CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022567Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology Review Amendment

NDA	22567
Submission Dates:	March 22, 2010, August 31, 2010
Brand Name:	Viibryd
Generic Name:	Vilazodone
Strength and Formulation:	10, 20, and 40 mg IR tablets
Sponsor:	PGxHealth, LLC
Indication:	Major Depressive Disorder (MDD)
Submission Type:	Original NDA (NME)
CP Reviewer Team:	Bei Yu, Ph.D., Huixia Zhang, Ph.D., Jee Eun Lee, Ph.D., Joga Gobburu, Ph.D. Atul Bhattaram, Ph.D. Yaning Wang, Ph.D., Issam Zineh, PharmD, MPH, FCCP., Li Zhang, Ph.D.

(b) (4)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

BEI YU
12/10/2010

JOGARAO V GOBBURU
12/10/2010

The original full OCP review was signed-off on 8Dec2010. This review amends a typo in the labeling recommendations.

ONDQA BIOPHARMACEUTICS REVIEW

NDA#:	22-567 (N-000)
Submission Date:	03/22/10, 10/29/10, and 11/30/10
Brand Name:	Pending
Generic Name:	Vilazodone HCl
Formulation:	Oral immediate-release (IR) tablets
Strength:	10, 20, and 40 mg
Sponsor:	PGx Health
Type of submission:	Original
Reviewer:	Tien-Mien Chen, Ph.D.

SUMMARY

Vilazodone HCl, is reportedly a dual-acting potent and selective serotonin reuptake inhibitor and 5-HT_{1A} receptor partial agonist. Vilazodone is a new chemical entity (NME) which was developed (up to 20 mg strength) by Merck and GSK as the first in a new class of antidepressants – the dual-acting serotonergic antidepressants.

On 03/22/10, PGx Health submitted NDA 22-567 (N-000) for Vilazodone HCl tablets. Vilazodone HCl is formulated as 10 mg, 20 mg, and 40 mg, film-coated IR tablets and is indicated for the treatment of major depressive disorder (MDD). The to-be-marketed (TBM) tablets are manufactured using a (b) (4) All 10, 20 and 40 mg tablets are (b) (4) and dose proportional and have been used in the Phase 3 clinical trials and several Phase 1 studies. Therefore, there is no biowaiver issue.

Six batches of Vilazodone HCl Tablets (three for each of 10 mg and 40 mg tablet strengths) are included in the primary registration stability study. These batches were manufactured using the intended commercial formulation, at the intended commercial facility using the intended commercial process. All batches were manufactured at (b) (4) scale, which is (b) (4) of the intended commercial scale (b) (4)). Each batch was packaged in container closures intended for commercial products. The 20 mg strength is bracketed and not included in the stability studies.

The dissolution data on the 10 and 40 mg TBM tablet strengths were submitted, however, the dissolution data on the 20 mg TBM tablet strength could not be located in this submission. On 10/15/10, an information request was sent to the sponsor and the sponsor responded on 10/29/10 and 11/30/10. The dissolution development report, dissolution methodology and data/profile for all three strengths, and the proposed dissolution specifications are, therefore, reviewed here.

The following dissolution method and the specifications are proposed as shown below.

USP Apparatus: **2 (Paddle) x 60 rpm**
Medium: **0.1% Acetic Acid (pH 3.1), 1000 mL at 37ºC**
Specifications: **Q=** (b) (4) **at 30 min**

The results of dissolution testing show that all three strengths meet the proposed dissolution specifications.

RECOMMENDATION

From the Biopharmaceutics perspective, the proposed dissolution methodology and specifications are acceptable. No further comments need to be sent to the sponsor.

BACKGROUND
Vilazodone HCl, is reportedly a dual-acting potent and selective serotonin reuptake inhibitor and 5-HT_{1A} receptor partial agonist. Vilazodone is a new chemical entity (NME) which was initially developed (up to 20 mg) by Merck (KGaA) and GSK as the first in a new class of antidepressants – the dual-acting serotonergic antidepressants.

CURRENT SUBMISSION
On 03/22/10, PGx Health submitted NDA 22-567 (N-000) for Vilazodone IR tablets. Vilazodone HCl is formulated as 10 mg, 20 mg, and 40 mg, film-coated IR tablets and is indicated for the treatment of major depressive disorder (MDD). The to-be-marketed (TBM) tablets are manufactured using a (b) (4) . The TBM tablet strengths, 10 mg (Formulation No. PG-10) and 20 mg (PG-20), were used in the second and third Phase 3 clinical trials (Nos. CLDA-07-DP-02 and CLDA-08-DP-04) as well as in several Phase 1 studies. The 40 mg (PG-40) was also used in the third Phase 3 clinical trial (CLDA-07-DP-04). Therefore, there is no biowaiver issue.

Six batches of Vilazodone HCl Tablets (three for each of 10 mg and 40 mg tablet strengths) were included in the primary registration stability study. These batches were reportedly manufactured using the intended commercial formulation, at the intended commercial facility using the intended commercial process. All batches were manufactured at (b) (4) scale, which is (b) (4) of the intended commercial scale ((b) (4) Each batch was packaged in container closures intended for commercial products. The 20 mg strength is bracketed and therefore, not included in the stability studies.

The dissolution development report and the dissolution data on TBM 10 and 40 mg IR tablet batches are included in the submission. During the pharmaceutical development of Vilazodone IR tablets, a rotational speed of 50 rpm was employed initially. However, the speed was changed to 60 rpm at a later time, therefore, for the stability batches, the dissolution data using 60 rpm are only available at one time point (at 30 min) at the 12th and the 18th month.

The dissolution data on the 20 mg TBM tablet strength, however, could not be located in this submission. On 10/15/10, an information request was sent to the sponsor and the sponsor responded on 10/29/10 and 11/30/10. The dissolution development report, dissolution methodology and data/profile, and the proposed dissolution specifications are, therefore, reviewed here.

FORMULATION COMPARISONS
The composition of the TBM formulation of vilazodone 10, 20 and 40 mg IR tablets is shown below. The 3 tablet strengths are (b) (4) and dose proportional.

Table 1. The Composition of the TBM Formulation of Vilazodone IR Tablets

Component	Quality Standard	Function	% w/w	mg/tablet		
				10 mg (PG-10)	20 mg (PG-20)	40 mg (PG-40)
						(b) (4)
vilazodone HCl	In-house standard					
Lactose Monohydrate	NF					
Microcrystalline Cellulose	NF					
Magnesium Stearate	NF					
Colloidal Silicon Dioxide	NF					
(b) (4)						
	In-house standard					
	In-house standard					
	In-house standard					
	USP					
Total Weight				103.0	206.0	412.0

[a] The compositions of the colorants are provided in (b) (4) Authorization to refer to this DMF on behalf of PGxHealth is provided in 1.4.1. (b) (4)

(b) (4)

DISSOLUTION METHODOLOGY AND SPECIFICATIONS

The dissolution method development was discussed with the Agency in the EOP2 meeting dated 09/28/09. The dissolution development report included the investigation on drug substance (b) (4)), apparatus, medium (water, 0.1% Acetic Acid, or pH 6.8 phosphate buffer; with or without surfactant), medium volume, and rotational speed (50, 55, 60, and 75 rpm). Please see the dissolution development report under Module 3.2.P.2.2.3.1 "Method History Report" for details.

The following dissolution method was selected based on the dissolution development report and the specifications are also proposed as shown below.

USP Apparatus: 2 (Paddle) x 60 rpm
Medium: 0.1% Acetic Acid (pH 3.1), 1000 mL at 37ºC
Specifications: Q= (b) (4) at 30 min

The mean dissolution data (n=6 tablets/batch) and profiles of vilazodone HCL 10 and 40 mg IR tablets using the proposed dissolution method are shown below.

Table 2. Mean Dissolution Data of Primary Stability Batches of 10 and 40 mg IR Tablets (n=6 tablets/batch)

Strength	Batch No	Mean Vilazodone HCL % Dissolved (n=6 Tablets/Batch					
		5 Min	10 Min	20 Min	30 Min	45 Min	60 Min
10 mg	MTSCL331E	(b) (4)					
	MTSCL332E						
	MTSCL333E						
40 mg	MTSCL328E						
	MTSCL329E						
	MTSCL330E						

Figure 1. Mean Dissolution Profiles of Vilazodone HCl 10 mg IR Tablets (3 Primary Stability Batches)

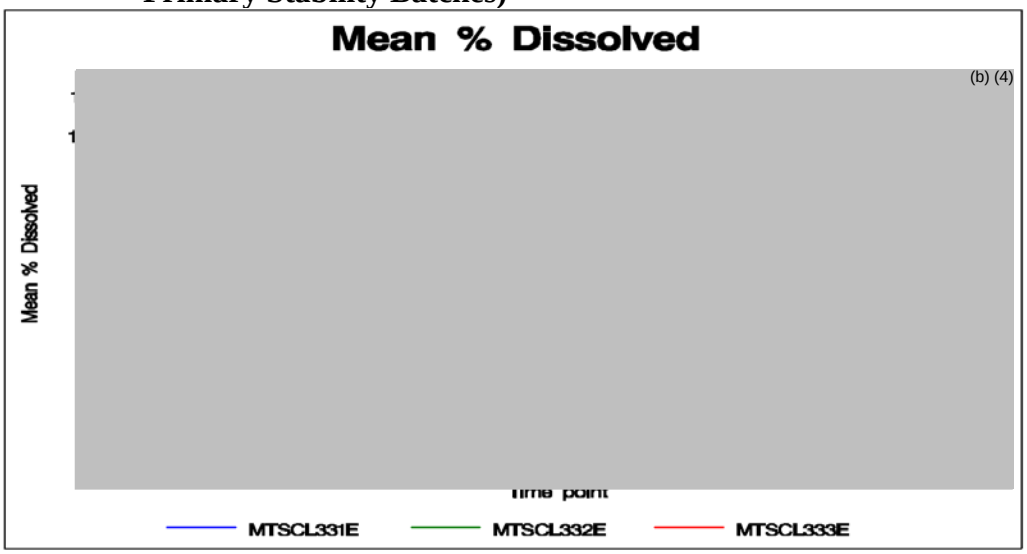
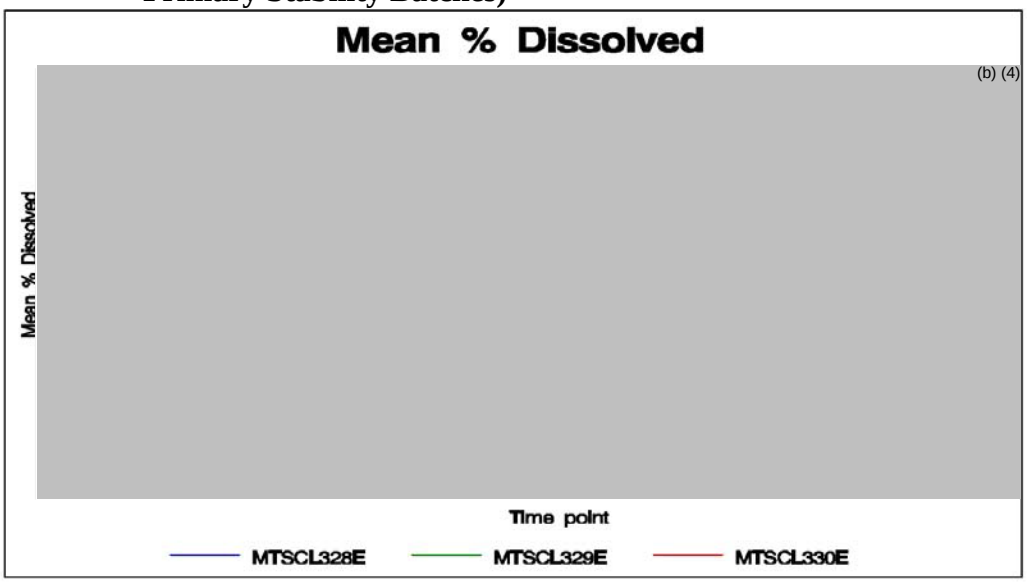


Figure 2. Mean Dissolution Profiles of Vilazodone HCl 40 mg IR Tablets (3 Primary Stability Batches)



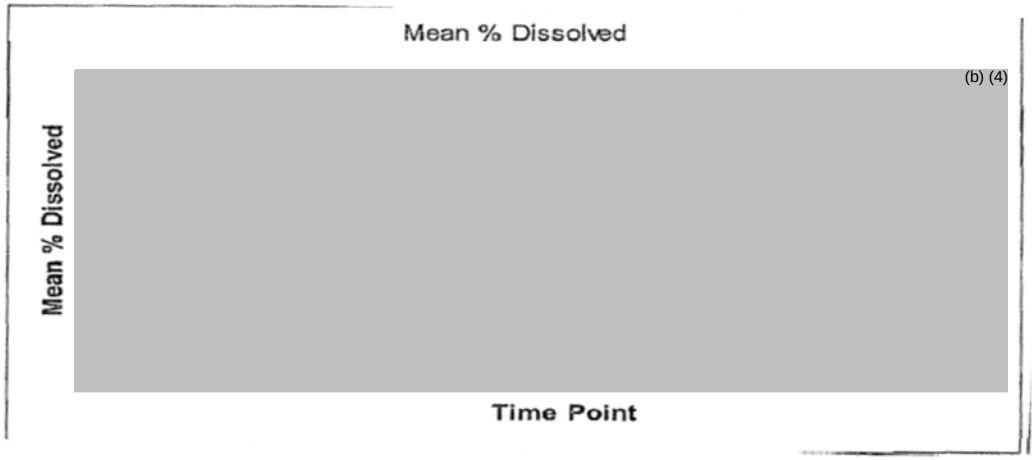
Upon request, the sponsor provided on 10/29/10 and 11/30/10 the dissolution data/profiles for vilazodone 20 mg IR tablets as shown below.

Table 3. Mean Dissolution Data of the Biobatch of 20 mg IR Tablets (n=12 tablets/batch)

Strength	Batch No	Mean Vilazodone HCL % Dissolved (n=6 Tablets/Batch					
		5 Min	10 Min	20 Min	30 Min	45 Min	60 Min
20 mg	07T6431*	(b) (4)					

*. The batch was used in a Phase-1 bioavailability study No. CLDA-07-DP-01.

Figure 3. Mean Dissolution Profiles of Vilazodone HCl 20 mg IR Tablets (3 Primary Stability Batches)



Please see individual and mean dissolution data in Appendix 2 for details.

Reviewer's Comment:
The proposed dissolution methodology and specifications are reviewed and found acceptable. The results of dissolution testing show that all three strengths meet the proposed dissolution specifications.

_____ 12/10/10

Tien-Mien Chen, Ph.D. Date
Reviewer
ONDQA Biopharmaceutics

_____ 12/10/10

Patrick Marroum, Ph.D. Date
ONDQA Biopharmaceutics

CC: NDA
Patrick Marroum, Angelica Dorantes, Tien-Mien Chen

7 Page(s) have been Withheld in Full as b4 (CCI/TS) immediately following this page.

Clinical Pharmacology Review

NDA	22567
Submission Dates:	March 22, 2010, August 31, 2010
Brand Name:	Viibryd
Generic Name:	Vilazodone
Strength and Formulation:	10, 20, and 40 mg IR tablets
Sponsor:	PGxHealth, LLC
Indication:	Major Depressive Disorder (MDD)
Submission Type:	Original NDA (NME)
CP Reviewer Team:	Bei Yu, Ph.D., Huixia Zhang, Ph.D., Jee Eun Lee, Ph.D., Joga Gobburu, Ph.D. Atul Bhattaram, Ph.D. Yaning Wang, Ph.D., Issam Zineh, PharmD, MPH, FCCP., Li Zhang, Ph.D.

Required office level OCP Briefing held on December 6, 2010.

Table of contents

1. Executive Summary 2
   - 1.1 Recommendations 2
   - 1.2 Post-Marketing Studies 6
   - 1.3 Clinical Pharmacology Summary 7
2. Question Based Review (QBR) 9
   - 2.1 Specific Questions 9
   - 2.2 Standard Questions 10
3. Individual Study Review 22
   - 3.1 Clinical Pharmacology Review 22

BA/BE STUDIES 22
IN VITRO METABOLISM STUDIES 28
PHARMACOKINETIC AND TOLERABILITY STUDIES 46
MASS BALANCE STUDY 60
PHARMACODYNAMIC STUDIES 68
INTRINSIC FACTORS 70
EXTRINSIC FACTORS 76
ANALYTICAL SECTION 88
   - 3.2 Pharmacometric Review 92
   - 3.3 Pharmacogenomic Review 99

1. Executive Summary

The sponsor is seeking approval Viibryd (vilazodone 40 mg QD with food after uptitration) for the treatment of patients with major depressive disorder (MDD) via 505 (b)(1) approach. Vilazodone HCl is an indolalkylamine new chemical entity purported to be related to its potentiation of serotonin activity in the CNS, through selective inhibition of serotonin reuptake and partial agonist activity at 5-HT_{1A} receptor.

Vilazodone 40 mg QD with food was shown to be superior to placebo in two registration trials. Early studies evaluated a dose range of 5 mg – 100 mg. Although not conclusive, 20 mg and higher doses result in similar effectiveness; however adverse events such as nausea, diarrhea, dizziness, somnolence, and excessive dreaming are dose-dependent. Hence a 20 mg dose will likely have a more favorable benefit-risk and is worth studying in future studies.

Vilazodone exhibits a dose-proportional PK with an absolute bioavailability of about 72% with food. Food approximately doubles vilazodone's AUC. About 40% of parent drug is possibly metabolized by carboxyl esterase and 60% by CYP pathways in which CYP3A4 is a major isoenzyme. Ketoconazole increases vilazodone AUC and Cmax by 50% - and thus a maximum dose of 20 mg is recommended.

1.1 Recommendations

The Office of Clinical Pharmacology has determined that there is sufficient clinical pharmacology and biopharmaceutics information provided in the NDA to support a recommendation of approval of vilazodone. The acceptability of specific drug information is provided below.

Decision	Acceptable to OCP?	Comment
Overall	☒ Yes ☐ No ☐ NA	Pending labeling and PMC agreements with sponsor.
Evidence of Effectiveness	☒ Yes ☐ No ☐ NA	2 positive registration trials; dose-response supportive
Proposed dose for general population	☒ Yes ☐ No ☐ NA	40 mg is acceptable; Effectiveness of 20 mg should be studied (PMC)
Proposed dose selection for others	☐ Yes ☒ No ☐ NA	Vilazodone dose should not exceed 20 mg when given with strong CYP3A4 inhibitors. Effect of CYP3A4 inducer on vilazodone should be studied (PMC) Severe hepatic impairment study (PMC)
Pivotal BE	☒ Yes ☐ No ☐ NA	
Labeling	☐ Yes ☒ No ☐ NA	Pending satisfactory agreement with sponsor.

3 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page.

1.2 Post-Marketing Studies

Office of Clinical Pharmacology proposes the following post-marketing studies.

PMC or PMR	Key drug development question	Rationale	Design summary
☒ PMC ☐ PMR	Does 20 mg exhibit a more favorable benefit-risk?	Effectiveness of 20 mg and 40 mg are likely similar; AEs are lower at 20 mg.	Study population: MDD Patients Study design: AWC, Parallel Sample size: 85% Power Dose(s): Placebo, 20 mg, and 40 mg fed (titrated from 10 mg) Study length: 8 wks Endpoints: MADRS Score (b) (4)
☒ PMC ☐ PMR	Should vilazodone dose be increased when taken with relevant CYP3A4 inducers? If so, by how much?	Vilazodone is metabolized by CYP3A4. Information on effect of induction was not submitted.	Study population: Healthy Subjects Study design: Crossover Sample size: 20% SE for Mean AUC Dose(s): 40 mg vilazodone single-dose, 400 mg daily carbamazepine (7 days) Study length: One week Endpoints: Vilazodone AUC, Cmax Submit protocol by: Jul-11 Start study by: Oct-11
☒ PMC ☐ PMR	(b) (4)		
☒ PMC ☐ PMR	(b) (4)		

			(b) (4)
☒ PMC ☐ PMR	Is vilazodone a Pg-P substrate, or inhibitor?	CYP3A4 substrates typically also effect or affected by Pg-P	Method: A bi-directional assay in Caco-2 cells. Submit protocol by: (b) (4) Start study by: Dec-10

1.3 Clinical Pharmacology Summary

The current submission consisted of 24 clinical pharmacology studies including a thorough QTc study and 9 in vitro studies.

Figure 1. Mean (± SD) vilazodone plasma concentration versus time profile after oral single dose of 40 mg vilazodone tablet under fed conditions.

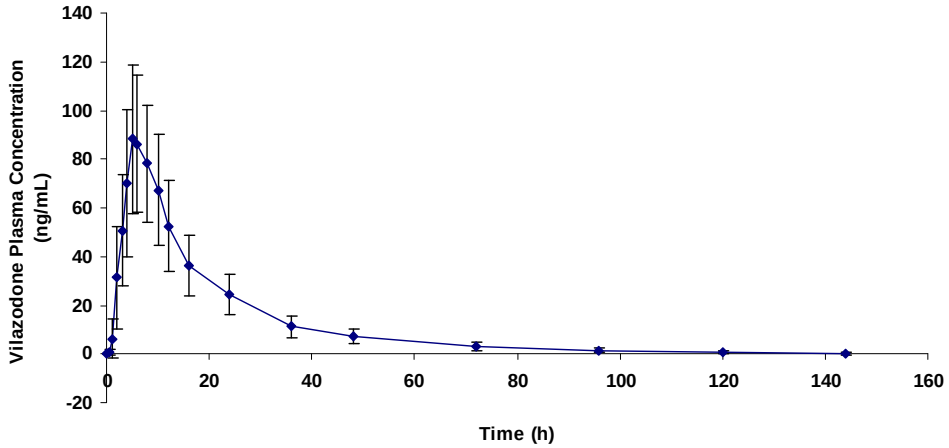


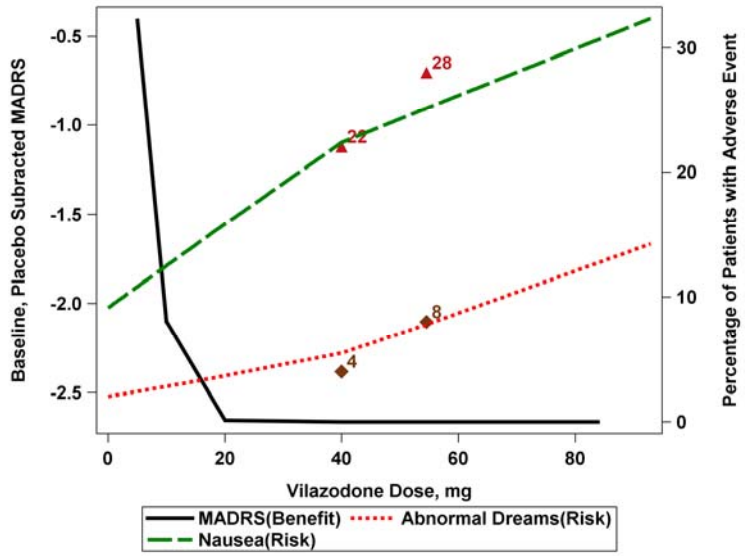
Table 1. **Important PK properties of vilazodone**

PK Property	PK Parameter	
Dose-proportionality	PK dose-proportional for doses 5 – 80 mg	
Absorption	Tmax (median), hour	4-5
	T1/2, hour	~25
	Absolute Bioavailability (with food), %	72
	Food Effect	High fat/light meal increased C_{max} and AUC by ~2-fold.
Distribution	Protein Binding, %	96-99
Metabolism	Pathways	CYP (3A4 is the primary isoenzyme, with the minor contributions from

		CYP2C19 and 2D6) and non-CYP (possibly by carboxylesterase). No active metabolites.
Excretion	A mass balance study for vilazodone showed ~85% of the administered radioactivity was recovered in the urine (~20%) and feces (~65%) combined, while ~ 3% of the administered dose of vilazodone was recovered as unchanged drug (~1% in urine, and ~2% in feces).	

