are shown in Figures 1 and 2 (as provided by the Sponsor) and Table 5 (as calculated by the reviewer). A bioequivalence comparison for values with and without food is shown in Table 6, below. The results are generally in agreement with those determined by the Sponsor. Pharmacokinetic parameters were determined using noncompartmental analysis.

Figure 1. Mean Plasma Concentration Time Course for α -HTBZ After Oral Administration of 25 mg under Fed and Fasting Conditions to Healthy Volunteers (as provided by Sponsor)



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Tetrabenazine

Figure 2. Mean Plasma Concentration Time Course for β -HTBZ After Oral Administration of 25 mg under Fed and Fasting Conditions to Healthy Volunteers (as provided by Sponsor)

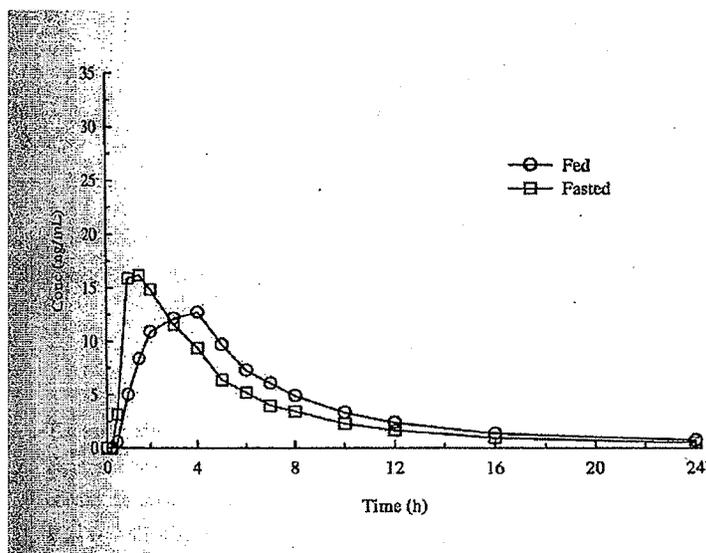


Table 5. Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ (Study TBZ 103,003)

	Fed TREATMENT A (% CV) n=25	Fasted TREATMENT B (% CV) n=25
α-HTBZ		
t_{max} (h) ^a	2.0 (1.0-5.0)	1.0 (1.0-4.0)
C_{max} (ng/mL)	30.6 (33)	32.2 (40)
AUC ₀₋₁ (ng*h/mL)	196.2 (42)	175.9 (43)
AUC _{0-∞} (ng*h/mL)	214.5 (49)	199.3 (47)
λz (hr ⁻¹)	0.123 (30)	0.101 (38)
$t_{1/2}$ (h)	6.1 (29)	8.1 (51)
β-HTBZ		
t_{max} (h) ^a	2.0 (1.0-5.0)	1.5 (1.0-4.0)
C_{max} (ng/mL)	17.1 (71)	17.8 (79)
AUC ₀₋₁ (ng*h/mL)	95.5 (118)	87.6 (118)
AUC _{0-∞} (ng*h/mL)	105.4 (136)	95.8 (133)
λz (hr ⁻¹)	0.206 (34)	0.259 (46)
$t_{1/2}$ (h)	3.9 (48)	3.6 (69)

^a median (range)

Table 6. Bioequivalence Assessment for Study TBZ103,003

	Geometric Mean		Ratio of Geometric Means	90% CI for the Ratio of Geometric Means
	Treatment A	Treatment B		
α-HTBZ				
C _{max} (ng/ml)	29.1	29.6	98.8	(0.86, 1.13)
AUC _{0-t} (ng*h/ml)	179.7	161.4	111.7	(1.04, 1.20)
AUC _{0-∞} (ng*h/mL)	192.6	180.2	107.2	(1.01, 1.15)
β-HTBZ				
C _{max} (ng/ml)	14.3	14.4	99.9	(0.83,1.20)
AUC _{0-t} (ng*h/ml)	66.8	62.0	108.3	(0.95, 1.24)
AUC _{0-∞} (ng*h/mL)	70.87	65.7	108.4	(0.96, 1.23)

Food effect – Although the mean C_{max} was slightly lower, AUC was slightly higher, and median t_{max} slightly later when TBZ was given with food, these results show no statistically significant effect of a high-fat, high calorie meal on C_{max} or AUC of tetrabenazine metabolites α -HTBZ and β -HTBZ.

Stereoselectivity in the pharmacokinetics of the HTBZ enantiomers was observed, with exposure to α -HTBZ greater than exposure to β -HTBZ. The mean C_{max} for α -HTBZ was approximately 1.8x greater than mean C_{max} for β -HTBZ under either fed or fasted conditions and mean AUC was approximately 2x greater for α -HTBZ than for β -HTBZ under either fed or fasted conditions. The elimination rate constant was approximately 2-fold greater for β -HTBZ than for α -HTBZ. Variability in the stereoselectivity was observed with a ratio for α -HTBZ to β -HTBZ of up to approximately 5 in C_{max} and approximately 8 in AUC.

The effect of gender on pharmacokinetics was evaluated. The means by gender are shown in the table below. The differences by gender for the α -metabolite were less than 20%. For the β -metabolite, C_{max} and AUC were approximately 20-50% greater for females than for males, although substantial variability was observed, especially in the females.

Table 7. Pharmacokinetic parameters (arithmetic mean, %CV) for α -HTBZ and β -HTBZ by Gender (Study TBZ 103,003)

	Fed TREATMENT A		Fasted TREATMENT B	
	Female	Male	Female	Male
α-HTBZ				
C _{max} (ng/mL)	31.8 (35)	29.7(32)	35.3 (42)	29.7 (38)
AUC _{0-t} (ng*h/mL)	207.3 (52)	188.1 (33)	186.7 (53)	166.7 (34)
AUC _{0-∞} (ng*h/mL)	235.9 (58)	199.1 (36)	219.8 (58)	177.6 (38)
t _{1/2} (h)	6.73 (32)	5.39 (26)	7.81 (37)	6.06 (29)
β-HTBZ				
C _{max} (ng/mL)	18.9 (83)	15.6 (56)	21.8 (83)	14.7 (62)
AUC _{0-t} (ng*h/mL)	115.9 (142)	77.6 (63)	107.9 (136)	69.1 (75)
AUC _{0-∞} (ng*h/mL)	144.3 (149)	77.6 (66)	143.7 (138)	72.5 (77)
t _{1/2} (h)	4.00 (58)	3.12 (37)	4.11 (70)	2.90 (43)

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Safety

The Sponsor states that there were no deaths, SAEs, or clinically significant AEs reported during the course of the study. Five AEs occurred in four subjects. These AEs included itching and rash that were considered mild and unrelated to study drug. One patient developed a headache that was reported 47 hours after oral administration of study drug and resolved spontaneously. This was considered unrelated to study drug. Two subjects developed drowsiness considered as possibly related to study drug.

One subject had abnormal elevation of ALT and AST (54 and 73 IU/L, respectively; normal range considered to be 0-40) that persisted on repeat test 11 days after study conclusion (73 and 71 IU/L, respectively). One subject had abnormal platelets but did not return for follow-up. There were no changes in vital signs that were considered to be clinically significant and related to study drug.

CONCLUSIONS:

There was no significant effect of food on the pharmacokinetics of α -HTBZ or β -HTBZ after administration of 25 mg tetrabenazine in healthy subjects. TBZ was not detectable in most subjects.

With respect to pharmacokinetics of TBZ in general, there is some evidence for stereoselective pharmacokinetics, with lower exposure to the β -HTBZ. There is a small difference in pharmacokinetics related to gender. However, this is primarily for the β -HTBZ and the variability was large, especially in women.

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4.2.14 SINGLE DOSE PROLACTIN PD STUDY

EFFECT OF A SINGLE ADMINISTRATION OF TETRABENAZINE ON PROLACTIN BLOOD LEVELS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSS-OVER STUDY IN HEALTHY VOLUNTEERS

Study Investigators and Site:

/ /

Protocol Number: TBZ 202,001

OBJECTIVES:

The primary objective was to determine the effect of a single 12.5 mg oral dose of TBZ on the serum prolactin concentration in healthy, adult, male volunteers.

The secondary objectives were 1) to determine serum concentration of TBZ and its metabolites α - and β -HTBZ, after oral administration of a single 12.5 mg dose of TBZ to healthy adult, male volunteers and 2) to evaluate the tolerability and safety of a single 12.5 mg dose of TBZ administered to healthy male volunteers.

FORMULATION:

Table 1. Product used in TBZ 202,001

	Batch No.	Exp. Date (Dates of Study)
Tetrabenazine 12.5 mg tablet	6573804	6/30/2003 (3/11/03-4/9/03)

STUDY DESIGN:

This study was a single center, randomized, placebo-controlled, cross-over study. Eligible subjects were randomized to one of 2 sequence groups on or before Day-1 (D-1) as shown in the table below.

Table 2. Treatment Sequence in TBZ 202,001

Sequence Number	Dosing Period	
	1	2
1	TBZ (12.5 mg oral dose)	Matching Placebo
2	Matching Placebo	TBZ (12.5 mg oral dose)

Eligible subjects were admitted to the clinical research unit at 8AM on D-1 of each dosing period, after having fasted beginning at 9PM on the prior evening. On the morning of Day 1

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(D1), when subjects were in a fasting state, blood samples were taken from each subject at approximately 7AM and 7:30 AM to be used in determining two baseline serum prolactin concentrations. In addition, subjects had a 12-lead ECG and vital signs. Subjects were then administered study drug (either TBZ or placebo) orally at 8AM with 200 ml water. Subjects were not allowed any food intake for at least 4 hours post-administration. Additional blood samples were drawn to determine prolactin, TBZ, α - and β -HTBZ at 1 hour, 1.5 hours, 4 hours, 10 hours, and 24 hours post-dose. Safety was monitored throughout the study. There was a minimum 7-day washout separating Period 1 and Period 2.