PK properties of vilazodone are summarized in Table 1. Food (light or high fat meal) significantly increased systemic exposure (AUC) of vilazodone. Vilazodone is extensively metabolized through CYP and non-CYP pathways, and mainly excreted through feces.

The figure below depicts the dose-MADRS and AE relationships. The depression symptoms decrease with increasing dose. Doses beyond 20 mg do not offer additional reduction in MADRS. However, the AEs increase such as abnormal dreams, nausea with dose between 5 mg – 80 mg.



Ketoconazole, a strong CYP3A4 inhibitor, increased vilazodone systemic exposure (AUC) by ~50%. CYP2C19 and 2D6 genotypes are not associated with different drug response. The PK profiles were comparable between hepatic impairment patients (mild and moderate only) and healthy subjects, and between renal impairment patients (mild and moderate only) and healthy subjects. There was no substantial PK difference between elderly subjects and young subjects. Although systemic exposure in female subjects is higher than that in male subjects, lower body weight in females was correlated with the higher exposure. Additionally, ethanol and proton pump inhibitor did not affect PK of vilazodone.

2. Question Based Review (QBR)

2.1 Specific Questions

2.1.1 Is there a need to assess effectiveness of vilazodone at doses < 40 mg?

Yes, effectiveness at 20 mg should be assessed in a definitive manner post-marketing. Patients who cannot tolerate 40 mg might benefit from the 20 mg dose. Two positive registration trials support the effectiveness and safety of the proposed 40 mg dose. Based on the Phase 2 studies, 20 mg consistently demonstrated numerically similar placebo-corrected changes in MADRS to those of 40 mg. More over, these changes across 5 mg – 80 mg exhibit a clear dose-response relationship. Doses closer to 5 mg have small changes in MADRS, and the changes are larger at higher doses. Doses beyond 20 mg do not offer additional reduction in symptoms. Although the Phase 2 studies allowed flexible dosing, a clear dose-response is reassuring about effects at 20 mg i.e. it follows an expected trend. Further, adverse events such as dizziness, somnolence, excessive dreaming, nausea, and diarrhea are clearly dose-dependent. Hence a 20 mg dose will likely have a more favorable benefit-risk especially in patients who cannot tolerate 40 mg and is worth studying in future studies.

2.1.2 Should dose of vilazodone be adjusted with concomitant use of strong, moderate, and mild CYP3A4 inhibitors?

The dose of vilazodone should be adjusted from 40 mg to 20 mg when vilazodone is coadministered with strong CYP3A4 inhibitors (e.g., ketoconazole).
Vilazodone dose should be reduced to 20 mg for patients with intolerable adverse events when the drug is co-administered with moderate inhibitors of CYP3A4.
No dose adjustment is recommended when vilazodone is co-administered with mild inhibitors of CYP3A4.

In vivo data showed that strong CYP3A4 inhibitor (i.e., ketoconazole) increased the AUC of vilazodone by 50%. This increase for 40 mg of proposed dose is equivalent to a dose of "60 mg". Due to dose-dependent AEs for vilazodone, the dose should be adjusted (details see Section 2.2.4.8.1). The AUC of vilazodone can be increased with concomitant use of moderate CYP3A4 inhibitors, but the increase can not exceed by 50%.

2.1.3 Is a drug-drug interaction study of vilazodone with CYP3A4 inducer needed?

Vilazodone is a low extraction ratio drug. Its systemic clearance is about one fifth of total hepatic blood flow. Under inducing conditions, its clearance could potentially be increased four fold, which could then lead to decreased exposure to vilazodone.

A random two-way crossover design in healthy subjects treated with 20 mg vilazodone under basal conditions and after pretreatment with 400 mg carbamazepine once daily for 7-14 days is recommended.

2.1.4 Is a drug-drug interaction study of vilazodone with CYP2C8 substrate needed?

No.

In-vitro experiment with hepatocytes indicates that vilazodone does not have a strong potential to inhibit CYP2C8 in-vivo. However, *in vitro* experiment with microsomes indicates that the [I]/Ki = 0.72 which is greater than 0.1 and suggests a need for in vivo evaluation. As the hepatocyte experiment represents a more physiologically relevant system, an *in vivo* study is not necessary. PBPK simulations also support no drug-drug interaction between vilazodone and CYP2C8 substrate, rosiglitazone.

2.1.5 Should dose be adjusted in patients with severe renal impairment?

No.

Vilazodone eliminated primarily by hepatic metabolism with 2% unchanged drug in feces and 1% unchanged drug in urine. Vilazodone PK did not show difference between mild/moderate renal impaired patients and healthy subjects. Additionally, mild/moderate hepatic impairment did not show increased systemic exposure of vilazodone. It's unlikely there is a clinically relevant elevation in vilazodone systemic exposure in severe renal impairment. However, vilazodone should be used with caution in severe renal impaired patients.

2.2 Standard Questions

2.2.1 What are the PK characteristics of the drug?

2.2.1.1 What are the single and multiple dose PK parameters of parent drug and relevant metabolites in healthy adults?

Single dose

The single dose PK of vilazodone between 20 mg and 80 mg was characterized mainly in two Phase I studies. Table 2 shows the single dose PK parameters following different doses of vilazodone. In study CLDA-07-DP-01 with 44 healthy subjects exposed to vilazodone, the inter-subject variability (% CV) was 29% for Cmax and AUC.

Table 2. Single dose PK parameters following different doses of vilazodone in healthy subjects

Study	**CLDA-07-DP-01**	**GPP-007-CLN-CP1-1997-232**		
	BE study	Dose rising		
Formulation	Tablet (PG-20*/T-20)	Capsule (C-10/C-40)		
Dose (mg)	40	20	40	80
Diet	Fed	Fasted		
Tmax (median, rang), h	5 (4-10)	5 (3.5 -5.5)	4.8 (2 – 8)	4.8 (2-8)
Cmax (mean (CV%)), ng/mL	96.96 (28.83)	28.5 (45.96)	36.8 (38.59)	58.4 (47.09)
T1/2 (mean (CV%)), h	24.57 (36.18)	24.1 (14.94)	21.4 (15.89)	20.4 (11.76)

AUCinf (mean (CV%)), ng h/mL	1711.82 (29.11)	502 (30.88)	741 (28.88)	1195 (30.38)

*To-be-marketed formulation.

Multiple doses

The multiple dose PK of vilazodone between 10 mg and 80 mg was characterized in two Phase I studies. After multiple daily doses, the Cmax and AUC of vilazodone increased and steady-state plasma levels of vilazodone were reached on the third day of multiple dosing. The accumulation factor is ~1.8 folds. Table 3 shows vilazodone PK parameters at steady state following different doses of vilazodone in healthy subjects.

Table 3. Multiple dose PK parameters following different doses of vilazodone in healthy subjects

Study	**GPP-007-CLN-CP1-1998-230**			**PGX-08-P1-06 (TQT study)**			
	Dose rising			Dose rising			
Formulation	Capsule (C-10/C-40)			Tablet (PG-10/PG-20*)			
Dose (mg)	10	20	40	20	40	60	80
Diet	Fasted			Fed			
Tmax (median, rang), h	3.5 (2.5-5)	4.5 (2.5-5)	4 (2-6)	4 (3-10)	4 (0-12)	4 (3-8)	4 (0-8)
Cmax (mean (CV%)), ng/mL	24.8 (21.37)	45.5 (38.9)	59.5 (36.97)	70.09 (43.13)	156.28 (43.23)	253.1 (44.67)	315.38 (53.75)
T1/2 (mean (CV%)), h	30.9 (22.65)	29.5 (15.59)	28.9 (11.07)	–	–	–	–
AUC0-24 (mean (CV%)), ng.h/mL	293 (27.65)	528 (35.61)	755 (33.11)	776.83 (45.31)	1645.25 (43.81)	2506.1 (44.19)	3269.76 (52.60)
Accumulation**	1.78 (23.6)	1.54 (21.43)	1.64 (26.83)	–	–	–	–

*To-be-marketed formulation.

** AUC0-24 on Day 16/AUC0-24 on Day 1.

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

Dose proportional increase in Cmax and AUC was observed in healthy subjects after single and multiple-dose administration of vilazodone dose ranging from 5 mg to 80 mg.

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

PK does not vary with time. Refer to Section 2.2.1.1 Multiple Doses.

2.2.1.4 What are the general ADME (Absorption, Distribution, Metabolism and Elimination) characteristics of vilazodone?

- **What are the characteristics of drug absorption?**

The absolute bioavailability of vilazodone is 72% under fed conditions. The maximum concentration is reached at a median of 4-5 hours after drug administration.

- **What are the characteristics of drug distribution?**

The protein binding was determined in vitro human serum at vilazodone concentrations of 0.05 – 5 ug/mL. Vilazodone is approximately 96-99% protein bound. Vilazodone is widely distributed with a volume of distribution of 605 L after 5 mg IV infusion for 4 hours.

- **Does the mass balance study suggest renal or hepatic as the major route of elimination?**

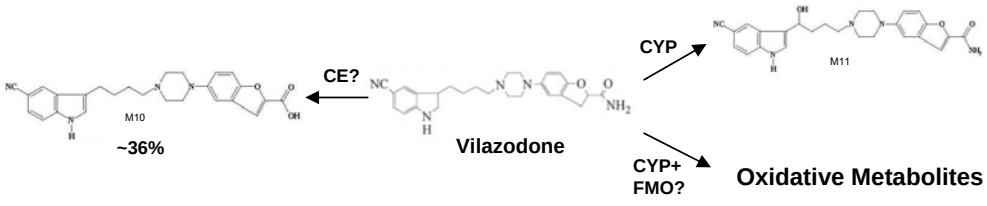
Fecal excretion is the major route of vilazodone elimination. During the collection interval of 14 days, approximately 85% of the administered radioactivity was recovered in the urine (~20%) and feces (~65%) combined, while approximately 3% of the administered dose of vilazodone was recovered as unchanged drug (~1% in urine, and ~2% in feces).

- **What is the percentage of total radioactivity in plasma identified as parent drug and metabolites?**

In the mass balance study, vilazodone metabolites were rapidly formed and peak radioactivity levels for the metabolites occurred at about 5 hours (vilazodone Tmax 4 hours). The AUC for radioactivity is approximately 2.5 times the value for vilazodone (2071 h.ng/mL v.s., 850 h.ng/mL), i.e., ~40% of total radioactivity in plasma was identified as parent drug. Vilazodone and the radioactivity were eliminated at a similar rate (half-life of 27 hours for vilazodone v.s., 32 hours for radioactivity).

- **What are the characteristics of drug metabolism?**

Figure 2. Proposed metabolic pathways of vilazodone in humans (OCP's figure)



The proposed metabolic pathway is provided in Figure 2. Vilazodone is extensively metabolized through CYP and non-CYP pathways (possibly by carboxylesterase (CE)). CYP3A4 is the primary isoenzyme for vilazodone metabolism among CYP pathways, with minor contributions from CYP2C19, and CYP2D6.

In vitro studies with human microsomes and human hepatocytes indicate that vilazodone is unlikely to inhibit or induce the metabolism of other CYP (except for CYP2C8, 2C19, and 2D6 inhibition) substrates, and an in vivo study with probe substrates for 2D6 and 3A4 showed vilazodone did not alter pharmacokinetics of the probe substrates. However, an in vivo study showed a minor induction of CYP2C19.

Vilazodone is not a potent inhibitor of CYP2C8 in hepatocytes. *In vitro* studies have shown that vilazodone is a moderate inhibitor of CYP2C19 and CYP2D6.

2.2.2 Exposure-Response

2.2.2.1 What are the design features of the clinical studies used to support dosing or claims?

Study	Design Features
GNSC-04-DP-02, CLDA-07-DP-02 *Registration trials*	Well-controlled, randomized, double-blind, placebo-controlled, MDD patients, 40 mg QD (fed) for 8 weeks, primary endpoint of MADRS
GPP-007-CLN-CP2-2001-244, GPP-007-CLN-CP2-2001-245	Well-controlled, randomized, double-blind, placebo-controlled, MDD patients, 5-100 mg QD (fed) for 8 weeks, primary endpoint of HAM-D; MADRS also collected
GPP-007-CLN-CP2-2003-248, GPP-007-CLN-CP2-2003-246, GPP-007-CLN-CP2-2003-247	Well-controlled, randomized, double-blind, placebo-controlled, MDD patients, 5-20 mg QD (fed) for 8 weeks, primary endpoint of HAM-D; MADRS also collected

2.2.2.2 What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology studies?

Standard endpoints for an MDD claim accepted by the Agency were employed. The primary effectiveness endpoint was the mean change from baseline to end of treatment (EOT) on MADRS (the Montgomery-Asberg Depression Rating Scale) for the registration trials. HAM-D was used for the early studies (listed above). Routine safety was collected in these trials.

2.2.2.3 What are the characteristics of the exposure-response relationship for efficacy? What is the time to the onset and offset of the desirable pharmacological response or clinical endpoint?

Based on the data from registration trials and early dose finding studies it appears that a lower dose of 20 mg will also have similar benefit as 40 mg based on change of MADRS score (details see Section 3.2 Pharmacometric Review).

2.2.2.4 What are the characteristics of the exposure-response relationships for safety? What is the time to the onset and offset (or duration) of the undesirable pharmacological response or clinical endpoint?

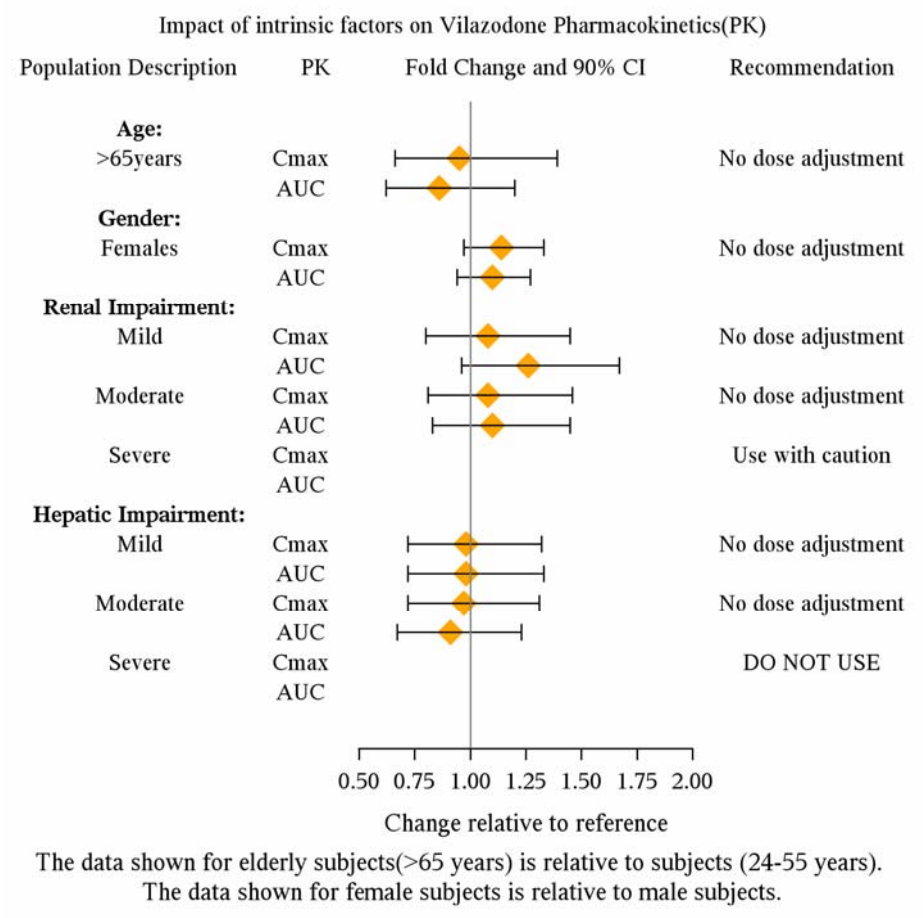
Most of the adverse events occurred during the initial titration phase (10 mg/day for 7 days (Days 1-7), 20 mg/day for 7 days (Days 8-14) in comparison to maintenance phase (40 mg/day for 42 days (Days 15-56)). Adverse events are dose dependent (details see Section 3.2 Pharmacometric Review).

2.2.2.5 Does this drug prolong QT/QTc Interval?

No, vilazodone does not meaningfully prolong QTc.（Refer to Dr. Joanne Zhang's Thorough QT Study Review for details).

2.2.3 Intrinsic Factors

Figure 6. Impact of intrinsic factors on vilazodone PK.



2.2.3.1 What intrinsic factors influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Higher body weight correlated with lower Cmax and AUC. Elderly and young subjects showed similar PK profiles for vilazodone. After adjustment for body weight, the systemic exposures (AUC) between males and females are similar. See Figure 6 above.

2.2.3.2 Effect of Renal Impairment

The effect of mild and moderate renal impairment on the single-dose PK of orally administered vilazodone 20 mg tablet was evaluated. The effect of severe renal impairment on PK of vilazodone has not been evaluated.

The PK profiles were similar between patients with mild and moderate renal impairment and normal healthy subjects. See Figure 6 above.

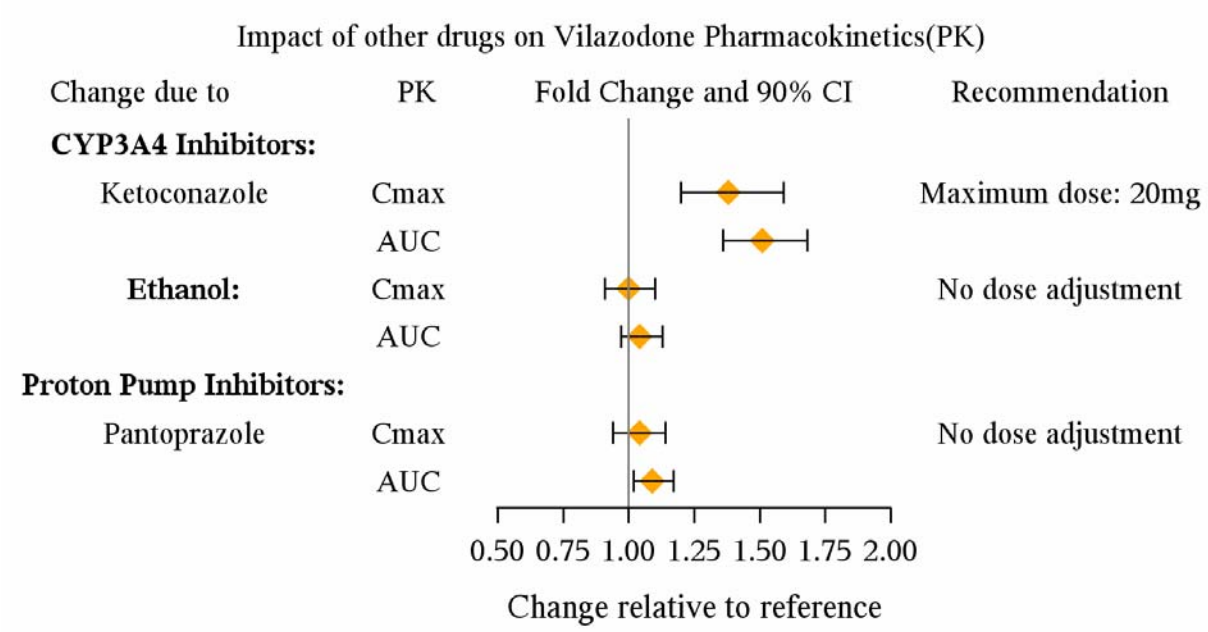
2.2.3.3 Effect of Hepatic Impairment

The effect of mild and moderate hepatic impairment on the single-dose PK of orally administered vilazodone 20 mg tablet was evaluated. The effect of severe hepatic impairment on PK of vilazodone has not been evaluated.

The PK profiles were similar between patients with mild and moderate hepatic impairment and normal healthy subjects. See Figure 6 above.

2.2.4 Extrinsic Factors

Figure 7. Impact of other drugs on vilazodone PK.



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

Food Effect
The effect of high fat (high calorie) and light meal (low calorie) on single-dose PK of orally administered vilazodone 20 mg tablet was evaluated.

The type of food (e.g., high fat or light meal) did not appear to be influential. Meal significantly increased systemic exposure (AUCinf) of vilazodone by 64-85%, and increased Cmax of vilazodone by 147-160%.

The sponsor proposed that vilazodone should be taken with food in the label based on the higher systemic exposure under fed conditions.

Ethanol Effect
The effect of ethanol (30 mL) on single-dose PK of orally administered vilazodone at 40 mg tablet was evaluated. The co-administration of 30 mL of ethanol had no significant effect on the absorption of vilazodone. See Figure 7 above.

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

In vitro metabolism studies in human liver microsomes indicated that vilazodone is mainly metabolized by CYP3A4/5, with minor contribution from CYP2C19, CYP2D6, and possibly non-CYP enzymes. CYP1A2, CYP2A6, CYP2C9, and CYP2E1 have minimal contribution to the metabolism of vilazodone.

2.2.4.3 Is the drug a substrate of CYP enzymes?

Vilazodone is mainly metabolized by CYP3A4/5, with minor contribution from CYP2C19, and CYP2D6. CYP1A2, CYP2A6, CYP2C9, and CYP2E1 have minimal contribution to the metabolism of vilazodone. Several CYPs are identified in the biotransformation of vilazodone using specific chemical inhibitors (troleandomycin 50 μM for CYP3A4, quinidine 25 μM for CYP2D6, S-mephenytoin 500 μM for CYP2C19, furafylline 5 μM for CYP1A2, coumarin 2.5 μM for CYP2A6, suphaphenazole 24 μM for CYP2C9, and diethyldithiocabamate 50 μM for CYP2E1) in human liver microsomes (study GPP-007-CLN-ANR-1997-028). Incubation of radioactive vilazodone in human hepatocytes also identified metabolites from non-CYP pathways (about 27% of vilazodone metabolism). Studies with CYP3A inducers should be conducted in vivo.

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

In vitro inhibition

Yes, vilazodone is a moderate inhibitor for CYP2D6 (IC50 = 2.8 μM) in human liver microsomes.

Human liver microsomes were incubated with selective CYP450 substrates in the presence of vilazodone (Study GPP-007-CLN-ANR-1997-028). IC50 value for CYP3A4 and CYP2D6 were 68 μM, and 2.8 μM, respectively. The mean steady state concentration Cmax for vilazodone following 40 mg once daily dosing was 0.33 μM in a pivotal trial (PGX-08-P1-06).

Study GPP-007-CLN-ANR-1998-029 demonstrated that vilazodone is a weak inhibitor for other CYPs in pooled human liver microsomes with Ki values of 81.7 μM, 24.0 μM,

114 μM for CYP1A2, CYP2C9, and CYP3A4, respectively. It showed moderate inhibition toward CYP2C19 with a Ki value of 7.37 μM.

***In vitro* induction**

No, vilazodone is not a potent inducer for major CYPs based on a human hepatocyte study.

Noticeable increase in mRNA expression was only observed for CYP2D6 (2.4x), and CYP3A4 (2.2x) for 60 hr incubation with hepatocytes from donor HB386 among all the incubations for the CYPs. Little/no increases were observed in mRNA expression for CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1, and CYP3A5. Significant induction of CYPs by vilazodone is not expected in vivo.

2.2.4.5 Is the drug a substrate, an inhibitor and/or an inducer of transporter processes?

No studies were performed by the Sponsor to evaluate if vilazodone is a substrate, inhibitor, or inducer of the transporters (e.g., P-glycoprotein).

2.2.4.6 Are there other metabolic/ transporter pathways that may be important?

None that are known

2.2.4.7 Influence of vilazodone on other drugs

A cocktail study to evaluate the effect of vilazodone (20 mg QD for 8-10 days) at steady-state on substrates of five CYP isoenzymes (3A4, 2D6, 2C19, 2C9, and 1A2) was conducted by the sponsor.