Inclusion criteria included healthy males, between the ages of 18 and 45 years of age (inclusive), with baseline prolactin no greater than 25 ng/ml. (Women were excluded to minimize the confounding effect of the cyclic nature of serum prolactin measurements). Subjects were not to consume alcohol, tobacco, grapefruits or grapefruit juice, quinine-containing products or xanthine-containing food or beverages during confinement in the study center (Day-1 through the morning of Day 2 of each period). With the exception of acetaminophen, no concomitant medications were allowed. Exclusion criteria included intake of more than 40 g/day of alcohol during the study, smoking more than 10 cigarettes per day, excessive intake of coffee, tea, or chocolate (more than 6 cups per day on average) and those with excessive intake of caffeine-containing beverages (more than 6 glasses per day), intake of any medication within 5 half-lives of that drug prior to study drug intake, or the existence of any surgical or medical condition which, in the judgment of the investigator, might interfere with the absorption, distribution, metabolism, or excretion of the study drug.

Safety monitoring included adverse events, vital signs, laboratory values, 12-lead ECG, and physical exam.

ASSAY:

Plasma concentrations were measured for TBZ, α - HTBZ, and β -HTBZ using a validated LC/MS/MS method — 1266/1).

Table 3. Performance of Analytical Method for TBZ 202,001

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.2-200 ng/ml	r > 0.997	0.2	0.5	8.8	0.2
					25.0	2.2	-1.88
					100.0	2.1	2.00
α - HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.996	0.5	1.0	7.5	2.8
					25.0	4.1	-3.5
					100.0	1.2	0.0
β -HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.994	0.5	1.0	9.7	-1.0
					25.0	4.3	0.2
					100.0	5.4	-2.1

Two calibration curves and duplicate QC samples were analyzed with each batch of study samples. Study samples were stored nominally at -80° C. Samples were analyzed (between June 18th and June 28th 2003) within the 321 day period for which the samples are stable at -80° C. The performance of the assays for all analytes is considered acceptable.

Serum prolactin concentrations were determined by the _____ . The analytical method was an immunometric technique involving a luminescent reaction produced by prolactin-sheep antibody complex. The kit is a product of _____ , and the Sponsor provided a copy of the package insert. A normal female reference interval is 64-395 mIU/L (approximately 3-19 ng/ml) and a normal male reference interval is 78-380 mIU/L (approximately 3.7-18 ng/ml). The sensitivity is 30 mIU/L (approximately 1.4 ng/ml). It has been characterized with respect to accuracy, precision, and specificity as well. (Results in ng/ml = (result in mIU/L) x0.047).

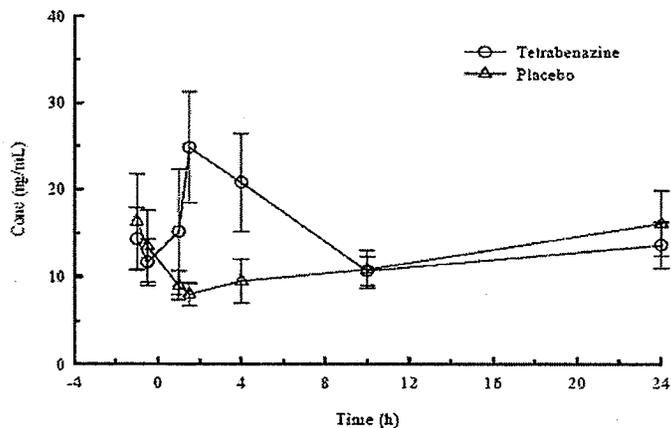
RESULTS:

Demographics

Six healthy, male volunteers were enrolled and completed the study. The mean age was 31 years and the range was 22-36 years. The mean weight (SD) was 78 ± 7 kg. Race was not provided and the Sponsor states elsewhere in the submission that data on race are not collected in France. Two of the six subjects were tobacco users. There was no use of concomitant medications.

Pharmacodynamics

Serum prolactin determination was based on samples from the second baseline prior to drug administration, on Day 1. Serum prolactin concentrations at each sampling time were compared between TBZ and placebo using ANOVA. The prolactin concentration time course results are shown in the figure below.



Mean \pm standard deviation serum prolactin concentrations after administration of a single 12.5 mg dose of tetrabenazine or placebo to healthy male volunteers – Protocol TBZ 202,001

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The maximum mean (SD) serum prolactin concentration was 24.73 (6.4) ng/ml and was at 1.5 hours after the dose of TBZ. At 1, 1.5, and 4 hours after the dose, the serum prolactin concentrations were significantly higher in the TBZ-treated group compared with the placebo group ($p < 0.05$).

Pharmacokinetics

TBZ concentrations were less than the LOQ in all subjects at all time points.

The plasma concentration time course for α - and β -HTBZ and the summary of PK parameters are shown in the figures and tables below, as provided by the Sponsor. There was an approximate 1.4-fold greater exposure to α -HTBZ than to β -HTBZ. The PK results are in agreement with the PK results seen in other studies submitted to this NDA.

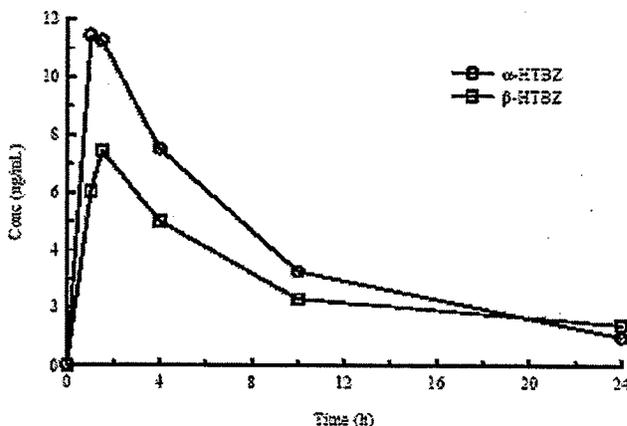


Table 4. Summary of parameters α - and β -HTBZ after oral administration of a single 12.5 mg dose of TBZ in Study TBZ 202,001. **Figure 2.** Mean plasma concentrations of α - and β -HTBZ after oral administration of a single 12.5 mg dose of tetrabenazine to healthy volunteers – linear - Protocol TBZ 202,001. **PK for**

Parameter*	α -HTBZ	β -HTBZ
C_{max} (ng/mL)	12.7 \pm 1.99	8.15 \pm 5.28
T_{max} (h)	1.25	1.25
AUC_{0-t} (h*ng/mL)	91.8 \pm 56.5	66.8 \pm 90.4

Source: Appendix 16.2.6.5

AUC_{0-t} = area under curve up to the last measured point; C_{max} = maximum concentration; α -HTBZ = alpha-dihydratetrabenazine; β -HTBZ = beta-dihydratetrabenazine; T_{max} = time to maximum concentration.

* Arithmetic mean \pm standard deviation except for T_{max} for which the median is reported.

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PK/PD

The relationships between plasma concentrations of α - and β -HTBZ and serum prolactin concentrations are shown in the figures below, as provided by the Sponsor. There is a positive correlation between change in serum prolactin concentrations and plasma concentrations of α - and β -HTBZ.

Figure 3. Relationship between the difference in serum concentrations of prolactin, between TBZ and placebo, and plasma concentrations of α -HTBZ after administration of a single dose of 12.5 mg TBZ.

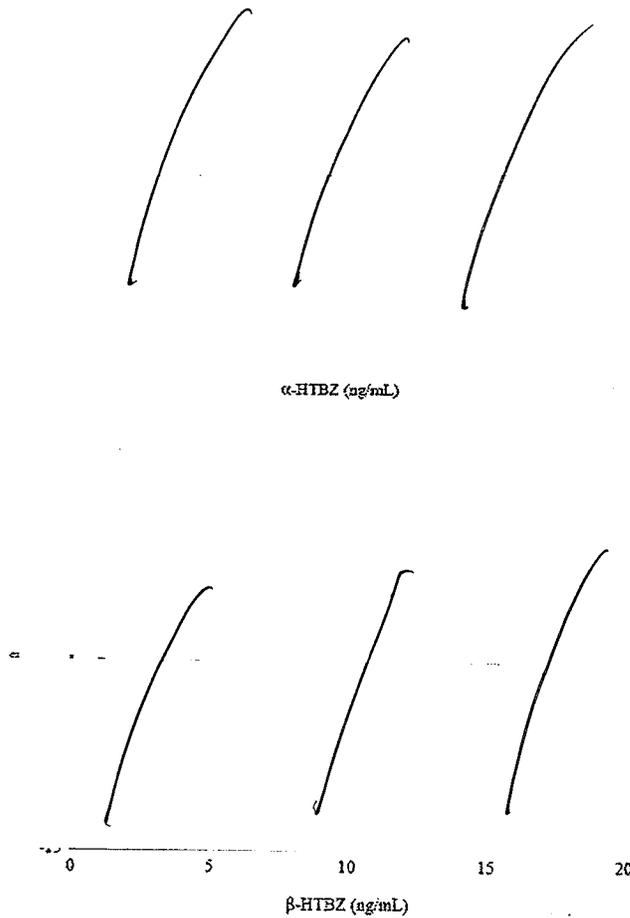


Figure 4. Relationship between the difference in serum concentrations of prolactin, between TBZ and placebo, and plasma concentrations of β -HTBZ after administration of a single dose of 12.5 mg TBZ.