Vilazodone had no effect on the pharmacokinetics of caffeine, flurbiprofen, nifedipine or debrisoquine, probes for CYP1A2, CYP2C9, CYP3A4 and CYP2D6, respectively. However, vilazodone coadministration with mephenytoin to healthy subjects resulted in a small (11%) increase in mephenytoin biotransformation, suggestive of a minor induction of CYP2C19.

2.2.4.8 Influence of other drugs on vilazodone

2.2.4.8.1 DDI with Ketoconazole

The effect of strong CYP3A4 inhibitor ketoconazole (200 mg QD at steady-state) on single-dose PK of vilazodone at 5 mg or 10 mg was evaluated.

200 mg Ketoconazole increased AUC of vilazodone by ~50%. Ketoconazole did not change the mean/median half-life of vilazodone – indicating that inhibition of pre-systemic metabolism is likely the principal mechanism. The absolute bioavailability of vilazodone is 72% with food. Although, FDA recommends 400 mg QD of ketoconazole, the OCP reviewer concludes that 200 mg provided almost complete inhibition of pre-systemic metabolism rendering the absolute bioavailability to be ~100%. Additionally, vilazodone is metabolized through multiple pathways, i.e., non- CYP (~40%) and CYP (~60%) pathways. Also, multiple isoenzymes were identified to metabolize vilazodone although CYP3A4 is major isoenzyme. Further, PBPK simulations indicated that the inhibition could be 85% at 200 mg BID ketoconazole. The PBPK simulations depict a

worst-case scenario that might not reflect the observed results after 200 mg ketoconazole inhibition. The simulations assume that after 400 mg ketoconazole all the inhibition occurs systemically. Whereas, the observed results at the 200 mg dose support that a majority of inhibition occurs pre-systemic. Nevertheless, the simulations at the 400 mg dose support the lack of a need for another inhibition study. Vilazodone dose in either case would be limited to 20 mg. See Figure 7 above.

2.2.4.8.2 DDI with PPI

The effect of proton pump inhibitor (PPI), pantoprazole (40 mg QD for a week), on single-dose PK of vilazodone at 40 mg was evaluated. The systemic exposure is similar between vilazodone alone and vilazodone combined with pantoprazole. See Figure 7 above.

2.2.5 Does genetic variation impact exposure, efficacy, and safety profiles?

2.2.5.1 Are drug metabolizing enzyme (CYP2C19, CYP2D6) gene variants associated with variable response to vilazodone?

No. CYP2C19 genotype was assessed in one pivotal study and showed no consistent pharmacogenetic effect. CYP2C19 genotype did not appear to associate with vilazodone discontinuation rates or failure to reach target dose. CYP2D6 genotype was assessed in two pivotal studies and showed no association with differential drug response. The pharmacogenetics of CYP3A4, the major metabolic pathway, were not reviewed due to small numbers of patients with genetic variants and genetic redundancy with CYP3A5.

2.2.5.2 Effect of Vilazodone on MADRS by CYP2C19 genotypes

CYP2C19 genotype did not influence vilazodone response in pivotal study GNSC-0402. PMs and UMs (who genetically represent the expected extremes of the response spectrum) achieved similar responses to vilazodone (33.3% vs. 25%) as well as similar changes in MADRS total score. There was no gene-dose effect for CYP2C19 and MADRS response. Though the numbers were small, none of the 6 PMs required dose reductions or discontinuation of vilazodone. These neutral results may be explained, in part, due to the minor metabolic contribution of CYP2C19 on vilazodone metabolism.

2.2.5.3 Effect of Vilazodone on MADRS by CYP2D6 genotypes

No significant differences were seen in proportion or magnitude of MADRS response among the different CYP2D6 genotypes in study GNSC-0402.

2.2.5.4 Are ACE gene variants associated with variable response to vilazodone?

No. Initial associations showing a differentially greater response in certain ACE genetic subgroups did not replicate when performing responder analyses in the second pivotal study CLDA-0702. Pooled analyses of the studies did not show a robust association between ACE genotypes and 8-week change in MADRS from baseline as a continuous dependent variable.

2.2.6 Does vilazodone significantly affect metabolic and stress hormone concentrations (e.g., growth hormone, prolactin, ACTH, and cortisol)?

No. In early phase clinical studies, transient elevation in growth hormone concentrations, a known effect of 5-HT1A agonists, was seen at the individual level. Transient stimulation in cortisol was also observed. However, the vilazodone phase 3 and long-term safety databases do not suggest meaningful drug-associated adverse events that could be attributable to sustained growth hormone or cortisol elevations such as hypertension, dysglycemia/diabetes, hypokalemia, and musculoskeletal pain. Of note, palpitations occurred at a significantly higher rate in vilazodone-treated patients.

2.2.7 General Biopharmaceutics

2.2.7.1 Based on the biopharmaceutic classification system principles, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?

BCS classification was not sought and determined by the sponsor. Vilazodone has relatively high aqueous solubility (water: 32 mg/100 mL) and the absolute bioavailability is 72% with food.

2.2.7.2 How is the proposed to-be-marketed formulation of vilazodone linked to the clinically used formulation?

Early clinical studies (1996-2001) for vilazodone used capsule formulations varying in strength from 1 mg to 80 mg. Certain of the subsequent clinical studies used both 10 mg and 20 mg IR tablet formulations (T-10 and T-20) manufactured using a (b) (4) process. Formulations T-10 and T-20 were used in the first Phase 3 placebo-controlled study (GNSC-04-DP-02), which was conducted by PGxHealth.

The to-be-marketed formulation of vilazodone is IR tablet made using a (b) (4) process in 3 strengths at 10, 20, and 40 mg (PG-10, PG-20, and PG-40), which are (b) (4) through the 3 strengths. PG-10 and PG-20 were used in the second placebo-controlled study (CLDA-07-DP-02), and PG-40 was used in the long-term safety study. The sponsor also conducted a BE study which showed T-20 formulation and PG-20 formulation were equivalent:

Parameter N=44	PG-20 Tablet	T-20 Tablet	% Mean Ratio	90% CI
AUC_{0-t} (hr*ng/mL)	1586	1748	91	87 - 95
$AUC_{0-\infty}$ (hr*ng/mL)	1627	1790	91	87 - 95
Cmax (ng/mL)	93	103	90	85 - 96

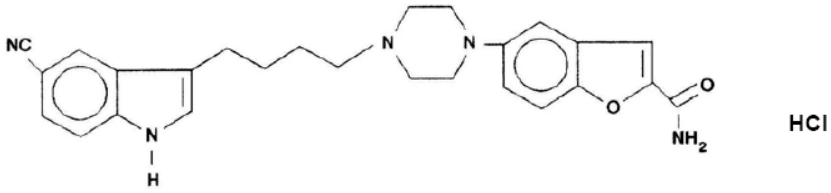
2.2.7.3 What is the effect of food on the bioavailability of the drug when administered as solution or as drug product?

See Section 2.2.4.1.

2.2.8 General Attributes of the Drug

2.2.8.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

Vilazodone HCl is a new chemical entity indolalkylamine. The full chemical designation is 2-benzofurancarboxamide, 5-[4-[4-(5-cyano-1*H*-indol-3-yl) butyl]-1-piperazinyl]-, hydrochloride (1:1) with a molecular weight of 477.99 g/mol. The structure is presented in the figure below:



Vilazodone HCl is formulated as film-coated immediate-release tablets with three dosage strengths of 10 mg (PG-10), 20 mg (PG-20) and 40 mg (PG-40). The core tablets of the three strengths for vilazodone are (b) (4).

The intended commercial tablets, PG-10 and PG-20, were used in one of two pivotal studies, CLDA-07-DP-02.

2.2.8.2 What are the proposed mechanism of action and therapeutic indications?

Vilazodone combines the established mechanisms of serotonin reuptake inhibition with partial agonism of 5-HT$_{1A}$ receptors, which is thought to optimize regulation of 5-HT circuitry at both pre- and postsynaptic sites to robustly augment 5-HT neurotransmission, thereby producing an antidepressant effect. Vilazodone is indicated for the treatment of major depressive disorder (MDD).

2.2.8.3 What are the proposed dosages and routes of administration?

Vilazodone is administered orally. The recommended dose is 40 mg QD with food after titration, starting with an initial dose of 10 mg once daily for 7 days followed by 20 mg once daily for an additional 7 days.

2.2.9 List the in vitro and in vivo Clinical Pharmacology and Biopharmaceutics studies and the clinical studies with PK and/or PD information submitted in the NDA

The current submission consisted of 24 clinical pharmacology studies including a thorough QTc study and 9 in vitro studies (Ref: OCP NDA Filing and Review Form). The 5 Phase II studies in the submission were not considered supportive of efficacy claim. The Phase III program consisted of 3 studies: 2 for well-controlled 8-week pivotal studies assessing the efficacy and safety of vilazodone in major depressive disorder (MDD), and 1 for uncontrolled long-term (52-week) safety study.

2.2.10 Analytical Section

2.2.10.1 Are the active moieties in plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes. HPLC with fluorescence detection and LC-MS/MS were developed for the determination of vilazodone, the active moiety, in plasma/urine/feces in healthy subjects.

2.2.10.2 What bioanalytical methods are used to assess concentrations and is the validation complete and acceptable?

During the development of vilazodone, there were three assays used for determination of vilazodone concentrations in human biomatrix samples:

- HPLC assay with fluorescence detection (developed by Merck, as part of the original development program).
- LC-MS/MS assay (developed by GSK in support of their development program).
- LC-MS/MS assay (developed for PGxHealth by (b) (4)

The analytical method developed for the analysis of vilazodone was adequately validated and acceptable. For the detail information, please see section of Analytical Analysis under Section 3 Individual Study Review ANALYTICAL SECTION.

(b)

3. Individual Study Review

3.1 Clinical Pharmacology Review

BA/BE STUDIES

Study 1, *Pivotal BE Study*: CLDA-07-DP-01

Title: A relative bioequivalence study of 20 mg vilazodone (b) (4) tablets versus 20 mg vilazodone (b) (4) tablets
Investigator: (b) (4)
Study center: (b) (4)

Study period: 07 July 07 - 17 August 07

Objectives	Primary: To compare the relative bioavailability (rate and extent of absorption) of 2 x 20 mg Vilazodone (b) (4) Tablets with 2× 20 mg of Vilazodone (b) (4) Tablets following a single oral dose in healthy adult volunteers
Study Design and Methodology	Double-blinded (open-label study in the protocol), randomized sequence, single-dose, two-way crossover bioequivalence study. In each study period, a single 40 mg dose (2×20 mg (b) (4) or (b) (4) tablets) was administered to all subjects following a light breakfast initiated 25 minutes prior to dosing. The subjects received the test product in one study period and the reference product in the other study period. Drug administration occurred according to the dosing randomization schedule. There was a 14-day washout interval between treatments.
Study population	One hundred and two healthy adult subjects were enrolled, 44 subjects were in PK analysis population
Investigational drug	

Treatment	Formulation identifier	Strength	Batch Number	Manufactured date
Test Drug (b) (4) Tablet)	PG-20	20 mg	07T6431	05/11/2007
Reference Drug (b) (4) Tablet)	T-20	20 mg	Merck 21238	08/25/2004

Dose	40 mg (2×20 mg single dose)
PK Assessment	During each study period, 19 blood samples were collected (6 mL each) from each subject within one hour prior to dosing (0 hour) and after dose administration at study hours 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120 and 144.
Study duration	The time from when the first subject was dosed to when the last subject completed the study (i.e., the date the last PK sample was taken) was 41 days

Formulations: Test product was manufactured by Merck in 2007 and Reference product was manufactured by Patheon in 2004. The detailed information of both products is given in the table below.

Product	Reference	Test
Treatment ID	A	B
Product Name	Vilazodone 20 mg (b) (4) (b) (4) Tablets	Vilazodone 20 mg (b) (4) (b) (4) Tablets
Manufacturer	Manufactured for Clinical Data, Inc. By Merck KGaA, Darmstadt, Germany	Manufactured for Clinical Data, Inc. By Patheon/MOVA, Caguas, Puerto Rico
Batch/Lot No.	21238	07T6431
Manufacture Date	08/25/04	05/11/07
Expiration Date		N/A
Strength	(20 mg	20 mg
Dosage Form	(b) (4) Tablet	
Dose Administered	2 x 20 mg	2 x 20 mg
Route of Administration	Oral	Oral
Cumulative Maximum Dose	40 mg	40 mg
Manufacturing Process:	(b) (4)	
Solid State (b) (4) content):		
Assay (% label claim):	(b) (4)	
Formulation:	Provided in Appendix 16.2.5.2	Provided in Appendix 16.2.5.2

Results: Of 102 subjects who enrolled the study, 44 subjects received a single dose of both reference and test product (reference product: 73 subjects; test product: 76 subjects) and completed the study. Fifty eight subjects were discontinued for AEs (50 subjects), abnormal laboratory results (3 subjects) or withdrew consent (5 subjects). Two subjects who received both treatments but vomited prior to 10 hours post-dose were not included in the PK analysis. The bioequivalence between vilazodone (b) (4) Tablets and vilazodone (b) (4) Tablets was demonstrated. The median Tmax was estimated to be 5 hours post-dose for both formulations.

Table 1. Summary statistics for vilazodone (b) (4) Tablets vs. vilazodone (b) (4) Tablets (source: Table 11.3 in the report)

Geometric Means, Ratio of Means, and 90% Confidence Intervals Log-Transformed Data Vilazodone N=44				
Parameter	Test Product (vilazodone (b) (4) Tablets)	Reference Product (vilazodone (b) (4) Tablets)	%Ratio	90% CI
AUC_{0-t} (hr*ng/mL)	1585.94	1747.45	90.76	86.63, 95.08
$AUC_{0-\infty}$ (hr*ng/mL)	1627.08	1789.94	90.90	86.89, 95.10
Cmax	92.93	102.89	90.32	85.00, 95.98

(ng/mL)				

No serious adverse events (SAEs) were reported over the course of the study. However, 83 subjects experienced a total of 265 adverse events over the course of the study. Two hundred (75.4%) AEs were of mild intensity and 65 (24.5%) were of moderate intensity.

Ref drug: Nausea - 29/73 (39.7%), vomiting - 24/73 (32.9%)
Test drug: Nausea – 34/76 (44.7%), vomiting – 27/76 (35.5%)

Overall, vilazodone administered as a single dose of 40 mg (2×20 mg (b) (4) Tablets or (b) (4) Tablets) showed poor gastrointestinal tolerability.

REVIEWER'S COMMENT:
1. Note: the protocol was written as an open-label study but a change in study conduct occurred at the request of the sponsor. The protocol deviation is listed in the protocol deviation log in Table 10.3 of the report.
2. The to-be-marketed formulation and the formulation used in the first phase 3 clinical trial (GNSC-04-DP-02) were found to be bioequivalent.
3. A single dose of 40 mg (2×20 mg) vilazodone administration showed poor gastrointestinal tolerability. This study was initiated with 60 subjects (36 males and 24 females). However, the sponsor had to replace 42 subjects due to emesis. Among total of 102 subjects, 44 subjects completed the study.

==========

Study 2*, Pivotal Food Effect Study with To-Be-Marketed Formulation*: PGX-08-P1-05

Title: Open Label, Single-Dose, Randomized Sequence, 3-Period Cross-Over Study to Determine the Effect of Food on the Relative Bioavailability of the Vilazodone Oral Tablet Formulation in Healthy Volunteers
Investigator: Melanie Fein, M.D.
Study center: Comprehensive Phase One, Fort Myers, 3745 Broadway Ave. Fort Myers, FL 33901
Study Period: 14 October 2008 – 14 July 2009

Objectives	Primary: To determine if a high fat (high calorie) meal affects the relative bioavailability of oral vilazodone (b) (4) tablets in healthy volunteers; To determine if light (low calorie) meal affects the relative bioavailability of oral vilazodone (b) (4) tablets in healthy volunteers Secondary: To evaluate the effect of food on the safety and tolerability of oral vilazodone in healthy volunteers.
Study Design	Phase I, open-label, randomized sequence, 3-period crossover, multiple-dose (3 single doses with a 7-day washout period between doses), PK study to assess the effect of coadministration of food (light meal or high fat meal) on the absorption of vilazodone in healthy subjects. Subjects were enrolled and randomized to 1 of the 6 treatment sequences. For the dose with a meal, the vilazodone was administered within 30 minutes of starting the light or high fat breakfast. The washout period between treatments was 7 days. Subjects who experienced emesis within 10 hours after a vilazodone dose were to be

<table>
<tr><td></td><td>withdrawn from the study. The effect of food on the plasma PK as well as on the safety and tolerability of vilazodone in healthy subjects was also examined.</td></tr>
<tr><td>Study population</td><td>Twenty subjects (9 females, 11 males; aged 20-55 years) were enrolled and 18 subjects were included in PK analysis.</td></tr>
<tr><td>Investigational drug and Treatment</td><td>
<table>
<tr><th>Treatment Sequence</th><th>Dose</th><th>Formulation Identifier</th></tr>
<tr><td>Fasting→light meal →high fat meal</td><td>20 mg</td><td>PG-20</td></tr>
<tr><td>Fasting →high fat meal →light meal</td><td>20 mg</td><td>PG-20</td></tr>
<tr><td>Light meal →high fat meal →fasting</td><td>20 mg</td><td>PG-20</td></tr>
<tr><td>Light meal →fasting →high fat meal</td><td>20 mg</td><td>PG-20</td></tr>
<tr><td>High fat meal→light meal →fasting</td><td>20 mg</td><td>PG-20</td></tr>
<tr><td>High fat meal →fasting →light meal</td><td>20 mg</td><td>PG-20</td></tr>
</table>
</td></tr>
<tr><td>Dosage</td><td>Three-single doses of vilazodone 20 mg with 7-day washout between doses</td></tr>
<tr><td>PK Assessment</td><td>Blood samples for PK assessments were drawn at 5 minutes pre-dose, and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, and 144 hours post-dose.</td></tr>
<tr><td>Safety Assessment</td><td>AEs were assessed daily basis for 7 days post-dose in each period.
Vital signs (respiratory rate, sitting radial pulse rates, and sitting systolic and diastolic blood pressures) were collected at all study visits and on dosing days (Days 1, 8, and 15) at 30 minutes pre-dose and 4 and 8 hours post-dose. 12-lead ECGs were performed at Screening, pre-dose (Days 1, 8, and 15) and 24 hours post-dose (Days 2, 9, and 16), and at early termination, as applicable. A urine drug screen and an alcohol breath test (breathalyzer test conducted at the investigational site) were performed at Screening and upon clinic admission (Days -1, 7, and 14).
A physical examination was performed at Screening, upon readmission to the clinic (Days 7 and 14), prior to discharge (Days 2, 9, and 16), and at end of study (Day 21) or early termination.</td></tr>
</table>

Results: The results are shown in the table below.

Table 1. Statistical analysis of plasma vilazodone PK parameters (Source: Table 8 in the report)

	Comparison	Ratio of Least Square Geometric Means[a]	90% CI[a]
$AUC_{0\text{-}tlqc}$	Light meal/Fasting	1.69	1.56, 1.83
	High fat meal/Fasting	1.92	1.77, 2.08
	High fat meal/Light meal	1.13	1.04, 1.22
$AUC_{0\text{-}\infty}$	Light meal/Fasting	1.64	1.51, 1.76
	High fat meal/Fasting	1.85	1.70, 1.99
	High fat meal/Light meal	1.13	1.04, 1.21
Cmax	Light meal/Fasting	2.47	2.19, 2.77
	High fat meal/Fasting	2.60	2.31, 2.92
	High fat meal/Light meal	1.05	0.93, 1.18

[a] Log-transformed PK parameters are analyzed using an ANOVA with fixed effect treatment group, treatment sequence, and period. Ratio and Confidence Intervals were based on the exponentiated treatment differences.

REVIEWER'S COMMENTS:

1. There was a significant food effect on vilazodone PK following single dose of 20 mg vilazodone (PG-20). Administration of vilazodone with food had little effect on Tmax or $T_{1/2}$.
2. The type of food (e.g., high fat meal or light meal) did not appear to be influential. The sponsor states in the proposed label that the dose administered with a light meal is bioequivalent to that administered with a high fat meal.
3. The incidence of AEs was lower when vilazodone 20 mg (b) (4) tablets were administered under fasting conditions than when taken with food in healthy adult subjects.

==========

Study 3*, Absolute BA Study*: PGX-08-P1-08

Title: Open label, single-dose, randomized sequence, 2-period cross-over study to determine the relative** bioavailability of the vilazodone oral tablet formulation vs. intravenous (IV) formulation in healthy volunteers

Study Period: 13 October 2008 – 10 November 2008

Objectives	To determine the relative** bioavailability of vilazodone oral tablet formulation vs. an IV formulation in healthy volunteers; To evaluate the safety and tolerability of vilazodone administered as oral and IV formulations in healthy volunteers.
Study Design	Phase 1 open-label, single-dose, randomized sequence, 2-period crossover pharmacokinetic (PK) study to assess the effect of oral vs. IV administration on the bioavailability and safety and tolerability of vilazodone in healthy subjects. Subjects were randomized to one of two treatment sequences: • A 20 mg oral dose of vilazodone followed 7 days later by a 5 mg IV dose of vilazodone • A 5 mg IV dose of vilazodone followed 7 days later by a 20 mg oral dose of vilazodone (single 20 mg tablet) with 240 mL of water. Dosing occurred in the morning with light breakfast. Dose #1 was administered on Day 1, and subjects were discharged on Day 2. Subjects were re-admitted to the inpatient unit on Day 7 for Period 2 and had all baseline evaluations repeated. Dose #2 was administered on Day 8, subjects were discharged on Day 9, and outpatient follow-up continued on Days 10 to 14. Six subjects completed PK assessments and were therefore evaluable for each treatment sequence. Subjects who experienced emesis within 10 hours after their first vilazodone dose were to be discontinued from the study. Replacement subjects were enrolled until at least 12 subjects completed both treatment periods. Replacement subjects were assigned to treatment sequence to maintain the balanced design.
Study population	Twenty one healthy subjects were screened and 12 subjects (7 males and 5 females; aged 19-44 years) completed the study and were included in PK assessment.
Investigational drug	<table><tr><th>Treatment</th><th>Dose*</th><th>Formulation</th></tr></table>

	Sequence | | Identifier Oral-IV | 20 mg-5 mg | PG-20 IV-Oral | 5 mg-20 mg | IV * IV dosing was 5 mg (205 mL) infused over 4 hours
Dose	Two doses of vilazodone in two sequences: 5 mg of IV with 205 mL infusion for 4 hours or 20 mg oral single dose with 7-day washout between treatments
PK Assessment	Blood samples for PK assessment were drawn in Period 1 at 5 minutes pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120 and 144 hours post-dose. Plasma samples were analyzed by LC/MS/MS for the concentration rage of 0.8119 to 270.6 ng/mL of vilazodone HCl.
Safety Assessment	AEs were assessed on a daily basis until 7 days post dose in each period. Vital signs and concomitant medication were assessed at Screening, Days -1, 1, 2, 3, 4, 5, 6, and 7 in each period. 12-leave ECG was performed at Screening, Days 1 and 2. Safety laboratory test was performed at Screening, Days -1, 1, and 2. TSH, T3 and T4 assessment were performed at Screening and End of Study/Early Termination.

****Note:** Although the absolute bioavailability is investigated in this study, the sponsor uses "relative bioavailability" in its report.

Results: The bioavailability of the oral vilazodone formulation relative to the IV formulation was 72% to 81% based on dose-normalized AUC ($AUC_{0-\infty}/D$). The PK parameters after IV administration and oral administration of 20 mg are summarized in the table below. The most common AEs were nausea reported by 3 (25%) subjects and diarrhea by 2 (16%) subjects. No reports of any SAEs.

Figure 1. Mean Vilazodone HCl Concentrations after IV infusion and Oral administration (source: Figure 2 in the report)

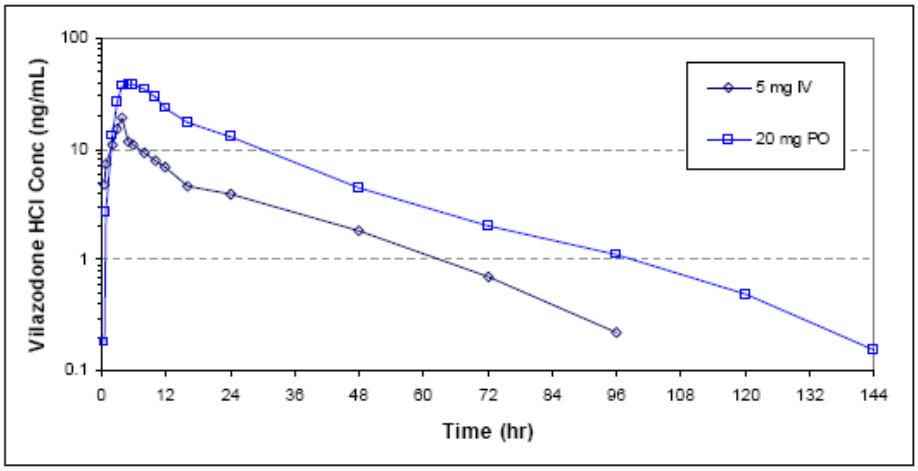


Table 1. Summary of Vilazodone HCl PK parameters (Source: Table 5 in the report)

Parameter[a]	5 mg IV over 4 hr (n=12)	20 mg Oral (n=12)
Cmax (ng/mL)	19.1 (35.5)	43.4 (37.2)

$AUC_{0-\infty}$ (ng*hr/mL)	320 (37.1)	920 (34.6)
Tmax (hr)[b]	4.0 (3.0-4.0)	5.0 (3.0-8.0)
Cmax/Dose (ng/mL/mg)[c]	3.82 (35.5)	2.17 (37.2)
$AUC_{0-\infty}$/Dose (ng*hr/mL/mg)	64.0 (36.9)	46.0 (34.7)
CL [CL/F for oral] (L/hr)	17.7 (38.2)	24.3 (34.5)
V [V/F for oral] (L)	605 (46.1)	864 (54.1)
$T_{1/2}$ (hr)	25.4 (42.2)	26.5 (53.3)

[a] Arithmetic Mean (%CV)
[b] Median (range)
[c] Cmax for IV infusion could be biased because it was influenced by the infusion time

Table 2. Bioavailability Analysis (Source Table 6 in the report)

Parameter	Oral/IV Ratio (%)	90% CI
Cmax/Dose	56.0	49.9-62.7
AUC_{0-t}/Dose	80.7	72.7-89.6
$AUC_{0-\infty}$/Dose	72.3	65.7-79.6

REVIEWER'S COMMENTS: The sponsor used the actual sample collection time and estimated actual dose for PK analysis. There were differences in nominal dose and the actual dose for both oral dose and IV dose. The average concentration of the prepared solution was estimated to be 4.686 mg (nominal dose 5 mg) and the oral dose was adjusted for vilazodone potency as reported on the Certificate of Analysis (assay, 98.7%) resulting in an estimated actual dose of 19.74 mg (nominal dose was 20 mg). This is acceptable.

==========

IN VITRO METABOLISM STUDIES

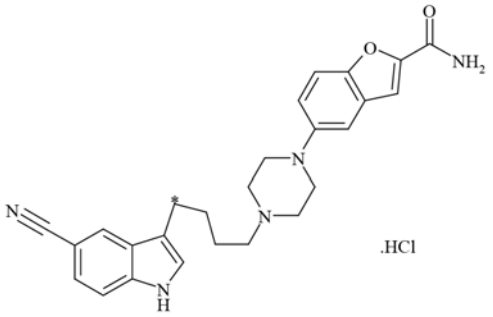
Study 1, *In vitro Metabolism of 14C-EMD 68843 in Hepatocytes*

The *in vitro* metabolism of [^{14}C]SB-659746-A in the rat, mouse, female rabbit, dog, cynomolgus monkey and man

Study Number: GPP-007-NCD-PKM-2002-034
Note: only human data was reviewed in this report
Objective: To provide preliminary information on the likely metabolic routes of SB-659746 in the preclinical animals and human using in vitro systems.
Materials:

[^{14}C]SB-659746-A (hydrochloride salt, EMD 68843 batch 7, specific activity 116 uCi/mg, stated radiochemical purity 99.7%, chemical purity 99.8%) was supplied as a solid by Merck KGaA, Germany.

The structure of [^{14}C]SB-659746-A is shown as follows:



* Denotes position of [^{14}C]radiolabel

A stock solution of [^{14}C]SB-659746-A (nominally 5 mM pure free base) was prepared by dissolution in dimethyl sulphoxide (DMSO). This was subsequently added to hepatocyte preparations to achieve final incubation concentrations of 10 and 50 uM.

Methods:

Human hepatocyte incubations (n=6) were conducted with hepatocyte monolayers maintained within Williams Medium E incubation media supplemented with antibiotics. 10 and 50 uM [^{14}C]SB-659746-A (free base) was incubated with human hepatocytes for 0, 4 and 24 hours. Appropriate controls without hepatocytes and without drug were performed in parallel over the same time periods. 7-Ethoxycoumarin was incubated with hepatocytes at 25 uM in parallel with compound incubations (0, 1, 2, 3 and 4 hour) as a positive control. Following incubations, all samples were subsequently stored at *ca.* -80°C prior to analysis and when not in use.

Radioassay of Samples

For all incubations, the media (*ca.* 0.6 mL) was removed and 1 mL of methanol added to each well and the cells were scraped from the well surface. Then methanol was combined with the media and made up to a 1.8 mL with additional methanol. Each sample was centrifuged at approximately 7,500g at room temperature for 5 minutes and aliquots of the supernatant were radioassayed by liquid scintillation counting (LSC) following the addition of Ultima Gold scintillant, to determine the recovery of radiolabelled material.

Quantitative Radio-HPLC Metabolite Profiling and LC/MS

Radiometabolite profiles were determined for 4 and 24 hour hepatocyte incubates at each concentration (10 and 50 uM). Supernatants (diluted 1:1 (v/v) with water) were analysed directly by on-line radio-HPLC. LC/MS or LC/MS/MS assay was used in parallel with radio-HPLC to assist the identification of radiolabelled metabolites and confirm peak assignments.

Results:

The average hepatocyte viability in this experiment was about 68.3%, though the method to demine cell viability was not specified in the report. The rates of disappearance of ethoxycoumarin (25 uM) in incubations with human hepatocytes were within the expected range. The mean radioactivity recovery was greater than 92%.

Table 1. Source, viability and ethoxycoumarin clearance of human hepatocyte preparations				
Hepatocyte	**Reference Date**	**Source**	**Viability (%)**	**EC-CLi mL/min/g**
Human 1	30-Aug-01	(b) (4)	76	NC
Human 2	02-Sep-01		62	NC
Human 3	29-Sep-01		81	NC
Human 4	11-Sep-01		68	0.16
Human 5	17-Oct-01		60	0.20
Human 6	20-Oct-01		63	0.43

Metabolism of [^{14}C]SB-659746 in Human Hepatocytes

Incubations at 10 uM SB-659746 for 24 hours were used to quantify the primary routes of metabolism of SB-659746 due to the greater extent of metabolism when compared to the 50 uM samples. For mass spectrometry analysis, the 50 uM incubation samples were used due to the greater concentration of drug-related material in each incubate (the radiometabolite profiles in the 50 uM samples were qualitatively similar to those in the 10 uM samples).

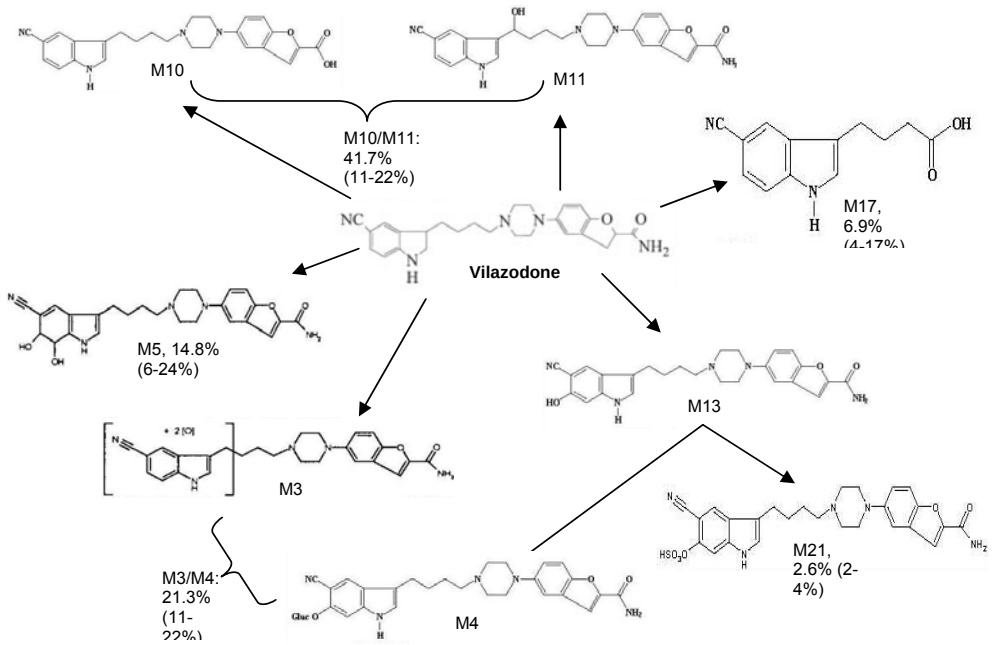
In all human (n=6) livers examined (10 uM, 24 hour), the principal metabolic components were M/10/M11, formed *via* hydrolysis of the amide moiety of SB-659746 and hydroxylation of SB-659746, respectively) and M5 (SB-659746 dihydrodiol) representing means of 42 and 15% of the total metabolism, respectively. Peaks M3 (dihydroxy SB-659746) and M4 (glucuronide of hydroxylated SB-659746), together accounted for a mean of *ca.* 21% of the total metabolism. Peak M17 (butyric acid derivative) was also observed in man and accounted for 4-17% of the total metabolism. Peak M21 (sulphate conjugate of hydroxylated SB-659746) accounted for a mean of 3% of the total metabolism.

Table 2: Metabolites of Vilazodone from Human Hepatocytes at 10 uM for 24 hrs (data expressed as % of total SB-659746 metabolized)					
	Mean % of Total Vilazodone Metabolized				
	M3/M4	M5	M10/M11	M21	M17
Human 1	18.8	11.0	22.1/17.4	2.3	11.0
Human 2	14.0	18.0	19.0/18.2	1.8	16.6
Human 3	21.9/11.1	5.9	11.6/23.3	1.9	-
Human 4	17.8	10.7	26.9/27.0	3.4	4.3

Human 5	10.5/14.4	24.0	39.0	2.5	4.4
Human 6	19.3	19.4	45.8	3.8	4.9
Mean	**21.3**	**14.8**	**41.7**	**2.6**	**6.9**

The total radio-activity of these identified metabolites accounts for 87.3% of the total radio-activity in the incubation system.

Figure 1. Metabolic scheme of vilazodone in human hepatocytes based on Study GPP-007-NCD-PKM-2002-034 _constructed by the reviewer



Reviewer's Comments

The sponsor has identified the metabolic pathways in human hepatocytes, however, enzymes involved in the biotransformation steps are not identified. An elucidated, clear human metabolic scheme for vilazodone need to be submitted with identified enzymes. Since validation report of the analytical method is not provided, acceptance of the data is based on the assumption that the method used is validated.

==========

Study 3*, CYP Enzyme Identification*

Identification of the cytochrome P450 isoforms involved in the metabolism of vilazodone

Study Number: GPP-007-CLN-ANR-1997-028 (Original Merck Study No. CLG 00301)

Objective: To identify CYP450 isoforms involved in the metabolism of vilazodone from pooled human liver microsomes.

Materials:

Non-radiolabelled EMD 68843 (release number EE 77185) was provided in powder and stored at 4ºC protected from light. ^{14}C-labelled EMD 68843 (batch number 3, Z7/215; specific activity 112 pCi/mg, 53.5 mCi/mmol) was received as a lyophilised powder and stored at 4ºC protected from light until reconstituted. Human liver microsomes (HHM-0220, pool of 5 individual livers) were obtained from the (b) (4)

Methods:

Pooled human liver microsomes were used to evaluate vilazodone metabolism in vitro. The following tables list the incubation conditions for the experiment.

Table 1. Incubation conditions in pooled HLM (0.5 mg/mL) for characterizing EMD68843 metabolism	
Total volume (uL)	250
NDAPH regenerating system (uL)	125
EMD68843 (uM)	1-16.7
Incubation time (min)	30

Inhibition experiments were conducted in the presence and absence of known CYP inhibitors or substrates, and the rate of conversion of vilazodone to its major metabolites was determined. The experimental conditions using EMD68843 as an inhibitor for CYPs were listed in Table 2. Inhibition of EMD68843 metabolism (^{14}C-labelled, final conc. 6.5 uM) in the presence of CYP inhibitors (Table 4) was conducted under the similar conditions for 30 min, except that CYP probes were replaced with CYP inhibitors. Reactions conducted in the presence of furafylline, DDC or TAO were preincubated with HLM and NADPH-regenerating system at 37^{o}C for 15 min, prior to the addition of ^{14}C-EMD68843. All reactions were terminated with 2 mL ice-cold ethanol.

Table 2. Incubation conditions in pooled HLM (0.5 mg/mL) using EMD68843 as an inhibitor			
	CYP3A	CYP2C19	CYP2D6
Total volume (uL)	500	200	250
NDAPH regenerating system (uL)	125	50	50
Probe substrate	^{14}C-testosterone	S-mephenytoin	bufuralol
Probe substrate concentration (uM)	65	100	10
Metabolite	6b-OH testosterone	4-OH mephenytoin	1'-OH bufuralol
EMD68843 (uM)	0.637-6.37 4.57-45.7	0.75-7.5	0.64-6.4
Incubation time (min)	10	30	30

Detection of ^{14}C-EMD68483 and metabolites, detection of CYP probes and metabolites were performed using on-line radio-HPLC analysis.

Results:

1. Vilazodone metabolism

Vilazodone was metabolized to 4 metabolites designated as M1, M2, M3, and M4 in the study report. The estimated kinetic parameters are listed in the following table.

Table 3. Estimation of Michaelis Menten kinetic parameters for EMD 68483 metabolites

Metabolite	Km (uM)	Vmax (pmol/min/mg)	Reduced Chi squared	Intrinsic clearace (uL/min/mg)
M1	24.6	587	38.2	23.9
M2	959	2376	16.3	2.48
M3	29.5	270	17.5	9.15
M4	40.2	171	1.06	4.25

2. CYP enzyme identification

Multiple CYP enzymes were involved in the metabolism of vilazodone at various degrees. Overall, vilazodone was predominantly metabolized by CYP3A. CYP2C19, CYP2D6, and/or CYP2E1 played a minor role in the biotransformation of vilazodone (Table 4).

Table 4. Inhibition of EMD68843 (6.5 uM) metabolite formation in the presence of CYP inhibitors/substrates in pooled HLM

CYP	CYP inhibitor/substrate	CYP inhibitor/substrate conc. (uM)	% Inhibition of metabolite formation (structure unknown)			
			# 1	# 2	# 3	# 4
CYP1A2	furafylline	5	1.83	0	2.71	1.04
CYP2A6	coumarin	2.5	3.23	11.2	0.759	0
CYP2E1	DDC	50	24.2	22.7	32.2	2.7
CYP2C9	sulphaphenazole	24	10.5	0	1.14	8.46
CYP2C19	S-mephenytoin	500	30.6	19.3	18.8	0
CYP2D6	quinidine	25	31.9	15.5	27.7	27
CYP3A4	TAO	50	91	62.1	93.3	78.9

3. Inhibition potential of EMD 68843

The effect of EMD 68843 on the metabolism of CYP model substrates was examined as shown in the table below.

Table 5. Effect of EMD68483 on the activity of CYPs

CYP	Marker activity	EMD68483 conc. (uM)	% Inhibition
CYP2C19	S-mephenytoin hydroxylase	7.5	0
CYP2D6	Bufuralol 1'-hydroxylase	6.4	59.0
CYP3A4	Testosterone 6b-hydroxylase	45.7	40.8

EMD68843 did not inhibit the activity of CYP2C19, but exhibited moderate inhibition potential toward CYP3A4 (Table 6) and CYP2D6 (Table 7).

Table 6. Effect of EMD68483 on the activity of CYP3A4	
EMD68483 conc. (uM)	6b-OH testosteronel formation % Inhibition of vehicle control
0.637	4.05
3.19	11.6
4.57	6.55
6.37	8.09
22.9	22.6
45.7	40.8

Based on the above results, an estimated IC_{50} of 68 uM for CYP3A was obtained.

Table 7. Effect of EMD68483 on the activity of CYP2D6	
EMD68483 conc. (uM)	1'-OH bufuralol formation % Inhibition of vehicle control
0.156	0
0.312	11.9
0.625	11.9
1.25	13.8
2.5	49.4
5	63.6
10	60.3
15	68.9
20	76.3
24	74.3

Based on the above results, an estimated IC_{50} of 2.8 uM for CYP2D6 was obtained.

Reviewers' Comments

To determine a Km value accurately, a range of substrate concentrations between 0.2Km and 5 Km that gives a wide variation in the rates of substrate turnover is recommended under optimized conditions (incubation time and protein concentrations). 0.5Km, Km, 2Km and 4Km are typically used for major P450 enzymes.

In this report, the sponsor characterized the Michaelis Menten kinetic parameters for the 4 metabolites identified. However, due to the poor design of the experiment (highest vilazodone concentration used in the incubation was less than the lowest Km obtained; probably not under optimized conditions), the obtained information probably is not accurate or reliable. In addition, the structures of the 4 identified metabolites need to be determined.

Validation report of the analytical method used in this study is not provided. Data generated can only be considered as supportive.

==========

Study 4, *Inhibition Potential of Vilazodone in Human Liver Microsomes*

Investigation of the potential inhibitory effect of EMD 68843 on the metabolism of cytochrome P450 (CYP) model substrates

Study Number: GPP-007-CLN-ANR-1998-029 (Original Merck Study No. CLG 00701)
Objective: To determine the potential of vilazodone to inhibit the metabolism of CYP1A2, CYP2C9, CYP2C19, and CYP3A4 substrates in human liver microsomes.
Materials:
EMD 68843 was provided in powdered form and stored at 4ºC protected from light.
Human liver microsomes (HHM-0219, pool of 4 individual livers) were obtained from the (b) (4)
Methods:
Pooled human liver microsomes were used to assess the inhibitory effect of vilazodone on CYP1A2, CYP2C9, CYP2C19, and CYP3A4 using respective probe substrates. Inhibition experiments were conducted in the presence and absence of known CYP substrates, and the rate of conversion of each probe substrate to its major metabolites was determined. The following tables list the incubation conditions for each experiment determining Ki values. All incubations were conducted at 37 ºC.

Table 1. Incubation conditions in pooled HLM using EMD68843 as an inhibitor

	CYP1A2	CYP2C9	CYP2C19	CYP3A4
Total volume (mL)	2.0	0.25	0.125	0.25
HLM Protein amount (mg)	0.1	0.3	0.1	0.1
NDAPH regenerating system (uL)	40	50	25	62.5
EMD68843 (uM)	15,30,40,50	20,40,80,100	15,30,60,100	16,32.5,65,100
Probe substrate	7-ethoxyresorufin	tobutamide	S-mephenytion	testosterone
Probe conc. (uM)	0.2,0.4,0.6,0.8	25,50,100,200	25,50,100,200	16,32,65,130
Probe metabolite	resorufin	4-OH tobutamide	4-OH S-mephenytion	6b-OH testosterone
Incubation time(min)	3	30	30	10

Detection of CYP probes and metabolites were performed using HPLC analysis, except for resorufin, a fluorescence spectrophotometer was utilized.
Results:
The inhibition constant of EMD68843 of each CYP enzyme tested was listed in the table below.

Table 2. Ki values (uM) of EMD68843 for CYPs

CYP	Marker activity	Ki. (uM)	Inhibition type
CYP1A2	Ethoxyresorufin O-deethylase	81.7	competitive
CYP2C9	tobutamide 1'-	24.0	Non-

	hydroxylase		competitive
CYP2C19	S-mephenytoin hydroxylase	7.37	competitive
CYP3A4	Testosterone 6b-hydroxylase	114	Non-competitive

Reviewers' Comments

To determine a Ki value accurately, a range of substrate concentrations between 0.2Km and 5 Km that gives a wide variation in the rates of substrate turnover and is in the non-linear part of the rate versus substrate concentration curve is recommended. 0.5Km, Km, 2Km and 4Km are typically used for major P450 enzymes. When a substrate concentration range between 0.2Km and 5Km is selected, the Eadie-Hofstee plot provides the largest spread of points on the entire graph, thus making the Ki value determination more accurate. Similarly, the appropriate range for the inhibitor is centered around the estimated Ki value, and spans 2 orders of magnitude: 0.4Ki, 1Ki, 3 Ki, 4Ki, and 10Ki.

Therefore, the experiments in the study were not well designed, and the Ki values obtained probably are not accurate. A better designed study is needed to get accurate estimation of the Ki values for major CYPs. In addition, in study GPP-007-CLN-PKM-2001-033, solubility issue occurred when EMD68843 concentration was 100 uM. This raises more suspicion towards the accuracy of the data obtained.

==========

Study 5*, Inhibition Potential of Vilazodone for CYP3A4/5 and 2C8*

An *in vitro* evaluation of the inhibitory potential of SB-659746-A on cytochrome P450 3A4/5 and 2C8

Study Number: GPP-007-CLN-PKM-2001-033
Objective: To determine the potential of SB-659746 to inhibit cytochrome P450 3A4/5 and 2C8 associated enzyme activities in human liver microsomes.
Materials:
SB-659746, supplied as the hydrochloride salt (SB-659746-A), was stored in the dark at ambient temperature. The chemical purity of the supplied test material (Batch No. EE 79685) was 99.89%. Each day an experiment was conducted, a SB-659746 stock solution was prepared by dissolving SB-659746 in dimethyl sulfoxide (DMSO) to obtain a concentration of either 3.0 or 100 mM. As appropriate, SB-659746 working solutions were then prepared from the stock solution.

Pooled human liver microsomes (HLM) was a pool prepared from nine individuals. Three individual human liver microsomes with low, medium, or high activity were also used.
Methods:
Using probe substrates, the following CYP3A4/5 associated enzyme activities were measured in the presence and absence of vilazodone: Lovastatin 6′β-hydroxylation, midazolam 1′-hydroxylation, nifedipine oxidation, and testosterone 6β-hydroxylation. To

assess for potential inhibition of CYP2C8, paclitaxel 6α-hydroxylation was measured in the presence and absence of vilazodone. Using a pooled human liver microsomal sample, the ability of vilazodone to directly and reversibly (metabolism-independent) inhibit each of these enzyme activities was evaluated and the corresponding inhibitory constants (IC_{50} and K_i values) were calculated. The potentials of vilazodone to act as a metabolism-dependent reversible inhibitor and as a metabolismdependent irreversible/quasi-irreversible inhibitor were evaluated using individual and pooled microsome samples. All incubations were conducted at 37 ºC.