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Safety

Only 1 adverse event was reported: a headache that began 3 hours after study drug (TBZ) dosing that was described as mild in severity and resolved within 8 hours.

CONCLUSIONS:

The PK results are in agreement with the PK results seen in other studies submitted to this NDA.

Administration of a single 12.5 mg dose of TBZ results in a statistically significant increase in serum prolactin levels that correlates with plasma concentrations of α - and β -HTBZ.

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NDA 21,894
Tetrabenazine

4.2.15 SINGLE AND REPEAT DOSE PROLACTIN STUDY

EFFECT OF SINGLE AND REPEATED DOSES OF TETRABENAZINE ON SERUM AND PLASMA CONCENTRATIONS OF VARIOUS HORMONES. A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSS-OVER STUDY IN HEALTHY MALE VOLUNTEERS

Study Investigators and Site:



Protocol Number: TBZ 203,008

OBJECTIVES:

The primary objective was to determine the pharmacodynamic effect of single and repeated doses of oral TBZ on the serum and plasma concentrations of prolactin, growth hormone, adrenocorticotrophic hormone, cortisol, thyroxin-stimulating hormone, arginine vasopressin, and testosterone in healthy male volunteers.

The secondary objectives were 1) to assess the PK characteristics of TBZ and its two metabolites α - and β -HTBZ after single and multiple dose administration and 2) to evaluate the tolerability and safety of single (25 mg) and repeated (25 mg bid) doses of TBZ in healthy male volunteers.

FORMULATION:

Table 1. Product used in TBZ 203,008

	Batch No.	Exp. Date (Dates of Study)
Tetrabenazine 12.5 mg tablet	6573872	5/22/2004 (1/13/04-5/22/04)

STUDY DESIGN:

This study was a single center, randomized, placebo-controlled, cross-over study. Eligible subjects were randomized to one of 2 sequence groups as shown in the table below, as provided by the Sponsor.

Table 2. Treatment Sequence for Subjects – Protocol TBZ 203,008

Sequence	First Treatment Period	Second Treatment Period
1	Two 12.5 mg tablets (25 mg) of tetrabenazine taken orally as a morning dose (Days 1 and 4);	Morning dose of two matching placebo tablets (Days 1 and 4);
	Two 12.5 mg tablets (25 mg) of tetrabenazine taken orally as a morning and evening dose (50 mg/day on Days 2 and 3)	Morning and evening doses of two matching placebo tablets (Days 2 and 3)
2	Morning dose of two matching placebo tablets (Days 1 and 4);	Two 12.5 mg tablets (25 mg) of tetrabenazine taken orally as a morning dose (Days 1 and 4);
	Morning and evening doses of two matching placebo tablets (Days 2 and 3)	Two 12.5 mg tablets (25 mg) of tetrabenazine taken orally as a morning and evening dose (50 mg/day on Days 2 and 3)

During the first treatment period, subjects received a single 25 mg oral dose of TBZ or matching placebo on Days 1 and 4 at approximately 9AM. On Days 2 and 3 subjects received a 25 mg oral dose of TBZ or matching placebo at approximately 9AM and 9PM. During the second treatment period, subjects received the alternate study drug according to the same schedule as the initial treatment period. Each treatment period was separated by a minimum of 7 days. On Days 1, 3, and 4 of each treatment period, blood samples were collected to measure serum or plasma concentrations of prolactin, growth hormone (GH), adrenocorticotropic hormone (ACTH), cortisol, thyroxin-stimulating hormone (TSH), arginine vasopressin, and testosterone. Samples were taken before the morning study drug dose (two samples within 30 minutes to establish a double baseline) and 1, 1.5, 2, 3, and 4 hours after the study drug dose.

Blood samples were drawn on Days 1 and 4 of each period to measure plasma concentrations of TBZ and α - and β -HTBZ. Samples were collected pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after the study drug dose.

Inclusion criteria included healthy males, between the ages of 18 and 45 years of age (inclusive), with baseline prolactin no greater than 25 ng/ml. (Women were excluded to minimize the confounding effect of the cyclic nature of serum prolactin measurements). Subjects were not to consume alcohol, tobacco, grapefruits or grapefruit juice, caffeinated beverage, bran and foods containing bran fiber, and subjects were to refrain from smoking during the assessment periods. With the exception of acetaminophen, no concomitant medications were allowed. Exclusion criteria included intake of more than 40 g/day of alcohol during the study, smoking more than 10 cigarettes per day, excessive intake of coffee, tea, or chocolate (more than 6 cups per day on average) and those with excessive intake of caffeine-containing beverages (more than 6 glasses per day), intake of any medication within 5 half-lives of that drug prior to study drug intake, or the existence of any surgical or medical condition which, in the judgment of the investigator, might interfere with the absorption, distribution, metabolism, or excretion of the study drug.