Table 1. Experimental conditions used to assess SB-659746 for metabolism-independent inhibition of cytochrome P450 3A4/5 and 2C8 in a pooled HLM

Experiment	Cytochrome P450 Enzyme	Activity Measured	[Probe Substrate] (uM)	Incubation Volume (uL)	Protein (ug/mL)	Incubation Time (min)	[SB-659746][a] (uM)
IC50	CYP3A4/5	Lovastatin 6′β-hydroxylation	2.0	1000	20	5.0	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100[b]
	CYP3A4/5	Midazolam 1′-hydroxylation	3.0	1000	50	5.0	0, 0.1, 0.3, 1.0, 3.0, 10, 30, 100[b]
	CYP3A4/5	Nifedipine oxidation	10	1000	100	5.0	0, 0.1, 0.3, 1.0, 3.0, 20, 30, 100[b]
	CYP3A4/5	Testosterone 6β-hydroxylation	100	500	100	10	0, 0.1, 0.3, 1.0, 3.0, 20, 30, 100[b]
	CYP2C8	Paclitaxel 6α-hydroxylation	15	1000	100	10	0, 0.1, 0.3, 1.0, 3.0, 20, 30, 100[b]
Ki	CYP3A4/5	Lovastatin 6′β-hydroxylation	0.6, 2.0, 6.0, 12, 20	1000	20	5.0	0, 7.3, 15, 30, 55, 70
	CYP3A4/5	Midazolam 1′-hydroxylation	0.9, 3.0, 9.0, 18, 30	1000	50	5.0	0, 10, 20, 40, 55[c], 70[c]
	CYP3A4/5	Nifedipine oxidation	3, 10, 30, 60, 100	1000	100	5.0	0, 4.9, 9.8, 20, 45, 70
	CYP3A4/5	Testosterone 6β-hydroxylation	30, 50, 75, 100, 200[d]	500	100	10	0, 5.6, 11, 22, 45, 70
	CYP2C8	Paclitaxel 6α-hydroxylation	4.5, 7.5, 11.25, 15, 20	1000	100	10	0, 0.46, 0.91, 1.8, 3.7, 7.3

Notes: Each incubation was performed in duplicate.
a: SB-659746 was dissolved in DMSO (final DMSO concentration in the incubation, 0.1% [v/v]).
b: Precipitate was visible in the 100 uM SB-659746 incubation tubes. The 100 uM SB-659746 samples were excluded from the data analyses and the highest SB-659746 concentration to be used in subsequent experiments was set at 70 uM.
c: Data from the 55 and 70 uM SB-659746 incubations were not reported because the observed inhibition was not concentration-dependent.
d: The 200 uM testosterone samples were not used in calculating the Ki value because of suspected testosterone insolubility.

Table 2. Experimental conditions used to determine IC50 values for SB-659746 inhibition of cytochrome P450 3A4/5 and 2C8 in individual HLM

CYP3A4/5	Lovastatin 6′β-hydroxylation	99, 140, 104	2.0	1000	20	5.0	0, 7.3, 15, 30, 55, 70
CYP3A4/5	Midazolam 1′-hydroxylation	99, 140, 104	3.0	1000	50	5.0	0, 10, 20, 40, 55, 70
CYP3A4/5	Nifedipine oxidation	99, 140, 104	10	1000	100	5.0	0, 4.9, 9.8, 20, 45, 70
CYP3A4/5	Testosterone 6β-hydroxylation	99, 140, 104	100	500	100	10	0, 5.6, 11, 22, 45, 70
CYP2C8	Paclitaxel 6α-hydroxylation	68, 132, 104	15	1000	100	10	0, 0.46, 0.91, 1.8, 3.7, 7.3

a: Human liver microsomal (HLM) sample numbers used in the evaluations. Each incubation was performed in duplicate.
b: SB-659746 was dissolved in DMSO (final DMSO concentration in the incubation, 0.1% [v/v]).

Table 3. Experimental conditions used to assess SB-659746 for reversible, metabolism-dependent inhibition of cytochrome P450 3A4/5 and 2C8 in individual HLM

Cytochrome P450 Enzyme	Activity Measured	HLM[a] Numbers	[Substrate] (uM)	Incubation Volume (uL)	Protein (ug/mL)	Preincubation Time (min)	Incubation Time (min)	[SB-659746][b] (uM)
CYP3A4/5	Lovastatin 6′β-hydroxylation	99, Pooled, 104	2.0	1000	20	15	5.0	14.3
CYP3A4/5	Midazolam 1′-hydroxylation	99, Pooled, 104	3.0	1000	50	15	5.0	10
CYP3A4/5	Nifedipine oxidation	99, Pooled, 104	10	1000	100	15	5.0	3.0
CYP3A4/5	Testosterone 6β-hydroxylation	99, Pooled, 104	100	500	100	15	10	3.0
CYP2C8	Paclitaxel 6α-hydroxylation	99, Pooled, 104	15	1000	100	15	10	0.3

a: Three human liver microsomal (HLM) samples with low, medium, or high activity, respectively, were used to assess the selected activity. Each incubation was performed in duplicate.
b: SB-659746 was dissolved in DMSO (final DMSO concentration in the incubation, 0.1% [v/v]).

Table 4. Experimental conditions used to assess SB-659746 for irreversible, metabolism-dependent inhibition of cytochrome P450 3A4/5 and 2C8 in HLM

		Preincubation				Incubation with Probe Substrate				
Cytochrome P450 Enzyme	Activity Measured	Volume (uL)	Protein (ug/mL)	Time (min)	[SB-659746][a] (uM)	Volume (uL)	Protein (ug/mL)	Time (min)	[SB-659746] (uM)	[Substrate] (uM)
CYP3A4/5	Lovastatin 6′β-hydroxylation	1000	200	15	100[b]	1000	20	5.0	10	2.0
CYP3A4/5	Midazolam 1′-hydroxylation	1000	500	15	70	1000	50	5.0	7.0	3.0
CYP3A4/5	Nifedipine oxidation	1000	1000	15	30	1000	100	5.0	3.0	10
CYP3A4/5	Testosterone 6β-hydroxylation	500	1000	15	30	500	100	10	3.0	100
CYP2C8	Paclitaxel 6α-hydroxylation	1000	1000	15	3.0	1000	100	10	0.3	15

Note: Three human liver microsomal samples with low, medium, or high activity (#99, Pooled, and #104, respectively) were used to assess the selected activity. Each incubation was performed in duplicate.
a: SB-659746 was dissolved in DMSO (DMSO concentration in the final incubation with the probe substrate, 0.1% [v/v]).
b: Due to an experimental error, 100 uM was used instead of the planned 70 uM. Because the data interpretation for these studies is only qualitative and the likelihood that the higher protein concentration in the preincubation would facilitate SB-659746 solubility, the Study Director judged that results of the experiment was acceptable.

For all incubations with SB-659746, only data obtained from concentrations up to 30 uM was used in analysis.

Detection of CYP probes and metabolites were performed using HPLC analysis.

Results:

1. Inhibition of CYP2C8

SB-659746 showed highly significant *in vitro* inhibition of CYP2C8 activity and little variability between the three individual microsomal samples. The inhibition was mostly competitive inhibition and there was no clear evidence that SB-659746 caused notable reversible or irreversible/quasi-irreversible metabolism-dependent inhibition of CYP2C8 activity.

2. Inhibition of CYP3A

In a pooled HLM sample, the metabolism-independent inhibition potential of SB-659746 on CYP3A4/5 was generally moderate and varied slightly with the probe substrate. For midazolam 1'-hydroxylation, the Ki value was approximately twice that measured for the other three CYP3A4/5 associated enzyme activities. Little sample-to-sample variability in the IC50 values was noted between three individual HLM samples.

Overall, in human liver microsomes, vilazodone was a significant inhibitor of CYP2C8 and a moderate inhibitor of CYP3A4/5 (Table 1). Comparable inhibitory constants were obtained for lovastatin 6′β-hydroxylation, midazolam 1′-hydroxylation, nifedipine

oxidation, and testosterone 6β-hydroxylation. No clear evidence was observed for notable metabolism-dependent inhibition of either CYP2C8 or CYP3A4/5.

Table 1 ***In vitro*** **inhibition of cytochrome P450 3A4/5 and 2C8 in human liver microsomes by SB-659746.**

Cytochrome P450 Enzyme	Activity Measured	Metabolism-Independent IC50 (uM) Pooled[b]	Metabolism-Independent IC50 (uM) Individual[c]	Metabolism-Independent Ki (uM)[b]	Metabolism-Independent Type of inhibition	Metabolism-Dependent[a] Reversible/Irreversible
CYP3A4/5	Lovastatin 6′β-hydroxylation	22	23, 15, 32	16	mixed	No effect
CYP3A4/5	Midazolam 1′-hydroxylation	41	70, 59, 60	53	noncompetitive	No effect
CYP3A4/5	Nifedipine oxidation	20	12, 8.5, 14	25	mixed	No effect
CYP3A4/5	Testosterone 6β-hydroxylation	29	29, 32, 55	24	competitive	No effect
CYP2C8	Paclitaxel 6α-hydroxylation	1.8	3.9, 1.5, 1.9	0.46	competitive	No effect

Notes: Values were calculated using the average data obtained from duplicates for each incubation condition. The IC50 and Ki values were calculated using GraFit software with simple weighting. HLM: Human liver microsomes.
a: Evaluated using the pooled HLM sample and two individual HLM samples.
b: Determined using the pooled HLM sample.
c: Values obtained from HLM samples with low, medium and high activity, respectively, for the selected enzyme.

Reviewer's Comments

To determine a Ki value accurately, the appropriate range of the inhibitor is centered around the estimated Ki value, and spans 2 orders of magnitude: 0.4Ki, 1Ki, 3 Ki, 4Ki, and 10Ki. However, because of the solubility issue of SB-659746 at a high concentration (100 uM), data analysis was done using data obtained from 0.1-30 uM SB-659746. Therefore, the IC_{50} and Ki values obtained probably was not accurate (too much extrapolation), especially those obtained using midazolam as a probe.

Since validation report of the analytical method is not provided, data generated can only be considered as supportive.

==========

Study 6, ***Inhibition Potential of Vilazodone towards CYP2C8 in Human Hepatocytes***

Determination of the potential for vilazodone to inhibit the activity of CYP2C8 in human hepatocyte suspensions

Study Number: PGX-08-PC-02
Objective: To examine the potential for vilazodone to inhibit the activity of CYP2C8 in cryopreserved human hepatocyte suspensions.
Materials:
Vilazodone (hydrochloride salt) was stored in the dark at ambient temperature. The chemical purity of the material (Lot No. EE 91085) was 97.8%. On the day of incubation, stock solutions were prepared in dimethyl sulfoxide (DMSO) at 1000x the

final concentrations. The stock solutions were diluted with incubation medium to obtain 2x doing solution.

Amodiaquine, a specific substrate for CYP2C8, was prepared as a 100x stock solution in water. Quercetin, a reversible inhibitor of CYP2C8, was prepared as a 1000x stock solution in DMSO and diluted with incubation medium to obtain a 2x dosing solution.

Pooled cryopreserved human hepatocytes, lot MLV, were obtained from the cyropreserved hepatocyte bank maintained at (b) (4) Hepatocytes were cultured following standard protocol. Trypan blue exclusion was used to determine hepatocyte yield and viability. The hepatocytes were diluted with incubation medium to prepare a 2x cell suspension of 2.0 x 10^6 viable cells/mL. Hepatocyte suspensions were pre-warmed at 37ºC for 5 min prior to use. The final cell density was 1.0 x 10^6 viable cells/mL.

Methods:

Hepatocytes were incubated with 0.21, 0.63, 2.1, 6.3, and 21 μM vilazodone and a specific substrate for CYP2C8, amodiaquine (final conc. 5 uM). To assess reversible inhibition, vilazodone and cryopreserved human hepatocyte suspensions were coincubated with amodiaquine. To assess time-dependent (i.e., mechanism-based) inhibition, vilazodone and cryopreserved human hepatocyte suspensions were pre-incubated for 30 minutes. Reactions were initiated with addition of amodiaquine. The mixtures were incubated at 37ºC for 10 minutes before termination by the addition of 500 uL ice-cold methanol. The rate of substrate metabolite formation was measured using a validated LC-MS/MS assay.

Vehicle control incubations were conducted to establish a baseline value for enzyme activity levels in cryopreserved human hepatocyte suspensions. Positive control incubations were conducted to evaluate the suitability of the test system for analysis of cytochrome P450 isoform inhibition. Test article interference control incubations were also included to ensure that neither vilazodone nor its metabolites caused chromatographic interference.

Results:

Slight inhibition by vilazodone at concentrations up to 6.3 μM was observed (see table below). Under co-incubation conditions assaying reversible inhibition there was 2% inhibition at 2.1 μM and 30% inhibition of CYP2C8 activity at 6.3 μM concentrations of vilazodone. Following preincubation conditions assaying time-dependent inhibition there was 18% inhibition at 2.1 μM and 40% inhibition at 6.3 μM concentrations of vilazodone. Since a precipitate was noted in a test article stock solution used to evaluate inhibition at 21 μM, the results (a lack of inhibition) from that experimental run are not used in estimating the IC_{50}. Based on these results, the theoretical IC_{50} would be greater than 6.3 μM (>3000 ng/mL) for both reversible and time-dependent inhibition of CYP2C8 by vilazodone.

Effect of Vilazodone on CYP 2C8 In Vitro – Study PGX-08-PC-02

	N-desethylamodiaquine Formation Percent of Vehicle Control	
Vilazodone Concentration (µM)	**Reversible Inhibition (Co-Incubation)**	**Time-Dependent Inhibition (Pre-Incubation)**
0	100±6.27	100±3.13
0.21	126±3.07	101±8.92
0.63	130±4.88	111±9.27
2.1	97.8±2.35	82.0±1.32
6.3	69.7±7.84	59.6±4.01
21	132±7.93	98.7±4.12

Note: Vilazodone C_{max} at steady-state with 40 mg qd dose is 0.33 µM (156 ng/mL) (PGX-08-P1-06).

Reviewer's Comments

The reviewer agrees with the sponsor's conclusion that vilazodone did not show signification inhibition towards CYP2C8 in cryopreserved human hepatocytes at concentrations lower than 6.3 uM. However, this result is not consistent with the result obtained in study GPP-007-CLN-PKM-2001-033. An attempt to explain the difference in the inhibition potential of vilazodone for CYP2C8 in the two incubation systems, namely human liver microsomes (Study GPP-007-CLN-PKM-2001-033) vs human hepatocyte (this study) would be nice if provided.

==========

Study 7, *Induction Potential of Vilazodone*

Quantitative RT-PCR Measurement of Cytochrome P-450s in Human Hepatocytes

Study Number: GPP-007-CLN-ANR-1999-027 (Original Merck Study No. PKM 40-99)
Objective: To determine the extent of induction of CYP isoenzymes by vilazodone in human hepatocytes.
Material:
EMD 68843 (hydrochloride salt) was stored in the dark at ambient temperature. The chemical purity of the material (Lot No. EE 79485) was 99.6%.

Methods:
Cell culture
Human hepatocytes were obtained from surgical liver biopsies (2-4 g) of two patients, after informed consent. Cells were isolated by microperfusion of the tissue sample with collagenase. Cellular viability was assessed by the trypan blue dye exclusion test and was usually 85-90%. Hepatocytes were seeded on fibronectin-coated plastic dishes (3.5 ug/cm^2) at a density of 8 x 10^4 viable cells/cm and cultured in Ham's F-12/Lebovitz L-15 (1:1) medium supplemented with 2% new-born calf serum, 50 U/ml penicillin, 50 p,g/rnl streptomycin, 0.2% BSA and 10^{-8} M insulin. One hour later the medium was changed,

and after 24 h the cells were shifted to serum-free hormone-supplemented medium (10^{-8} M dexamethasone and insulin).

Treatment of cultures

Four hours after culture medium change, human hepatocytes were exposed to EMD68483 (60 uM) for 24, 48 and 60 hours. Positive controls (methylcholantrene 2 uM, Phenobarbital 1 mM, and rifampin 50 uM) were also included to test if the system was working properly.

Isolation and purification of total RNA from human hepatocytes

Total RNA was extracted from 6cm culture plates using "Rnease Total RNA kits". The amount of purifed RNA was estimated by ribogreen fluorescence and its purity by the absorbance ratio 260/280 nm. RNA was incubated for 15 min at 23 ºC with DNase I (1 unit/ug) according to the recommendations of the supplier, followed by thermal inactivation of the enzyme (65 ºC for 10 min) in the presence of 2.5 mM EDTA and a rapid cooling down to 4ºC.

Reverse-transcription-PCR

Specific primer pairs for CYPs 1A1, 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5 were selected to specifically match each CYP mRNA, to have a very close annealing temperature, and to render PCR products of similar sizes.

The reverse transcriptase (RT) reaction mixture consisted of 20 ul of 1x reverse transcriptase buffer, 10 mM DTT, 500 uM dNTPs, 3 uM oligo d(T)14 primer, 60 U RNout and 250 U RNase H. One ug of RNA total and 0.01ng of luciferase mRNA was added to this mixture. The reaction was allowed to proceed for 60 min at 42ºC, followed by a 5 min heating at 95º C and a rapid cooling on ice. The cDNA was stored at-20ºC until use. The PCR reaction was conducted in small vessels each one containing 4 ul of appropriately diluted cDNA (problems or standards) and 36 ul of reaction mixture (20 mM Tris-HC1 buffer, pH S.4, 50 mM KC1, 1.5 mM $MgCl_2$, 50 pM of each deoxynucleotide triphospate, 1U Taq DNA polymerase and 0.2 uM of each specific primer). After 4 min at 94ºC, amplification was perforrned by 28-30 PCR cycles of 40 sec at 94ºC, 45 sec at 60ºC, 50 sec at 72ºC and a final extension of 4 min at 72ºC.

Measurement of human CYPs by fluorescence

Three different amounts of each of the CYP standards and aproppiate dilutions of the sample to be tested were used in each PCR assay. Appropriate cDNA dilutions were empirically determined to ensure that the PCR amplification of each CYP did not reach saturation. To quantify the amplified cDNA, 15 ul of the PCR reaction were diluted with TE buffer and stained with picogreen at a 1:400 final concentration. Fluorescence was measured in a micro-plate spectrofluorometer at 485 nm Excitation 538 nm Emission. A calibration curve was constructed with known amounts of λ-DNA. Fluorescence was found to be linear in the range of 0-90 ng/well. The concentration of specific cDNAs in samples was estimated from the amount of amplified product measured and the amplification ratio (cDNA content of standards before and after PCR amplification).

Results:
Positive control inducers: methylcholantrene (MC), pheonobarbital (PB) and rifampin (Rif) demonstrated inductive effect on CYP expression as expected (Table 1).

Table 1. Effects of model inducers on CYP mRNA expression

60h incub	MC	PB	Rif
Conc	2 µM	1 mM	50 µM
1A1	**7,4**	0,4	0,4
1A2	**30,2**	0,4	0,2
2A6	1,3	**1,9**	**3,7**
2B6	1,3	**3,6**	**4,1**
2C9	1,2	1,1	1,4
2C19	1,4	0,7	1,3
2D6	1,2	0,7	0,8
2 E1	1,4	0,9	0,7
3A4	0,9	**10,4**	**13,1**
3A5	1,1	**5,8**	**4,4**

Data indicate the relative increase of CYP mRNA (times induction over control cells).

Vilazodone did not induce CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1, or CYP3A5 isoforms (Table 2). It may be a very weak inducer of CYP2D6 and CYP3A4, since borderline effects (approximately 2-fold increases in mRNA expression) were observed in the longest incubation experiments (60 hours) for these 2 CYP isoforms.

Table 2. Effects of EMD68843 on CYP mRNA expression

EMD68843	HB386	HB388	HB386
Conc 60 µM	24h	48h	60h
1A1	1,0	1,0	0,7
1A2	1,4	0,6	0,4
2A6	0,5	1,0	1,0
2B6	0,7	1,3	1,5
2C9	1,3	1,1	1,2
2C19	0,6	1,2	1,4
2D6	1,1	1,3	2,4
2 E1	1,3	0,7	0,9
3A4	1,2	0,9	2,2
3A5	1,0	1,0	1,3

Data indicate the relative increase of CYP mRNA (times induction over control cells).

Reviewers' Comments

According to the Guideline, to determine the induction potential of a compound, experiments should be conducted with hepatocytes prepared from at least three individual donor livers. In this study, the induction potential was only evaluated in hepatocytes from one donor at each time point. It is not adequate and may not be representative of all isoenzymes from on individual. Therefore, data from at least another two donors would be needed. In addition, a modern technique, for example, real-time PCR, could be applied to monitor the changes of mRNA expression in the major CYPs.

==========

Study 8*, Protein binding*

EMD68843-serum protein binding in various animal species and in man

Study Number: GPP-007-NCD-PKM-1995-010
Note: only human data was reviewed in this report
Objective: To determine the extent of protein binding of vilazodone in serum from animals and humans
Material:
^{14}C-labelled EMD 68 843 was used in the measurement of serum protein binding. The purity of the substance was 98%.
Methods:
The protein binding was determined in vitro at concentrations of 0.05, 0.5, and 5ug/ml using pooled human serum samples (from healthy volunteers, (b) (4)

Appropriate amounts of EMD 68843, dissolved in 60% aqueous polyethylene glycol, were added to the serum samples under stirring, the solvent volume being 0.5% of the serum volume.
Ultracentrifugation
With the ultracentrifugation method, the protein and protein-bound drug was sedimented using an ultracentrifuge (245,070 g, Beckman, model TLA-100.3), with a fixed angle rotor. The unbound drug remaining dissolved in the protein-free supernatant. Serum samples were centrifuged in 1.5 ml polyallomer microcentrifuge tubes for 23 h at 4ºC.

After centrifugation, the protein-free supematant was carefully withdrawn with a syringe. The adsorption of EMD 68 843 to the microcentrifuge tube was checked.

Ultrafiltration
For ultrafiltration, the micropartition system MPS-1 was used. The MPS-1 consists of a membrane support base, sample reservoir with cap, O-ring, YMT membrane, a set of clips which secure the sample reservoir to the support base, and a filtrate collection cup. The serum was pressed through the protein imperrneable membrane at approximately 1400 x g in a fixed angle rotor of a centrifuge . The unbound (dissolved) fraction of the test substance can be determined in the protein-free filtrate. Centrifugation was carried out for 20 min at 4000 rpm and at room temperature. It was found that no radioactivity was bound to the filter membrane and to the polystyrol material.
Measurement of radioactivity
Radioactivity assays in serum and in protein-free supernatant or filtrate were carried out after addition of 0.1 and 0.2 ml, respectively, to 10 ml Omni-Szintiso10 (E. MERCK, Darmstadt Cat. No. 153S6) in liquid scintillation counters Tri Carb 460 CD and Tri Carb 1600 TR (PACKARD, Dreieich). Radioactivity was counted for 20 minutes.

Evaluation
The protein binding was calculated using the formula below.

$$\% \text{ bound} = 100 - 100 \times \frac{dpm/ml(\text{supernatant})}{dpm/ml(serum)}$$

Results:
Protein binding of vilazodone in human serum ranged from 96.1 to 99.4%, depending on the method employed. At concentrations between 0.05 and 5 ug/mL, no concentration dependence of the protein binding was found.

Table 1. Protein binding of vilazodone in human serum		
Total cerum conc. (ug/mL)	ultracentrifugation	ultrafiltration
0.05	99.2	96.3
0.5	99.1	96.2
5.0	99.1	96.1

Reviewers' Comments

Two different methods were used in determine the protein binding of vilazodone, and 2.9% difference was obtained (ultracentrifugation 99.1% vs ultrafiltration 96.2%).

==========

Studies 9 and 10, *Characterization of vilazodone and metabolites in humans*

Study Number: GPP-007-CLN-ANR-1997-019
Pilot studies on the metabolism in man: determination of EMD68843 and its metabolites M1 and M4 in urine of healthy volunteers after a single oral dose administration of 80 mg vilazodone

Study Number: GPP-007-CLN-ANR-2002-252
Preliminary characterization of vilazodone and metabolites in human urine and plasma after a single oral dose administration of 10 mg vilazodone

Objective: To obtain preliminary information on the metabolism of vilazodone in humans after single oral dose administration of vilazodone.