Safety monitoring included adverse events, vital signs, laboratory values, 12-lead ECG, and physical exam.

ASSAY:

Plasma concentrations were measured for TBZ, α -HTBZ, and β -HTBZ using a validated LC/MS/MS method (266/1).

Table 3. Performance of Analytical Method for TBZ 203,008

Analyte	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	Inter-assay CV (%)	Inter-assay Accuracy (%)
TBZ	LC/MS/MS	0.2-200 ng/ml	r > 0.990	0.2	0.5	8.5	-1.1
					25.0	5.3	1.9
					100.0	6.5	-0.2
α -HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.993	0.5	1.0	7.0	2.4
					25.0	6.0	1.9
					100.0	6.5	2.7
β -HTBZ	LC/MS/MS	0.5-200 ng/ml	r > 0.990	0.5	1.0	7.8	0.0
					25.0	6.4	2.8
					100.0	7.6	3.7

Two calibration curves and duplicate QC samples were analyzed with each batch of study samples. Study samples are stored nominally at -80° C, according to the procedures. Samples were analyzed within the 321 day period for which the samples are stable at -80° C. The performance of the assays for all analytes is considered acceptable.

Radioimmunoassays of ACTH, GH, and testosterone samples in human plasma were performed by _____ Plasma samples were maintained at -20 C. The assays were performed using the ELSA-ACTH RIA kit (_____) RAI kit for GH (_____) and the _____ RIA kit for testosterone _____ QC samples were prepared from human plasma or from controls provided with the kits. For each analyte, there were 9 calibration curves and 2 QC samples at each of 3 levels with each calibration curve. According to the SOP at the Biotec Center, the %CV on 6 series of analyses (n=12) had to be < 20%, and the assay met this requirement with CV < 15% for all assays.

Prolactin, cortisol, and TSH were measured at the _____ Plasma arginine vasopressin concentrations were measured at _____
Details were not provided.

RESULTS:

Demographics

Thirteen subjects were enrolled and 12 healthy, male volunteers completed the study. The mean age of all 13 subjects was 27.7 years and the range was 18-35 years. The mean weight (SD) was 73 ± 7 kg. Race was not provided. Five of the 13 subjects were tobacco users who smoked an average of 7 cigarettes per day.

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Tetrabenazine

The only concomitant medication use was azithromycin (Zithromax) in 1 subject during the tetrabenazine dosing. That subject did not complete the study and is not included in the PK or PD analysis.

Pharmacodynamics in Study 203,008

Plasma concentrations of prolactin, ACTH, cortisol, GH, testosterone, and TSH at each sampling time were compared between TBZ and placebo using ANOVA.

Table 6. Plasma Prolactin Concentration After Oral Administration of Morning Dose of 25 mg of Tetrabenazine – Protocol TBZ 203,008

Scheduled Time (h)	Prolactin Concentration (ng/mL)*					
	Day 1		Day 3		Day 4	
	Placebo	Tetrabenazine	Placebo	Tetrabenazine	Placebo	Tetrabenazine
-0.5	14.00 ± 5.66	16.67 ± 10.34	14.50 ± 6.29	15.25 ± 5.64	14.58 ± 6.15	15.25 ± 5.05
0.0	11.25 ± 4.56	13.83 ± 6.70	12.42 ± 5.11	12.75 ± 4.58	12.08 ± 4.72	13.67 ± 5.09
1.0	9.58 ± 3.73	31.67 ± 13.15	10.00 ± 3.10	18.83 ± 8.85	9.58 ± 3.40	29.75 ± 8.34
1.5	9.50 ± 3.94	50.67 ± 10.00	9.50 ± 3.55	28.58 ± 12.32	9.50 ± 4.34	40.67 ± 11.44
2.0	9.92 ± 3.92	44.73 ± 6.77	9.17 ± 3.24	30.58 ± 7.99	9.50 ± 3.66	38.17 ± 7.07
3.0	9.58 ± 4.12	34.67 ± 5.03	9.35 ± 3.82	30.17 ± 5.54	9.92 ± 5.00	29.17 ± 4.22
4.0	9.08 ± 4.17	27.42 ± 5.07	8.83 ± 2.62	25.58 ± 4.58	10.58 ± 4.93	23.75 ± 3.74

Source: Table 14.2.1 in Section 14.2

* Mean (± SD)

Statistically significant differences were observed between TBZ and placebo on Days 1 and 4 at each time point after study drug administration and on Day 3 beyond 1 hour after study drug administration.