Methods

Samples

Plasma and urine samples from clinical studies were extracted, reconstituted following standard practice.

Analytical method

In study GPP-007-CLN-ANR-1997-019, the determination of vilazodone and the metabolites M1 and M4 in urine was carried out by HPLC and fluorimetric detection. The method used was deemed validated based on the data provided. The lower limit of quantification of this method was set to be 10 ng/mL urine for the three compounds.

Table 1. Performance of the analytical method for urine samples in this study									
	Quality Control Samples (ng/mL), mean of two batches								
	vilazodone			M1 (carboxylic acid)			M4 (butyric acid)		
	20	100	500	20	100	500	20	100	500
Accuracy (% bias)	13.0	7.15	6.0	10.5	3.2	1.6	7.0	6.15	2.5
Precision (% CV)	4.6	7.5	3.2	4.35	1.85	1.15	11.6	3.4	0.85

In study GPP-007-CLN-ANR-2002-252, the validation for the analytical method was not provided. The structures of the analytes were confirmed by NMR analysis.

Results:

1. 10 mg administration

After a single oral dose administration of 10 mg vilazodone, the parent compound and the carboxylic acid derivative (amide hydrolysis product) were detected in both urine and plasma. No other metabolites were detected under the conditions used.

2. 80 mg administration

After a single oral dose of 80 mg vilazodone, in addition to vilazodone and the carboxylic acid metabolite, another metabolite, the butyric acid metabolite (N-dealkylation product) was also detected in the urine samples.

Table 2. Excretion [% of dose] of unchanged vilazodone and its metabolites M1 and M4 in 0-96 hr urine samples of healthy volunteers after single oral dose administration of 80 mg vilazodone. Data are presented as mean (%CV).

Vilazodone	M1 (carboxylic acid)	M4 (butyric acid)
0.163 (86.7)	1.05 (10)	0.546 (50.7)

Reviewers' Comments

Based on the results obtained from the two single oral dose studies, looks like the carboxylic acid metabolite is only metabolite detected in both plasma and urine after a low dose (10 mg) administration of vilazodone,. At a higher dose (80 mg), another metabolite, the butyric acid metabolite is also detected in urine. The parent compound vilazodone is detected in the urine at both low (10 mg) and high (80 mg) doses.

==========

PHARMACOKINETIC AND TOLERABILITY STUDIES

Study GPP-007-CLN-CP1-1996-231 (EMD 68843 – 001, Merck Study 001): Tolerability and pilot pharmacokinetics following single oral administration of different doses (2.5 to 80 mg) of EMD 68843.

A brief overview of some essential components of the study design is given below:

Study Design	Double-blind, placebo-controlled, randomized, ascending-dose
Objective(s)	To investigate the tolerability and pilot PK of different single oral doses (2.5 mg to 80 mg *) of EMD 68843 in healthy male subjects.
Study Investigator(s)	Dr. med. H. Achenbach
Study Site(s)	Department of Clinical Pharmacology Merck KGaA, Darmstadt, Germany
Study Period	March 29, 1996 – May 30, 1996
Study Population	N=30 Age: 24-49 years (mean 34 years) Gender: males

<table>
<tr><td></td><td>Weight: 70.8-109.4 kg (mean 84.6 kg)
Race: Caucasian</td></tr>
<tr><td>Treatment Group</td><td>5 groups (2.5 mg, 5 mg, 10 mg, 20 mg, and placebo groups)</td></tr>
<tr><td>Dosage and Administration</td><td>Three subjects were examined per day, and 6 subjects were randomized in each group:
<table>
<tr><th>Study day</th><th>1</th><th>2</th><th>3[1]</th><th>4[1]</th><th>5</th><th>6</th><th>7</th><th>8[1]</th><th>9[1]</th><th>10</th></tr>
<tr><th>Dose / Subj. No.</th><th>1- 3</th><th>4 - 6</th><th>7 - 9</th><th>10-12</th><th>13-15</th><th>16-18</th><th>19-21</th><th>22-24</th><th>25-27</th><th>28-30</th></tr>
<tr><td>0 mg</td><td>1</td><td>1</td><td></td><td></td><td>1</td><td>1</td><td>1</td><td></td><td></td><td>1</td></tr>
<tr><td>2.5 mg</td><td>2</td><td>1</td><td>2</td><td>1</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>5 mg</td><td></td><td>1</td><td>1</td><td>2</td><td>1</td><td>1</td><td></td><td></td><td></td><td></td></tr>
<tr><td>10 mg</td><td></td><td></td><td></td><td></td><td>1</td><td>1</td><td>1</td><td>2</td><td>1</td><td></td></tr>
<tr><td>20 mg</td><td></td><td></td><td></td><td></td><td></td><td></td><td>1</td><td>1</td><td>2</td><td>2</td></tr>
</table>
Batch no: Placebo capsule XN 1922
2.5 mg capsule XN 1919
10 mg capsule XN 1920
Diet:
The study medication was administered under fasted conditions.</td></tr>
<tr><td>Sampling: Blood</td><td>Pre-dose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours post-dose. Blood samples (10 ml each) were taken from selected three subjects at each group.</td></tr>
<tr><td>Urine</td><td>Pre-dose and post-dose (during 0-4, 4-8, 8-12, and 12-24 hours).
N=3 for each group. Urine samples were taken from selected three subjects at each group.</td></tr>
<tr><td>Feces</td><td>none</td></tr>
<tr><td>Analysis</td><td>Bioanalytical Facility
Institute of Pharmacokinetics and Metabolism of Merck KGaA

Analysis Date(s): May 28, 1996

Method: HPLC with fluorescence detection
<table>
<tr><th>Matrix</th><th>Analyte</th><th>LLOQ (ng/mL)</th><th>ULOQ (ng/mL)</th><th>Precision (%)</th><th>Accuracy (%)</th></tr>
<tr><td>Plasma</td><td>EMD 68843</td><td>0.3</td><td>20</td><td>2.45 – 8.00</td><td>-2 – 3</td></tr>
<tr><td>Urine</td><td>EMD 68843</td><td>5</td><td>250</td><td>1.74 – 10.2</td><td>-21.2 – -5.6</td></tr>
</table>
EMD 68843:
Linear range : 0.4-20 ng/mL in plasma, 10-250 ng/ml in urine.</td></tr>
<tr><td>PK Assessment</td><td>Plasma: AUC0-t, AUC inf, Cmax, Tmax, T1/2.
Urine: Amount accumulative unchanged drug (% dose).</td></tr>
<tr><td>Safety Assessment</td><td>blood pressure, heart rate, ECGs, lung function, adverse events, and laboratory tests etc</td></tr>
<tr><td>PD Assessment</td><td>None</td></tr>
</table>

* Due to dose-dependent occurrence of gastrointestinal and cardiovascular adverse events following single oral doses of 10 mg and 20 mg, the higher doses at 40 mg and 80 mg were not tested.

Pharmacokinetic Results:

EMD 68843 in plasma: dose-proportional increase in Cmax and AUC0-24 of EMD 68843 following single dose of EMD 68843 (Vilazodone) up to 20 mg was observed.

- Mean EMD 68843 (Vilazodone) concentration-time profiles at 4 dose levels are shown in the following figure (n=3 / group):
- Major PK parameters (mean ± SD) for EMD 68843 (Vilazodone) are shown in the following table:

Treatment Groups (n=3/group)	Cmax, ng/mL (%CV)	Tmax, hour (rang)	AUC 0-24, ng*h/mL (%CV)
2.5 mg	1.45 ± 0.14 (9.7)	4 – 6	22.64 ± 0.81 (3.6)
5 mg	5.6 ± 3.7 (66.1)	3 – 4	82.55 ± 47.23 (57.2)
10 mg	12.2 ± 1.84 (15.1)	3 – 5	165.68 ± 18.68 (11.3)
20 mg	28.9 ± 16.14 (55.8)	3 – 5	385.28 ± 185.8 (48.2)

EMD 68843 in urine: Overall, <1.2 % of unchanged drug excreted via kidney. Unchanged EMD 68843 was not detectable in the urine at 2.5 mg dose group.

Safety:

There was no SAE or death reported in the study.

The most common AEs were GI disorders (e.g., diarrhea) and cardiovascular disorders (e.g., orthostatic hypotension and collapse tendency). The incidence and severity of AEs were dose-dependent. The AEs started not earlier than 2 hours post dose and most of them lasted more than 5 hours.

Conclusions:

The study showed a linear increase in Cmax and AUC0-24 of vilazodone over the single dose range of 2.5-20 mg under fasted conditions.

Reviewer's comments: The bioanalytical method for measuring vilazodone plasma concentration at 20 mg dose level may not be reliable for this study by itself. However, results from other PK studies (e.g.: GPP-007-CLN-CP1-1997-232*)* which used a better bioanalytical method are consistent with the PK of 20 mg in this study.

==========

Study GPP-007-CLN-CP1-1997-232 (EMD 68843 – 002, Merck Study 002): A double-blind, randomized clinical study to investigate the tolerability and pharmacokinetics following single oral administration of different doses (20 - 80 mg) of EMD 68843.

A brief overview of some essential components of the study design is given below:

Study Design	Double-blind, placebo-controlled, randomized, single rising-dose
Objective(s)	To investigate the tolerability and PK of EMD 68843 following single oral escalating doses (20 mg to 100 mg*) in 48 (4 groups of 12) healthy young male subjects.
Study Investigator(s)	(b) (4)

<table>
<tr><td>Study Site(s)</td><td>(b) (4)</td></tr>
<tr><td>Study Period</td><td>December 12, 1996 – February 25, 1997</td></tr>
<tr><td>Study Population (completed)</td><td>N=36 (12 /group)
Age: 18-48 years (mean 25 years)
Gender: male
Weight: 62-94 kg (mean 77.4 kg)
Race: Caucasian</td></tr>
<tr><td>Treatment Group and Dosage and Administration</td><td>3 groups (20 mg, 40 mg, and 80 mg), active : placebo = 9:3 /group

Batch no: Placebo capsule XN 1922
10 mg capsule XN 1920
40 mg capsule XN 1921
Diet:
The study medication was administered under fasted conditions.</td></tr>
<tr><td>Sampling: Blood</td><td>Pre-dose, 0.25, 0.5, 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, 16, 20, 24, 30, 36, 48, 72 and 96 hours post-dose (5 mL each).</td></tr>
<tr><td>Urine</td><td>-12-0, 0-12, 12-24, 24-48, 48-72, and 72-96 hours post dose**.</td></tr>
<tr><td>Feces</td><td>none</td></tr>
<tr><td>Analysis</td><td>Bioanalytical Facility
(b) (4)
Analysis Date (s): December 1996 – January 1997
Method: HPLC with fluorescence detection.
<table>
<tr><th>Matrix</th><th>Analyte</th><th>LLOQ (ng/mL)</th><th>ULOQ (ng/mL)</th><th>Precision (%)</th><th>Accuracy (%)</th></tr>
<tr><td>Plasma</td><td>EMD 68843</td><td>0.400</td><td>200</td><td>2.8 – 7.8</td><td>-0.5 – 0.6</td></tr>
</table>
The concentrations of growth hormone and prolactin were calculated directly by the Immulite® system by referring to an internally stored calibration curve (master curve):
Growth hormone: 0.05 ug/L -40 ug/L (LLOQ 0 ULOQ), Precison 4.6-6.1%, accuracy 1.7-3.3%.
Prolactin: 0.5 ug/L – 150 ug/L (LLOQ – ULOQ), Precision 4.9-6%, accuracy -1.3 – 3.4%.</td></tr>
<tr><td>PK Assessment</td><td>Plasma: AUC0-t, AUC inf, Cmax, Tmax, T1/2, CL.</td></tr>
<tr><td>Phenotyping Analysis</td><td>2D6: EM (n=33), PM (n=3: one in 20 mg group and two in 40 mg group);
2C19: EM (n=36);
NAT2: EM (n=14), PM (n=22)</td></tr>
<tr><td>Safety Assessment</td><td>blood pressure, heart rate, ECGs, telemetry, spirometry, adverse events, and laboratory tests etc</td></tr>
<tr><td>PD Assessment</td><td>Growth hormone and prolactin in plasma at pre-dose, and 1, 2, 4 and 24 (only prolactin) hours post dose (4 ml each).</td></tr>
</table>

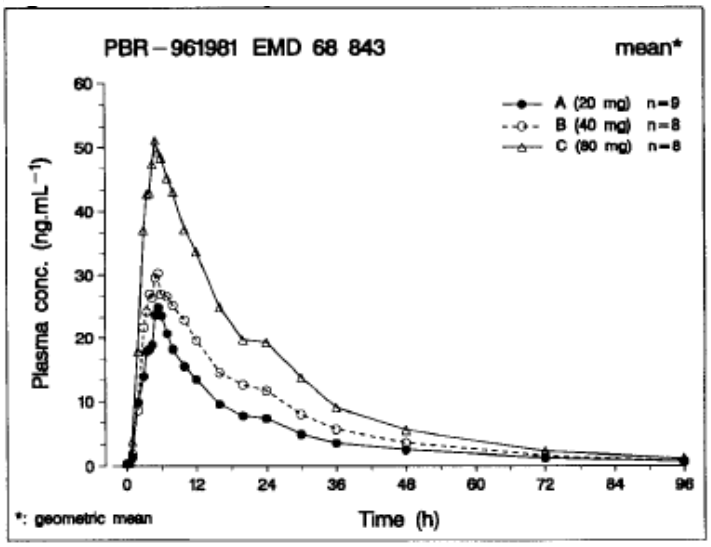
* Due to dose-dependent adverse events following single oral doses of vilazodone, the higher dose at 100 mg was not tested.

**There is no urine analysis available for the study report.

Pharmacokinetic Results:

EMD 68843 in plasma: dose-proportional increase in Cmax and AUCinf of EMD 68843 following single dose of EMD 68843 (Vilazodone) between 20 and 80 mg was observed.

- Mean EMD 68843 (Vilazodone) concentration-time profiles at 3 dose levels are shown in the following figure (n=8-9 / group):



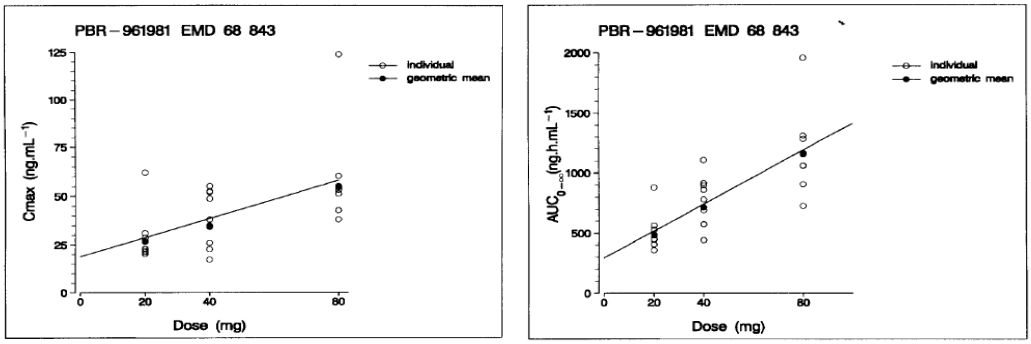
- Major PK parameters (mean (SD)) for EMD 68843 (Vilazodone) are shown in the following table:

Treatment Groups	Cmax, ng/mL	Tmax, h (rang)	T1/2, hour	AUC 0-t, ng*h/mL	AUC inf, ng*h/mL	CL, L/min
20 mg (n=9)	28.5 (13.1)	5 (3.5-5.5)	24.1 (3.6)	478 (153)	502 (155)	0.71 (0.16)
40 mg (n=8*)	36.8 (14.2)	4.8 (2-8)	21.4 (3.4)	712 (208)	741 (214)	0.97 (0.29)
80 mg (n=8*)	58.4 (27.5)	4.8 (2-8)	20.4 (2.4)	1159 (349)	1195 (363)	1.2 (0.34)

* One subject excluded due to vomiting

- Correlation between systemic exposures (Cmax and AUCinf) and doses of EMD 68843 are shown in the following figures:

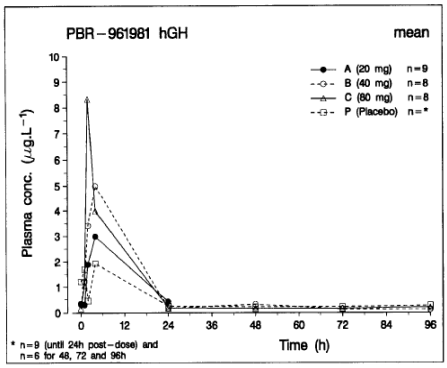
C_{max} AUC



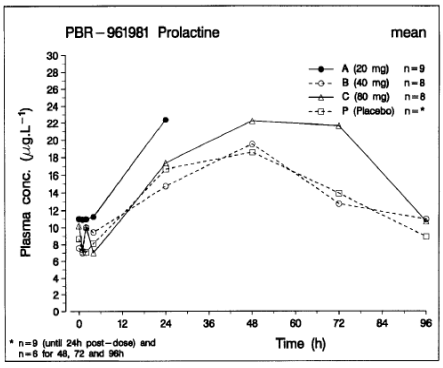
PD Results:

Growth Hormone and Prolactin: EMD 68843 showed some stimulating effect on growth hormone which is dose-dependent after single dose administration. All treatments including placebo showed an increase in plasma prolactin concentrations, which returned to baseline at 96 hours post dose.

Mean plasma growth hormone curves



Mean plasma prolactin curves



Safety:

There was no SAE or death reported in the study.

The incidence of AEs was increased along with the increase of dose: eighteen AEs were reported in 20 mg group; twenty eight AEs were reported in 40 mg group; thirty two AEs were reported in 80 mg group. The most frequently reported AEs were GI system disorders like diarrhea, nausea, stools loose and vomiting.

Effect of phenotype on PK, PD, and safety:

PK: there was no indication that CYP2D6 or NAT2 had effects on the PK profile of EMD 68843.

PD: No distinction was seen between PM and EM for NAT or CYP2D6.

Safety: 2 PM of CYP2D6 (#5 and #14) had no AEs reported, and one PM of 2D6 in 40 mg group (#22) had more AEs. There was no distinction in AEs between PM and EM for NAT.

Conclusions:
The study showed a dose-proportional increase in Cmax and AUCinf of vilazodone over the single dose range of 20-80 mg under fasted conditions.

Vilazodone showed a dose-dependent, transient increase in growth hormone and prolactin over the single dose range of 20-80 mg under fasted conditions, which is not clinically relevant.

==========

Study GPP-007-CLN-CP1-1998-230 (EMD 68843 – 003, Merck Study 003): Tolerability and pharmacokinetics of EMD 68843 in healthy subjects following repeated oral administration for 10 days of different daily doses (10-40 mg).

A brief overview of some essential components of the study design is given below:

Study Design	Double-blind, placebo-controlled, randomized within each group, multiple rising - dose
Objective(s)	To investigate the tolerability and PK of EMD 68843 following single and multiple oral escalating doses (20 mg to 80 mg*) in 45 (3 groups of 15) healthy young male subjects.
Study Investigator(s)	(b) (4)
Study Site(s)	(b) (4)
Study Period	March 7, 1997 – June 12, 1997
Study Population	N=45 (15 /group), 42 subjects completed the study. Age: 18-44 years (mean 26 years) Gender: male Weight: 59.5-95.8 kg (mean 75.4 kg) Race: Caucasian
Treatment Group	3 groups (10 mg, 20 mg, and 40 mg), active: placebo = 12: 3/group.
Dosage and Administration	15 subjects (12 active and 3 placebo) for each group were administered the study medication or placebo QD on Day 1, and Days 7-16. Batch no: Placebo capsule XN 1922 10 mg capsule XN 1920 40 mg capsule XN 1921 Diet: The study medication was administered under fasted conditions.
PK Sampling: Blood	Pre-dose, 0.5, 1, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 18, 24, 30, 36, 48, 72, 96, 120 and 144* hours post-dose on Days 1 and 16 (* Day 16 only), and prior to dosing on Days 7-15 (6 mL for each time point).
Urine	-12-0 predose on Day 1; 0-12, 12-24, 24-48, 48-72, and 72-96 hours post dose on Day 16.
Feces	One sample at predose on Day -1; 0-72h post dose on Days 1 and 16.
Analysis	Bioanalytical Facility (b) (4). Analysis Dates: April 1997 – June 1997

<table>
<tr><td></td><td>Method: HPLC with fluorescence detection.
<table>
<tr><th>Matrix</th><th>Analyte</th><th>LLOQ (ng/mL)</th><th>ULOQ (ng/mL)</th><th>Precision (%)</th><th>Accuracy (%)</th></tr>
<tr><td>Plasma Apparatus 3</td><td>EMD 68843</td><td>0.4</td><td>200</td><td>3.8 – 10.6</td><td>-8.8 – 0.6</td></tr>
<tr><td>Plasma Apparatus 5</td><td>EMD 68843</td><td>0.4</td><td>200</td><td>2.8 – 9.3</td><td>-3.7 – 2.1</td></tr>
</table>
No additional assay information on urine and feces provided.

The concentrations of growth hormone and prolactin were calculated directly by the Immulite® system by referring to an internally stored calibration curve (master curve):
Growth hormone: 0.05 ug/L -40 ug/L (LLOQ - ULOQ), Precison 5.6-8.7%, Accuracy -1 – 5.1%.
Prolactin: 0.5 ug/L – 150 ug/L (LLOQ – ULOQ), Precision 5.3 – 10.2%, Accuracy -4.4 – -0.8%.
ACTH: 12 – 1250 pg/mL (LLOQ – ULOQ), Precision 7.5 – 13.4%, Accuracy -9 - -3.4%.
Cortisol: 10 – 500 ug/L (LLOQ – ULOQ), Precision 5.5 – 12.1%, Accuracy -5.7 – 1.7%.</td></tr>
<tr><td>PK Assessment</td><td>Plasma: AUC0-24, Ctrough, Cmax, Cav, Cmax, Tmax, T1/2, CL/f, AUC inf (Day 16).</td></tr>
<tr><td>Phenotyping Analysis</td><td>2D6: EM (n=42), PM (n=3);
2C19: EM (n=44); no data for one subject.
NAT2: EM (n=27), PM (n=18)</td></tr>
<tr><td>Safety Assessment</td><td>Vital signs, ECGs, spirometry, adverse events, and laboratory tests.</td></tr>
<tr><td>PD Assessment</td><td>Growth hormone, prolactin, ACTH, and cortisol in plasma at pre-dose, and 1, 2, 4, 12, 24, 48, 72 and 96 hours after dosing on Days 1 and 16 (6 mL at each time point).</td></tr>
</table>

* Only dose levels at 10, 20, and 40 mg were tested in the study.

Pharmacokinetic Results:

EMD 68843 in plasma: dose-linear increase in Cmax, Cav, and AUC0-24 of EMD 68843 at steady state following multiple doses of EMD 68843 (Vilazodone) between 10 and 40 mg was observed. Steady state of EMD 68843 (vilazodone) was reached around 3 days after multiple doses of EMD 68843 under fasted conditions.

- Major PK parameters (mean (SD)) for EMD 68843 (Vilazodone) are shown in the following table:

Treatment Groups		Cmax, ng/mL	Tmax, h (rang)	T1/2, hour	AUC 0-24, ng*h/mL	Cav, ng/mL	Accumulation Factor**
10 mg (n=11-12[a])	D1	14 (3.8)	5 (2-6)	25.3 (6.5)	161 (40)	–	–
	D16	24.8	3.5	30.9	293	12.2	1.78

		(5.3)	(2.5-5)	(7)	(81)	(3.4)	(0.42)
20 mg (n=9-12*)	D1	29.9 (11)	5 (3-6)	23 (5.2)	336 (115)	–	–
	D16	45.5 (17.7)	4.5 (2.5 -5)	29.5 (4.6)	528 (188)	22 (7.8)	1.54 (0.33)
40 mg (n=11-12 [b])	D1	39.8 (13)	5 (2-5)	24.5 (5.2)	488 (143)	–	–
	D16	59.5 (22)	4 (2-6)	28.9 (3.2)	755 (250)	31.4 (10.4)	1.64 (0.44)

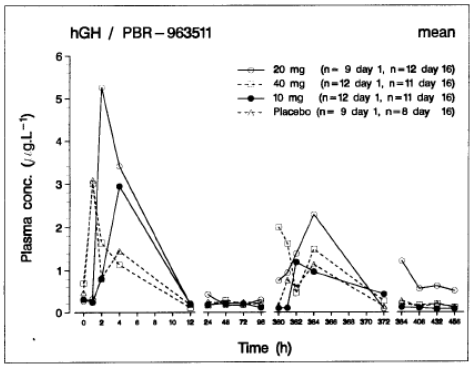
* n=9 on Day 1, three subjects were excluded due to vomiting
** Accumulation Factor = AUC0-24 on Day 16 / AUC0-24 on Day 1
a, N=11 on Day 16 due to an incomplete subject.
b, N=11 on Day 16 due to an incomplete subject.

PD Results:

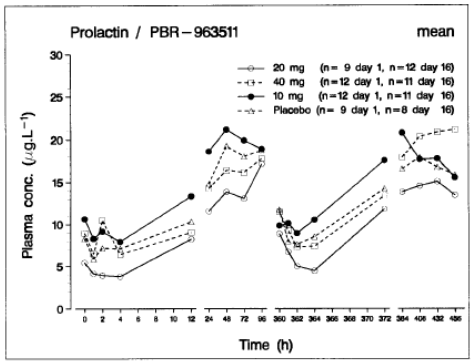
Growth Hormone and Prolactin: EMD 68843 showed some stimulating effect on growth hormone which is more prominent after single dose administration. This increase in plasma growth hormone is transient with a maximum effect between 1 and 4 hours after dosing, and the values returned to baseline at 24 hours after dosing.

All treatments including placebo showed an increase in plasma prolactin concentrations. This increase maintained even for 96 hours after dosing on Days 1 and 16. However, pre-dose values on Day 16 after multiple doses for 9 days were comparable to those at predose on Day 1.

Mean plasma growth hormone curves



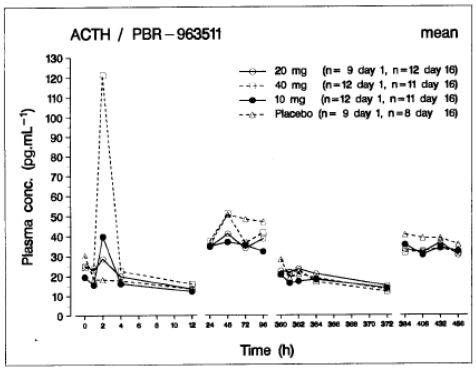
Mean plasma prolactin curves



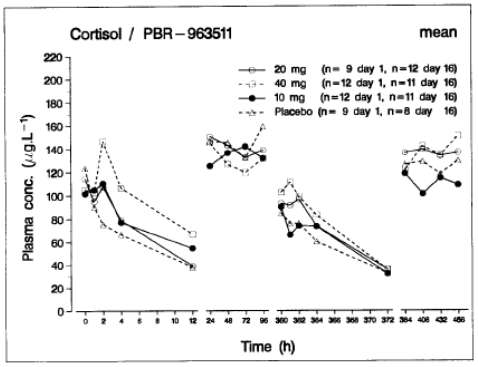
ACTH and Cortisol: There is a dose-dependent transient increase plasma ACTH between 1 and 4 hours after administration of vilazodone only on Day 1. Pre-dose values for ACTH on Day 1 and Day 16 were similar. However, ACTH values were about 1.5 times higher for 96 hours after dosing than the pre-dose values on Days 1 and 16 for all treatment groups including placebo.

EMD 68843 showed some dose-dependent stimulating effect on cortisol which is more prominent after single dose administration. This increase in plasma cortisole is transient with a maximum effect between 1 and 4 hours after dosing on Days 1 and 16. Pre-dose values on Days 1 and 16 were comparable for all treatments.

Mean plasma ACTH curves



Mean plasma cortisol curves



Safety:

There was no death reported in the study. One SAE occurred during the study (prolonged hospitalization for one subject in 40 mg dose group due to psychiatric and central nervous system related AEs during the study)

The incidence of AEs related to the study medication was increased along with the increase of dose: twenty five (25) AEs were reported in placebo; thirty seven (37) AEs were reported in 10 mg dose level; forty (40) AEs were reported in 20 mg dose level; one hundred thirty six (136) AEs were reported in 40 mg dose level. The most frequently reported AEs were central and peripheral nervous system disorders, gastro-intestinal system disorders and psychiatric disorders.

Effect of phenotype on PK, PD, and safety:

PK: there was no indication that CYP2D6 or NAT2 had effects on the PK profile of EMD 68843.
PD: No distinction was seen between PM and EM for NAT or CYP2D6.
Safety: there was no obvious indication that CYP2D6 had effects on the safety profile of EMD 68843.

Conclusions:

The study showed a dose-proportional increase in Cmax, Cav, and AUC0-24 of EMD 68843 at steady state following multiple doses of EMD 68843 (vilazodone) between 10 and 40 mg. Steady state of EMD 68843 (vilazodone) was reached by Day 3 after multiple doses of EMD 68843 under fasted conditions.
EMD 68843 showed some stimulating effect on growth hormone between 1 and 4 hours after dosing, which is more prominent after single dose administration. All treatments including placebo showed an increase in plasma prolactin concentrations, and this increase maintained even for 96 hours after dosing on Days 1 and 16. There is a dose-dependent transient increase plasma ACTH between 1 and 4 hours only after single dose.

EMD 68843 also showed some dose-dependent stimulating effect on cortisol which is more prominent after single dose administration between 1 and 4 hours after dosing.

==========

Study CLDA-07-DP-01 Addendum A (CLDA-07-DP-01A): The Effect of Emesis within the First 10 Hours Post-dose on the Extent of Vilazodone Absorption.

Objective: To gain insight into if a replacement dose should be administered if patients vomit within two times the median Tmax (first 10 hours) after ingesting a vilazodone dose.

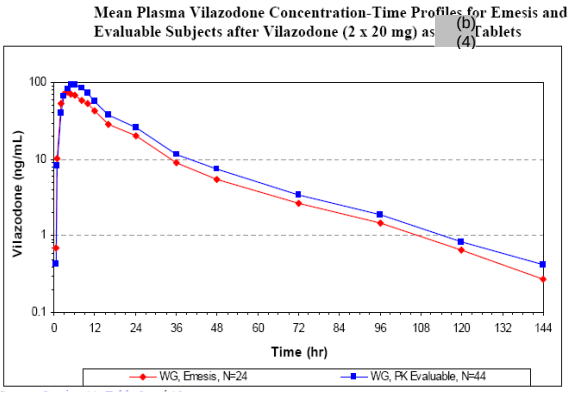
Study Design: This is a post-hoc analysis to evaluate Cmax and AUC0-t for the subjects participating in study CLDA-07-DP-01 who experienced emesis after 40 mg single dose compared to those subjects that completed the study that did not have any episodes of emesis. For the detailed information on study design, please see the individual study review for study CLDA-07-DP-01.

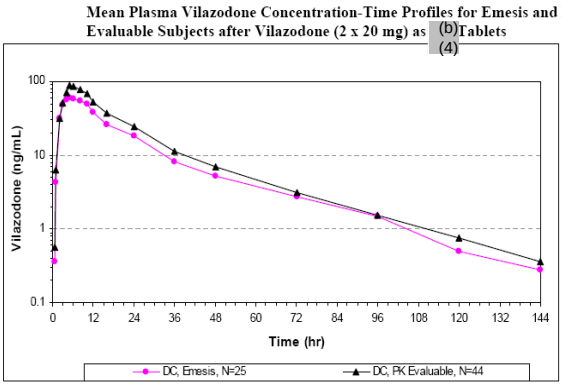
PK Results:

The effect of emesis on vilazodone PK: no replacement or "make-up" dose should be administered to subjects having emesis.

Emesis generally occurred prior to Tmax. The time to emesis averaged about 2.5 hours with range of 50 minutes to ~7 hours. On average, emesis reduced the absorption of an oral dose of 40 mg vilazodone by 22-30% as demonstrated using Cmax and AUC comparisons.

Mean drug plasma concentration-time profiles and mean PK parameters for (b) (4) tablets and (b) (4) tablets in subjects with emesis and without emesis are shown below.





The effect of gender on vilazodone PK:

Among the 49 subjects experiencing emesis, 27 (55.1%) were male and 22 (44.9%) were female. While the number of cases of emesis were approximately equivalent for the 2 sexes, a larger proportion of the enrolled female subjects (22/37, 59.5%), as compared to male subjects (27/65, 41.5%), experienced emesis.

For [redacted] (b) (4) tablets, Cmax and AUC of female subjects were increased by 37% and 34% compared to male subjects, respectively. For [redacted] (b) (4) tablets, Cmax and AUC of female subjects were increased by 50% and 36% compared to male subjects, respectively (see the table below). This higher rate of emesis in the female subpopulation might be associated with the smaller average body weight among the female subjects that participated in the study, and thus higher exposures in the female subjects (see figures showed below).

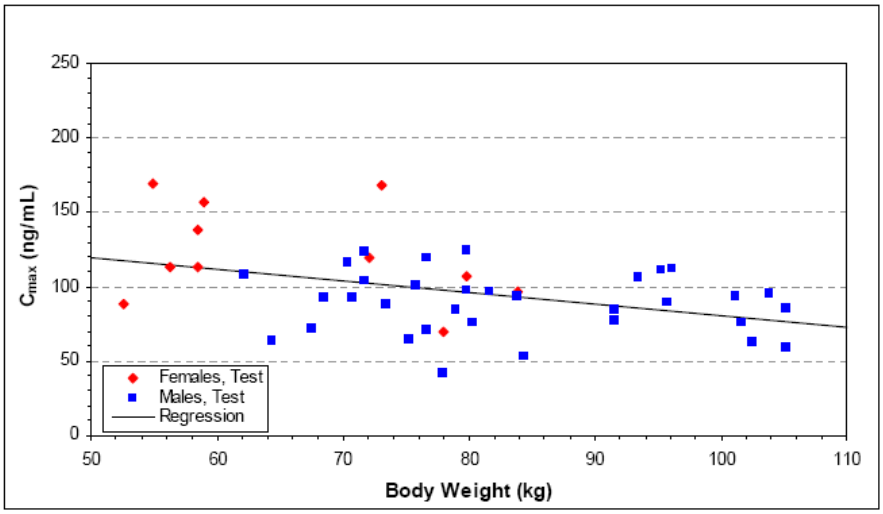
Summary of Standard and Dose-Normalized Exposure Parameters by Sex (PK Evaluable Subjects)

	[redacted] (b) (4) (test)		[redacted] (b) (4) (reference)	
	Males (N=33)	**Females (N=11)**	**Males (N=33)**	**Females (N= 11)**
	Mean ± SD (Male/Female)[a]	**Mean ± SD**	**Mean ± SD (Male/Female %)[a]**	**Mean ± SD**
C_{max} (ng/mL)	88.73 ± 20.85 (73.0%)	121.6 ± 32.8	95.90 ± 27.49 (66.5%)	144.3 ± 34.7
C_{max}/Dose[b] (ng/mL)(mg/kg)	184.3 ± 47.7 (93.1%)	198.0 ± 52.7	199.6 ± 64.0 (83.5%)	239.1 ± 69.5
AUC_{0-t} (ng•hr/mL)	1540 ± 453 (74.6%)	2064 ± 407	1675 ± 496 (73.7%)	2275 ± 362
AUC_{0-t}/Dose[b] (ng•hr/mL)/(mg/kg)	3217 ± 1081 (95.1%)	3384 ± 801	3496 ± 1204 (93.0%)	3761 ± 898

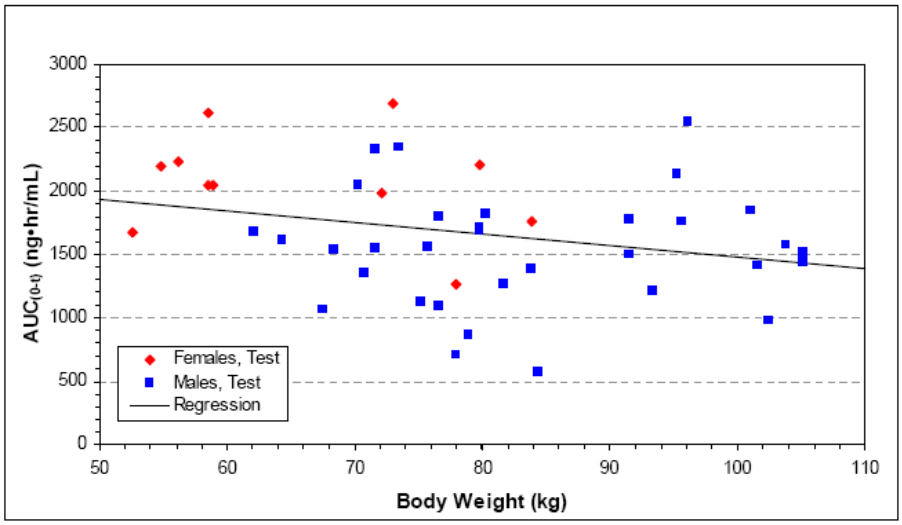
[a] Percentage value in parentheses is Males' value as a % of the Females' value

[b] Dose = 40 mg/body weight in kg

Vilazodone C_{max} Values in PK Evaluable Subjects following Oral Administration of the [redacted] (b) (4) (Test) Tablet (2x20 mg) as a Function of Body Weight



Vilazodone AUC_{0-t} Values in PK Evaluable Subjects following Oral Administration of the [redacted] (b) (4) (Test) Tablet (2x20 mg) as a Function of Body Weight



OCP Reviewer's Analysis:
Since two formulations, [redacted](b) (4), are bioequivalent, the Cmax and AUCinf in Forty-four subjects (33M/11F) with evaluable PK exposed to the two formulations were compared between males and females after adjustment of body weight:

Statistical Analysis of Body Weight Normalized PK Parameters following Single Dose of Vilazodone at 40 mg of [redacted](b) (4).		
Parameters	Ratio (F/M)	90% CI
Cmax/Dose*	114.12	97.28-133.87
AUCt/Dose*	110.04	94.81-127.72

* Dose=40 mg/body weight in kg.

Conclusions:
On average, emesis reduced the absorption of an oral dose of 40 mg vilazodone by 22-30% as demonstrated using Cmax and AUC comparisons. No replacement dose is needed to subjects having emesis.

After adjustment of body weight, vilazodone PK showed similar between females and males.
==========

Study PGX-08-P1-06: A Double-Blind Randomized Parallel Study to Define the ECG Effects of Vilazodone Using a Clinical and a Supratherapeutic Dose Compared to Placebo and Moxifloxacin in Healthy Volunteers: A Thorough ECG Study. (Vilazodone PK Review)

Refer to IRT-QT review for the study design details. The TQT study also assessed PK at 20 mg – 80 mg under fed conditions, which are presented here.

Pharmacokinetic Results:
EMD 68843 in plasma:

- PK parameters for EMD 68843 (Vilazodone) at different dose groups are shown in the following table:

Mean and median plasma vilazodone pharmacokinetic parameters (PK Evaluable Population)

	Vilazodone 20 mg (N = 56)	Vilazodone 40 mg (N = 55)	Vilazodone 60 mg (N = 55)	Vilazodone 80 mg (N = 56)
$AUC_{0\text{-}tlqc}$ (hr x ng/mL)				
Mean (SD)	776.8 (352.0)	1645.3 (720.9)	2506.1 (1107.4)	3269.8 (1720.0)
Median	780.5	1575.8	2557.7	3138.9
C_{max} (ng/mL)				
Mean (SD)	70.1 (30.2)	156.3 (67.6)	253.1 (113.1)	315.4 (169.5)
Median	68.7	143.5	240.9	312.0
T_{max} (hr)				
Mean (SD)	4.8 (1.6)	4.3 (1.7)	4.3 (1.1)	4.5 (1.4)
Median	4.0	4.0	4.0	4.0
CL/F (L/hr)				
Mean (SD)	30.5 (42.6)	34.0 (74.9)	35.8 (60.5)	59.3 (216.3)
Median	21.4	21.7	20.2	21.3

- Dose-proportional increase in Cmax and AUC0-t of EMD 68843 following three doses of EMD 68843 (Vilazodone) between 20 and 80 mg was observed.

Conclusions:
The study showed a dose-proportional increase in Cmax and AUC0-t of vilazodone over a dose range of 20-80 mg under fed conditions.
==========

MASS BALANCE STUDY

Study PGX-08-P1-07: Open label, single-dose, study to determine the absorption, distribution, metabolism, and excretion of 14C-vilazodone in healthy male subjects.

A brief overview of some essential components of the study design is given below:

Study Design	Open-label, single-dose study
Objective(s)	To determine the absorption, metabolism, and excretion of vilazodone in healthy male subjects by administering unlabeled and radiolabeled (14C-vilazodone) vilazodone; To evaluate the safety and tolerability of vilazodone.
Study Investigator(s)	(b) (4)
Study Site(s)	(b) (4)
Study Period	February 2, 2009 – February 16 2009
Study Population (completed)	N=7 Age: 24-56 years (mean 33.71 years)

<table>
<tr><td></td><td>Gender: male
Weight: 70.2-110.1 kg (mean 83.47 kg)
Race: Caucasian (n=6), and other (n=1).</td></tr>
<tr><td>Treatment Group</td><td>One group</td></tr>
<tr><td>Dosage and Administration</td><td>Single oral dose of vilazodone HCl aqueous solution (100 mL) containing 20 mg vilazodone HCl and approximately 37.6 µCi of radioactivity (from 14C-vilazodone) were administered to the subjects. After administration of the vilazodone dose, the dosing cup was rinsed twice with tap water (75 mL each rinse), and subjects consumed the rinse solution within 5 minutes after dosing.

Batch no: ~1.88 µCi/mg bulk powder of vilazodone (b) (4) 1837-1837-08-001)

Diet:
The study medication was administered under fed conditions (light meal).</td></tr>
<tr><td>Sampling: Blood</td><td>Pre-dose, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, and 144 hr after dosing (or at early termination).</td></tr>
<tr><td>Urine</td><td>-8 to 0 hr before dosing and 0-3, 3-6, 6-9, 9-12, 12-24, and 24-48 hr, and additional 24 hr intervals until the end of the study (i.e., 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288, 288-312, and 312-336 hr).</td></tr>
<tr><td>Feces</td><td>screening (Day -21 to -2) and over 24-hr intervals from Day 1 until the end of the study (i.e., 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288, 288-312, and 312-336 hr).</td></tr>
<tr><td>Analysis</td><td>Bioanalytical Facility
Unchanged vilazodone:
(b) (4)

Radioactivity:
(b) (4)

Analysis Dates: February 2009- March 2009 (radioqunatitation), May 2009 (plasma and urine), July 2009 (feces)

Method: LC-MS/MS with (b) (4) (plasma), LC-MS/MS (urine and feces).
<table>
<tr><th>Matrix</th><th>Analyte</th><th>LLOQ* (ng/mL)</th><th>ULOQ* (ng/mL)</th><th>Precision (%)</th><th>Accuracy (%)</th></tr>
<tr><td>Plasma</td><td>Vilazodone HCl</td><td>0.8119</td><td>270.6</td><td>1.2 – 5.1</td><td>11.5 – 12.8</td></tr>
<tr><td>Urine</td><td>Vilazodone</td><td>1.0</td><td>200</td><td>3.8 – 7.7</td><td>-0.1 – 1.7</td></tr>
</table></td></tr>
</table>

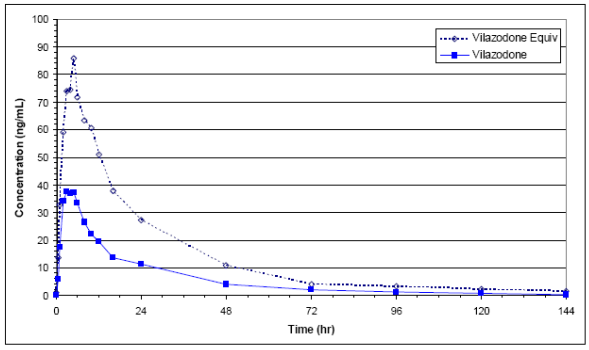
		HCl				
	Feces	Vilazodone HCl	2.5	80	2.6 – 3.5	-3.0 – -0.8
	*ng/g for Feces					
PK Assessment	Plasma: AUC0-24, AUC0-t, AUC inf, Cmax, Tmax, T1/2, CL/F, Vz/F, CLr. Urine: Amount recovered in urine (AU), % of dose recovered in urine. Feces: % of dose recovered in feces.					
Safety Assessment	Vital signs, ECGs, adverse events, and laboratory tests etc.					
PD Assessment	None					

Pharmacokinetic Results:

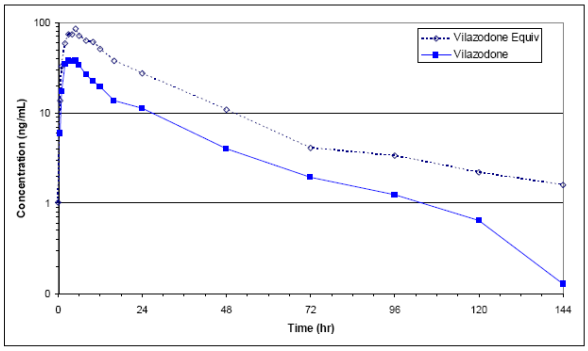
- Vilazodone metabolites were rapidly formed, as indicated by the rapid rise in radioactivity levels in plasma above those associated with parent drug. Peak radioactivity levels for the metabolites occured a median of 1 hour after the vilazodone T_{max} (5 hours v.s., 4 hours). The C_{max} and AUC values for radioactivity were approximately 2 to 2.5 times the values for vilazodone. This disproportionate contribution of the metabolites to the total radioactivity in the plasma suggested that the metabolites are more hydrophilic than vilazodone and have a smaller volume of distribution. Vilazodone and the radioactivity were eliminated at a similar rate, with a half-life of approximately 30 hours. Similar elimination rates for parent and metabolites suggested that the PK of metabolites is formation rate limited. Approximately 85% of the administered radioactivity was recovered in the urine (~20%) and feces (~65%) combined, while approximately 3% of the administered dose of vilazodone was recovered as unchanged drug (~1% in urine, ~2% in feces).