For ACTH, there was no change in plasma ACTH after dosing with TBZ except for the 1 hour sample on Day 3 that was significantly lower than placebo. For TSH there was no change in plasma TSH after dosing with TBZ except for the 1.5 and 2-hour samples on Day 1 in which values in the TBZ group were greater than in the placebo group ($p < 0.05$).

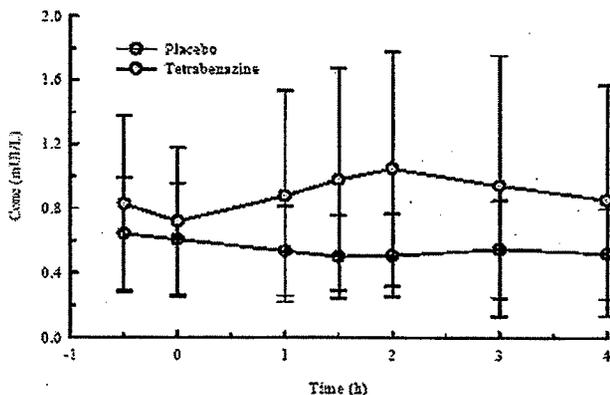


Figure 18. Mean (± SD) plasma concentrations of thyroxin-stimulating hormone after oral administration of tetrabenazine 25 mg or placebo to healthy volunteers, Day 1 - Protocol TBZ 203,008
Source: Table 14.2.1 in Section 14.2.

NDA 21,894
Tetrabenazine

There was no significant change in plasma cortisol, GH, testosterone, or arginine vasopressin.

In summary, statistically significant changes were seen in plasma prolactin concentrations after single and repeated doses of TBZ given 25 mg twice daily.

Pharmacokinetics in TBZ 203,008

Plasma concentrations of TBZ were less than the LOQ for most sampling times in all subjects. The highest measured concentration was — ng/ml. All other detectable concentrations of TBZ were less than 0.6 ng/ml.

The mean PK parameters for α - and β -HTBZ after oral administration on Days 1 and 4 are shown in the table below, as calculated by the Reviewer. These results are generally in agreement with the results provided by the Sponsor.

Table 5. Pharmacokinetic parameters (arithmetic mean) for α -HTBZ and β -HTBZ (Study TBZ104,012)

	Day 1 (% CV) n=12	Day 4 (% CV) n=12
α-HTBZ		
t_{max} (h) ^a	1.0 (1.0-2.5)	1.00 (0.5-2.00)
C_{max} (ng/mL)	32.7 (34)	51.1 (63)
AUC _{0-t} (ng*h/mL)	154.3 (56)	244.4 (64)
AUC _{0-∞} (ng*h/mL)	159.6 (60)	
λ_z (hr ⁻¹)	0.173 (31)	0.153 (30)
$t_{1/2}$ (h)	4.4 (32)	4.9 (31)
β-HTBZ		
t_{max} (h) ^a	1.0 (1.0-1.5)	1.0 (0.5-2.0)
C_{max} (ng/mL)	12.8 (62)	21.0 (96)
AUC _{0-t} (ng*h/mL)	43.4 (79)	74.2 (84)
AUC _{0-∞} (ng*h/mL)	43.9 (76)	
λ_z (hr ⁻¹)	0.313 (32)	0.258 (30)
$t_{1/2}$ (h)	2.4 (33)	3.0 (36)

^a median (range)

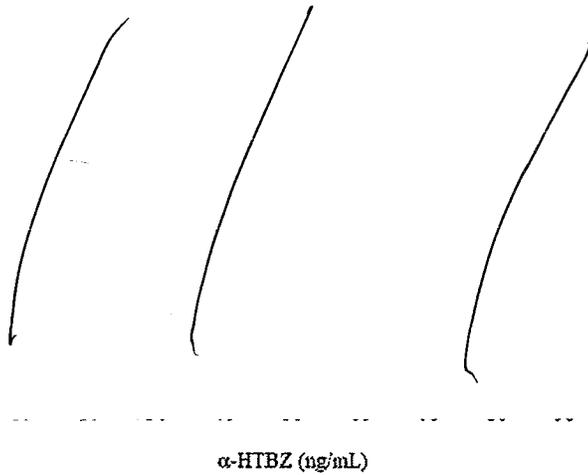
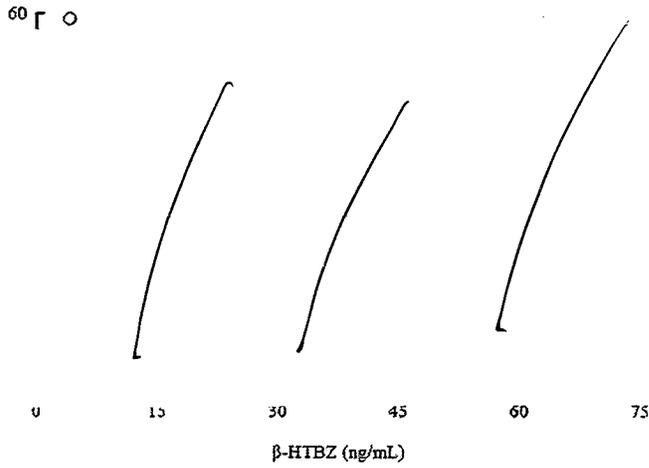
As observed in other PK studies, on average there was an approximate 3-fold to 4-fold greater exposure to α -HTBZ than to β -HTBZ.

The predicted accumulation after twice daily dosing, based on the half-life on Day 1, would be approximately 17% and 4% for α - and β -HTBZ, respectively. However, the observed accumulation (based on AUC) is approximately 58% for α -HTBZ and approximately 71% for β -HTBZ, and there was an approximate 56% increase in C_{max} for α -HTBZ and approximate 64% increase in C_{max} for β -HTBZ on repeated dosing. There was little change in elimination half-life. This suggests nonlinearity with repeated dosing.

NDA 21,894
Tetrabenazine

PK/PD

The relationships between plasma concentrations of α - and β -HTBZ and serum prolactin concentrations are shown in the figures below, as provided by the Sponsor. There was a statistically significant positive correlation for both α -HTBZ ($r^2=0.217$) and β -HTBZ ($r^2=0.164$).



NDA 21,894
Tetrabenazine

Safety

There were no deaths or serious adverse events reported. There were 7 adverse events in 5 study subject: 4 on placebo and 3 on TBZ. Single report of somnolence was reported in a subject receiving placebo. There were no reports of somnolence in subjects receiving TBZ. One subject on TBZ (and 2 on placebo) experienced transient asymptomatic orthostatic hypotension that did not require treatment. One subject developed urethritis necessitating the use of Zithromax and the subject did not complete the PK or PD portions of the study.

CONCLUSIONS:

TBZ given as a 25 mg single dose and repeated doses of 25 mg twice daily produced statistically significant increases in plasma prolactin concentrations in healthy male subjects that appeared to be positively correlated with the concentrations of α - and β -HTBZ.

Greater than predicted accumulation of α - and β -HTBZ was observed after twice daily dosing, suggesting nonlinearity in PK with repeated dosing.

APPEARS THIS WAY
ON ORIGINAL

4.2.16. Pgp DIGOXIN INTERACTION STUDY

**STUDY OF THE INTERACTION OF TETRABENAZINE WITH P-GLYCOPROTEIN
BASED ON DIGOXIN BIOAVAILABILITY IN HEALTHY SUBJECTS**

Study Investigators and Site:

/ /

Protocol Number: TBZ 203,009

OBJECTIVES:

The primary objective was to determine the effect of repeated doses of tetrabenazine (TBZ) (25 mg bid) on P-glycoprotein based on the bioavailability of digoxin, a P-glycoprotein substrate.

The secondary objectives were 1) to determine the PK characteristics of TBZ and its two metabolites α -dihydrotrabenazine (α -HTBZ) and β -HTBZ, in men and women, when co-administered with digoxin and 2) to evaluate the tolerability and safety of repeated doses of TBZ (25 mg bid for 4 days) co-administered with digoxin (0.25 mg once per day for 4 days) in subjects who had previously been administered a single, daily, oral dose of digoxin for 6 days (0.5 mg per day for 1 day followed by 0.25 mg per day for 5 days).

FORMULATION:

Table 1. Product used in TBZ 203,009

	Batch No.	Exp. Date (Dates of Study)
Tetrabenazine 12.5 mg tablet	6573804	6/8/2004 (1/28/04-4/11/04)
Digoxin 0.25 mg tablet	381394	04/8/04 (1/28/04-4/11/04)

STUDY DESIGN:

This study was a single-center, open-label study of the possible interaction of tetrabenazine with P-glycoprotein (Pgp) in healthy volunteers as determined by bioavailability of digoxin. Eligible subjects were admitted to the research center on the morning of the day before the start of study dosing (Day -1) and had been instructed to fast for at least 10 hours prior to their admission. Following additional screening, eligible subjects were admitted to the study. During the treatment and assessment period (10 dosing days plus 2 post-dosing observation days), the subjects received 6 days of oral digoxin (0.5 mg on the first day, 0.25 mg daily thereafter, Days 1-6), followed by 4 days of oral digoxin (0.25 mg) and tetrabenazine 25 mg bid (Days 7-10) and a 2 day post-dosing assessment period (Days 11 and 12). There was then a 7-10 day follow-up