Mean plasma concentration-time profiles for vilazodone HCl and radioactivity are shown below:

Linear Scale



Log-linear Scale



PK parameters for vilazodone and radioactivity are shown below:

Pharmacokinetic Parameters for Vilazodone HCl and Radioactivity (PK Evaluable Population)

Parameter	Statistic	Vilazodone HCl	Radioactivity
C_{max} (ng/mL)	Mean ± SD Median (Range) % CV	43.4 ± 17.0 39.5 (30.0, 79.8) 39.2	92.3 ± 20.7 81.1 (69.2, 120.0) 22.4
T_{max} (hr)	Mean ± SD Median (Range) % CV	4.14 ± 1.35 4.00 (2.00, 6.00) 32.5	4.29 ± 1.38 5.00 (2.00, 6.00) 32.2
AUC_{0-24} (hr·ng/mL)	Mean ± SD Median (Range) % CV	496 ± 138 512 (360, 757) 27.8	1168 ± 197 1028 (1003, 1476) 16.8
AUC_{0-t} (hr·ng/mL)	Mean ± SD Median (Range) % CV	811 ± 211 826 (563, 1119) 26.0	1996 ± 289 1950 (1670, 2310) 14.5
$AUC_{0-\infty}$ (hr·ng/mL)	Mean ± SD Median (Range) % CV	850 ± 213 867 (589, 1146) 25.1	2071 ± 292 2160 (1690, 2370) 14.1
λz (1/hr)	Mean ± SD Median (Range) % CV	0.0274 ± 0.0079 0.0303 (0.0186, 0.0396) 28.7	0.0290 ± 0.0104 0.0315 (0.0075, 0.0372) 36.0
$T_{1/2}$ (hr)	Mean ± SD Median (Range) % CV	27.2 ± 7.9 22.9 (17.5, 37.3) 29.0	31.8 ± 27.2 22.0 (18.6, 93.0) 85.3
CL/F (L/hr)	Mean ± SD Median (Range) % CV	24.9 ± 6.39 23.1 (17.5, 34.0) 25.7	9.85 ± 1.46 9.26 (8.45, 11.9) 14.8
Vz/F (L)	Mean ± SD Median (Range) % CV	966 ± 308 1070 (440, 1402) 31.9	442 ± 357 314 (228, 1240) 80.6
CLr (L/hr)	Mean ± SD Median (Range) % CV	0.253 ± 0.126 0.284 (0.101, 0.406) 50.0	Not calculated
% Dose Recov in Urine	Mean ± SD Median (Range) % CV	1.08 ± 0.68 1.05 (0.47, 2.29) 62.2	19.91 ± 2.69 19.87 (16.01, 23.85) 13.5
% Dose Recov in Feces	Mean ± SD Median (Range) % CV	1.76 ± 0.38 1.84 (1.15, 2.25) 21.5	65.27 ± 13.98 68.23 (35.73, 76.92) 21.4

- Metabolite Profile:

    1. Plasma Metabolite Profile: The mean sum of four analytes, M16, M10, M11, and M17, in plasma accounted for at maximum of ~79% of the total vilazodone related material in the plasma samples at time range of 10-24 hours (see the table below). The rank order of contributions in plasma was

M16 (vilazodone) >M17 (butyric acid of the indole fragment of the N-dealkylation product of vilazodone) >M10 (carboxylic acid derivative of vilazodone) >M11 (aliphatic hydroxylation), with M16 (vilazodone) contribution being about ~ 50%.

Summary of Concentrations of Vilazodone Related Substances in Plasma

Subject ID	Time Range (h)	Concentrations in ng-eq/mL of Vilazodone free base, and as % of Total Vilazodone-derived Material in the Pooled Sample											
		M16		M10		M11		M17		Sum of 4 Analytes		Mean ^{14}C Concentration in Pooled Plasma Sample	
		Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total	Conc	Percent of Total		
Mean	0.5-4	12.75	(28.4%)	2.41	(5.3%)	0.57	(1.3%)	4.82	(10.7%)	20.55	(45.7%)	46.9	(100%)
Mean	5-8	22.62	(33.5%)	7.77	(11.8%)	0.58	(0.9%)	11.85	(17.9%)	42.82	(64.1%)	68.0	(100%)
Mean	10-24	16.58	(41.6%)	5.71	(14.2%)	0.38	(0.9%)	8.65	(21.7%)	31.32	(78.9%)	40.7	(100%)

2. Urine Metabolite Profile: Urine samples for each subject were pooled across multiple collection intervals to creat 3 pooled samples per subject (0-9 hours, 9-24 hours, and 24-96 hours). The rank order of contributions in urine was M10 > hydroxyl-M16 ≥ M16> the remaining metabolites. M11 was the leasat prevalent in all 7 subjects (see the table below).

Metabolites Quantified in Urine expressed as Percent of the Administered Dose and Arranged by Mean Prevalence

	Subject ID							
Quantified Metabolite	0701-002	0701-003	0701-004	0701-005	0701-007	0701-010	0701-011	Mean
M10	2.46	3.22	2.98	2.81	5.93	3.18	6.78	3.91
hydroxy M16	1.49	3.03	3.03	1.71	3.30	4.01	1.93	2.64
M16	2.00	1.34	1.99	2.76	3.85	1.33	2.72	2.28
dihydroxy M16	0.71	0.72	0.84	0.77	2.13	0.41	0.95	0.93
M17	0.61	0.27	1.45	0.82	1.18	0.84	1.12	0.90
M4/M6	0.63	1.09	0.90	0.81	1.34	0.51	0.91	0.89
unidentified	0.68	0.38	0.83	0.75	0.94	0.43	0.58	0.66
M11	0.36	0.23	0.57	0.41	0.91	0.17	0.44	0.44
Total of Quantified Peaks as % of Dose	9.0	10.3	12.6	10.8	19.6	10.9	15.4	12.7
Total of Quantified Peaks as % of the Fraction of the Dose in the Assayed Urine Samples	96.4	86.3	93.2	89.7	95.8	95.9	86.3	92.0

Note: Shading is used to group the quantified metabolites into the prevalence "tiers" discussed in the text.

M4/M6: glucuronide conjugates of hydroxyl M16

3. Fecal Metabolite Profile: The most prevalent vilazodone –derived species recovered in the feces was M10. M16 was second in prevalence at about half that of M10 (see the table below).

Metabolites Quantified in Feces Expressed as Percent of the Administered Dose and Arranged by Mean Prevalence

	Subject ID							
Quantified metabolite	0701-002	0701-003	0701-004	0701-005	0701-007	0701-010	0701-011	Mean
M10	14.40	10.07	17.64	29.40	19.10	30.64	13.04	19.18
M16	7.83	8.27	11.07	12.36	12.34	8.69	4.71	9.32
M10 N-oxide	5.72	6.90	8.11	6.43	4.00	3.05	2.88	5.30
M10-taurine	13.88	9.40	0.00	0.00	3.44	0.63	0.00	3.91
M16 N-oxide	3.65	5.24	3.14	4.11	2.92	0.90	6.24	3.74
dihydroxy M16	3.48	2.36	0.22	1.68	0.79	0.13	0.32	1.28
M10-taurine N-oxide	4.29	3.42	0.00	0.00	0.73	0.00	0.00	1.21
M10 N-oxide	1.32	2.21	1.31	0.00	2.28	0.00	1.12	1.18
dihydroxy M10	0.59	0.52	1.13	0.97	1.18	1.82	0.43	0.95
hydroxy M16	0.95	0.68	0.41	2.70	0.29	0.63	0.00	0.81
M16 N-oxide	0.00	0.96	0.00	1.57	2.41	0.00	0.00	0.71
M10-glycine	0.00	0.00	0.00	0.00	1.25	2.02	0.76	0.58
hydroxy M16	0.88	1.64	0.64	0.40	0.00	0.00	0.00	0.51
trihydroxy M16	1.16	1.31	0.00	0.00	0.00	0.00	0.10	0.37
Total of Quantified Peaks as % of Dose	58.2	53.0	43.7	59.6	50.7	48.5	29.6	49.0
Total Percent of Dose in Soluble Extract	61.9	65.3	48.4	62.1	54.6	49.7	31.0	53.6
Total of Quantified Peaks as % of the Fraction of the Dose in the Soluble Extract	94.0	81.1	90.2	96.0	92.9	97.6	95.4	92.5

Note: Shading is used to group the quantified metabolites into the prevalence "tiers" discussed in the text.

Additionally, the metabolite profiling results suggested that oral dosing of vilazodone HCl resulted in the formation of 8 primary metabolites, 4 secondary metabolites including conjugates of the primary metabolites, and a number of tertiary metabolites formed by multiple Phase 1 and 2 reactions. The metabolites, the biomatrix from which they were isolated, and their retention times with the HPLC separation method employed are summarized in the table below:

Relationship Summary for the Quantified Metabolites

Stage	Metabolite	Retention Time (min)	Biomatrix			Postulated Enzyme Class(es)
			Plasma	Urine	Feces	
Parent	M16	32.5	x	x	x	N/A
Primary	hydroxy M16	23.2		x		CYP
Primary	hydroxy M16	26.3			x	CYP
Primary	M11	27.0	x	x		CYP
Primary	M16 N-oxide	27.6			x	CYP/FMO
Primary	hydroxy M16	28.9			x	CYP
Primary	M10	29.3	x	x	x	CE
Primary	M16 N-oxide	33.6			x	CYP
Primary	M17	34.0	x	x		CYP
Secondary	dihydroxy M16	21.0		x		CYP
Secondary	M4/M6	22.0		x		CYP + UGT
Secondary	M10 N-oxide	25.5			x	CE + CYP/FMO
Secondary	M10 N-oxide	29.8			x	CE + CYP/FMO
Tertiary	dihydroxy M10	15.2			x	CE + CYP
Tertiary	trihydroxy M16	20.0			x	CYP
Tertiary	M10 glycine	30.1			x	CE + AAC
Tertiary	M10 taurine	30.8			x	CE + AAC
Tertiary +	M10 taurine N-oxide	31.1			x	CE + AAC + CYP/FMO

AAC - amino acid conjugation; CE - carboxylesterase; CYP - cytochrome P450;
FMO - flavin monooxygenase; N/A - Not applicable; UGT - UDP-glucuronosyltransferase

Safety Results:
No death or SAE was reported.

Five AEs occurred in 4 of 7 subjects. The most common AEs are GI disorders in 3 of 7 subjects with mild in intensity.

Conclusions:

- The metabolites are more hydrophilic than vilazodone and had a smaller volume of distribution. Vilazodone and the radioactivity were eliminated at a similar rate, with a half-life of approximately 30 hours. Similar elimination rates for parent and metabolites suggested that the PK of metabolites is formation rate limited. Approximately 85% of the administered radioactivity was recovered in the urine (~20%) and feces (~65%) combined, while approximately 3% of the administered dose of vilazodone was recovered as unchanged drug (~1% in urine, ~2% in feces).

- M16, the parent molecule, was the most prevalent drug related species in the plasma, and the second most prevalent drug related species in the urine and feces. In the urine, 1% to 2% of the dose was recovered as unchanged M16, while recovery in the feces accounted for 2% to 9% of the dose. (The range in values observed for fecal recovery suggests that the sample processing and/or analytical

procedures used with the fecal samples converted some metabolite(s) of M16 back to M16.)

- M10, a carboxylic acid metabolite of M16, was the most prevalent drug related species in the urine and feces, accounting for approximately 4% of the dose in the urine and approximately 19% in the feces. M10 was present at approximately one third the concentration of M16 in the plasma. Secondary and tertiary metabolites of M10 in the feces accounted for approximately another 13% of the administered dose, suggesting that more than one third of the administered dose was converted the acid prior to elimination or further metabolism.

- M17, the butyric acid derivative of the N-dealkylation product of M16, was the most prevalent metabolite in the plasma, being present at approximately half the concentration of M16. However, its recovery in the urine was 1% or less of the dose, and it was not among the 14 consistently quantifiable metabolites in the feces. No secondary or tertiary metabolites of M17 were detected.

- M11, M16 hydroxylated at the aliphatic carbon adjacent to the indole moiety, is the most pharmacologically potent of the vilazodone metabolites, but its contribution to serotonin related actions was estimated to be less than 0.1% that of the parent molecule. It is also not expected to contribute to off-target pharmacological activities related to other commonly targeted CNS receptors. The possible contributions of M10 and M17 to pharmacological activity were similar to, or less than, the projected contribution from M11.

- Eight primary metabolites of vilazodone were identified in the plasma, urine and/or feces and consisted of 4 hydroxylated and 2 N-oxide derivatives of the parent molecule, a carboxylic acid derivative (M10) and an N-dealkylation product (M17). In addition 4 secondary metabolites and 4 tertiary metabolites were quantified.

- Multiple parallel elimination pathways are likely to be involved in the elimination of vilazodone; CYP dependent metabolism is expected to form the hydroxyl and N-dealkylation products, carboxylesterase may play a role in formation of the acid (M10), and FMO enzyme may play a role in N-oxide formation, while biliary secretion may also clear some of the parent molecule. N-oxide formation, glucuronidation, conjugation to amino acids, hydroxylation and biliary secretion all appear to contribute to the clearance of the primary and secondary metabolites. The likely multiplicity of parallel elimination pathways suggest a reduced impact on the clearance of vilazodone by alteration in intrinsic factors (renal and hepatic impairment) or the addition of extrinsic factors (drug-drug interactions).

==========

PHARMACODYNAMIC STUDIES

Study GPP-007-CLN-CP1-2001-255 (EMD 68843 – 011): A PET Study to determine the occupancy of central serotonin 5-HT1A receptors by EMD 68 843

A brief overview of some essential components of the study design is given below:

<table>
<tr><td>Study Design</td><td>Single-center, single-dose, two-scan study</td></tr>
<tr><td>Objective(s)</td><td>To determine of binding potential of EMD 68 843 for 5-HT1A receptors</td></tr>
<tr><td>Study Investigator(s)</td><td>(b) (4)</td></tr>
<tr><td>Study Site(s)</td><td>(b) (4)</td></tr>
<tr><td>Study Period</td><td>January 7, 1998 – April 9, 1998</td></tr>
<tr><td>Study Population</td><td>N=6 (2D6 EM)
Age: 25-50 years (mean 34.3 years)
Gender: males
Weight: 73-99.4 kg (mean 88 kg)
Race: Caucasian</td></tr>
<tr><td>Methodology</td><td>Positron Emission Tomography (PET) and [11C]-WAY-100635 were used to measure the occupancy of central 5-HT1A receptors by EMD 68 843.</td></tr>
<tr><td>Dosage and Administration</td><td>The study medication, 20 mg (N=2, SD) and 40 mg (N=4, SD), was administered on Day 0 and on Day 7, respectively.

Batch no: 20 mg capsule 5311S3
40 mg capsule 5312S3

Diet:
The study medication was administered under fed conditions.</td></tr>
<tr><td>Sampling: Blood</td><td>Pre-dosing and 2, 4, 5, 6, 8, 12 and 24 hours after dosing.</td></tr>
<tr><td>Analysis</td><td>Bioanalytical Facility
Institute of Pharmacokinetics and Metabolism of Merck KGaA

Analysis Dates: April 5, 1998 – May 5, 1998

Method: HPLC with fluorescence detection
<table>
<tr><th>Matrix</th><th>Analyte</th><th>LLOQ (ng/mL)</th><th>ULOQ (ng/mL)</th><th>Precision (%)</th><th>Accuracy (%)</th></tr>
<tr><td>Plasma</td><td>EMD 68843</td><td>1</td><td>100</td><td>2.4- 5.3</td><td>2.4 – 4.7</td></tr>
</table></td></tr>
<tr><td>PK Assessment</td><td>Plasma concentration</td></tr>
<tr><td>Safety Assessment</td><td>blood pressure, heart rate, ECGs, adverse events, and laboratory tests</td></tr>
</table>

	etc
PD Assessment	5HT1A Receptor Occupancy

PD Results:
For the two subjects treated with 20 mg EMD 68843 p.o. there was little conclusive evidence for occupancy of the 5-HT1A receptor. Three of four subjects treated with 40 mg EMD 68843 p.o. the MT occupancy showed 15-35% for 5-HT1A receptor occupancy.

Difference between scans (%)
[(Baseline BP – Drug BP) / Baseline BP] × 100

	20 mg Subjects		40 mg Subjects			
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Medial temporal						
Time–activity curve	–5.31	13.16	17.30	37.03	36.17	–32.03
Voxel by Voxel	–1.09	8.73	15.20	32.15	34.54	–25.63
Insula						
Time–activity curve	–0.36	14.23	6.60	27.36	36.74	–19.67
Voxel by Voxel	0.38	7.24	8.20	24.14	36.15	–14.26
Anterior cingulate						
Time–activity curve	8.50	–21.00	10.97	11.05	34.28	–15.29
Voxel by Voxel	5.63	–22.75	14.11	10.98	32.33	–8.52
Raphe nuclei						
Time–activity curve	4.88	–7.07	15.97	89.93	48.18	3.16
Voxel by Voxel	7.13	–1.25	19.56	57.76	46.50	–4.37

Since the PET scan under EMD 68 843 was performed approximately six hours after the intake of the study medication, and the scan itself lasted 1.5 hours, the most interesting plasma concentrations of EMD 68 843 were those measured from 6 to 8 hours after intake of the drug. Plasma concentration of the study drug is presented below:

Plasma concentrations of EMD 68 843 [ng/ml] during the drug scan

	20 mg Subjects		40 mg Subjects			
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Time after dosing						
6 hours	23.3	23.3	11.7	91.7	58.2	11.0
8 hours	20.8	18.6	17.0	82.8	64.8	11.8

Safety Results:
No death and SAE was reported.

Two AEs of mild nausea were reported in 2 subjects with taking 40 mg study drug.

OCP reviewer's comments: the PET study is one of the rationales for use of 40-mg dose of vilazodone as a therapeutic target dose. This study showed a dose-dependent occupancy of the 5-HT1A receptor with vilazodone, with measurable occupancy observed following a single 40-mg oral dose of vilazodone in 3 of 4 subjects (15-35%), while no measurable occupancy of the receptor was observed following a single 20-mg oral dose. However, the single dose occupancy study can not support the rationale for the dose selection due to the relatively long half life of the vilazodone (~26 hours) with accumulation factor at ~1.8. An occupancy study after multiple doses of vilazodone at steady state can properly elucidate the dose-occupancy profile but not single dose study.
==========

INTRINSIC FACTORS

Hepatic Impairment Study:

Report # ***Study PGX-08-P1-02*** | **Study Period:** ***June 20, 2008 – March 3, 2009***

Title ***: A Phase I, open label, study of vilazodone tolerability and pharmacokinetics in subjects with mild and moderate hepatic impairment compared to matched control subjects with normal hepatic function***

Study Design

Single-Dose	Non-Randomized	Open-Label	Parallel	Multi-Center	
No. of Groups	☑Normal	☑Mild	☑Moderate	☐Severe	Total
No. of Subject /Completed	16	8	8	NA	**32**
Males/Females	6/3, 6/2	5/3	6/2	NA	23/10
Age, Mean(range)	44.3 (29-53), 55.3 (47.4-64.4)	48.5 (39.3-55.1)	57.8 (54.4-60.9)	NA	-
Dose (mg)	20	20	20	NA	20
Diet	Fed (light meal)				-
Tablet Lot #	07T10772			NA	-

- Sampling Times:
  - PK, plasma: pre-dose, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, and 144 hours post dose and early termination.
  - PD, plasma: NA
  - Protein Binding: ☐All ☑Limited (24 subjects: 8 with mild, 8 with moderate and 4 healthy subjects, 4 healthy subjects from renal impairment study)
    Sampling Times: 15-60 min pre-dose, 6 and 12 hours post-dose.
    Method: validated equilibrium dialysis
- Classification of hepatic function is consistent with the FDA Guidance Recommendations:

☑ Yes ☐ No

- Hepatic function was determined via Child-Pugh classification ☑ Yes ☐ No
- Hepatic function was determined at: ☑ Screening ☑Baseline

- The control group is adequate ☑ Yes ☐ No
- The groups are matched by☑ Age ☑ Sex ☑ BMI ☐ Smoking Status ☐ Race
- The selected dose is acceptable ☑ Yes ☐ No
- Dosing is long enough to obtain steady state ☐ Yes ☐ No☑ Not Applicable
- Sample size was determined based on statistical analysis ☐Yes ☑ No
- The overall study design acceptable: ☑ Yes ☐ No

Analytical Method (Study Samples Analysis)

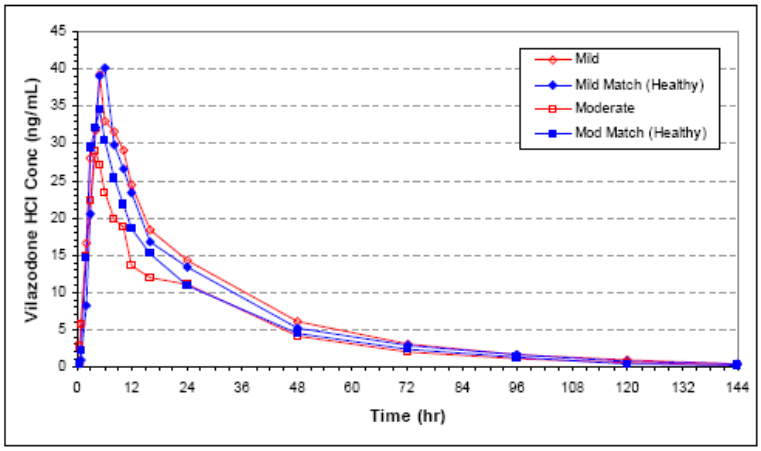
- Study samples were analyzed within the established stability period: ☑ Yes ☐ No
- Quality control samples range is acceptable ☑ Yes ☐ No
- Internal standard was used ☑ Yes ☐ No
- Method was validated prior to use ☑ Yes ☐ No
- Chromatograms were provided ☑ Yes ☐ No
- Overall performance is acceptable ☑ Yes ☐ No

Analyte	Vilazodone HCl	Vilazodone HCl	Vilazodone HCl	Vilazodone HCl
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Matrix	Plasma	Urine	Protein Binding Donor Cell	Protein Binding Receiver Cell
LLOQ-ULOQ (ng/mL)	0.8119 – 270.6	1 – 200	2.706 – 108.3	0.1083 – 5.413
Precision (%)	5.8 – 10.1	4.1 – 9.4	2.6 – 7.8	4.8 – 9
Accuracy (%)	5 – 5.4	-1.8 – 5.2	-1.9 – 2.8	5.6 - 6

Pharmacokinetics

Hepatic Impairment	**Geometric Mean Ratio (Cmax) Renal Impairment/ Healthy Volunteers**	
	Point Estimate	**90% CI**
Mild	**0.98**	**0.72-1.32**
Moderate	**0.97**	**0.72-1.31**
Hepatic Impairment	**Geometric Mean Ratio (AUCinf) Renal Impairment/ Healthy Volunteers**	
	Point Estimate	**90% CI**
Mild	**0.98**	**0.72-1.33**
Moderate	**0.91**	**0.67-1.23**

Figure 1. Mean PlasmaVilazodone HCl Concentration Profiles (linear scale).



Safety

Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA

Conclusions

Should the dose be adjusted in subjects with hepatic impairment (mild and moderate)?

☐ Yes ☑ No

==========

Renal Impairment Study:

Report # ***PGX-08-P1-01***	**Study Period**: ***May 30 2008-March 23 2009***

Title: A phase I, open label, study of vilazodone tolerability and pharmacokinetics in subjects with mild and moderate renal impairment compared to matched control subjects With normal renal function.

Study Design

Single-Dose	Non-Randomized	Open-Label	Parallel	Multi-Center	
No. of Groups	☑Normal	☑Mild	☑Moderate	☐Sever	☐ESRD
No. of Subject /Completed	16	8	8	NA	NA
Males/Females	11/5	5/3	6/2	NA	NA
Age, Mean(range)	56.4 (25.4-66.7); 59 (55.7-62.8)	58.6 (31.4-71.6)	63.9 (55.2-71)	NA	NA
Dose (mg)	20	20	20	NA	NA
Diet	Fed (light meal)			NA	NA
Plasma Sampling Times: pre-dose, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, and 144 hours post-dose and early termination. Urine Sampling Times: 0-3, 3-6, 6-9, 9-12, 12-24, 24-48, 48-72, and 72-96 hours post-dose.					

- Classification of renal function is consistent with the FDA Guidance Recommendations:

☑ Yes ☐ No

- Renal function was determined based on estimated GFR via ☐ G-C formula ☑ MDRD formula
- Renal function was determined at: ☑ Screening ☑Baseline
- The control group is adequate ☑ Yes ☐ No
- The groups are matched by☑ Age ☑ Sex ☑ Body Weight ☐ Smoking Status ☐ Race
- The selected dose is acceptable ☑ Yes ☐ No
- Protein Binding: ☐All ☑Limited (in all subjects)
  Sampling Times: 15-60 minutes pre-dose, and 4 and 12 hours post-dose.
  Method: Validated equilibrium dialysis
- Dosing is long enough to obtain steady state ☐ Yes ☐ No☑ Not Applicable
- Sample size was determined based on statistical analysis ☐Yes ☑ No
- The overall study design acceptable: ☑ Yes ☐ No

Analytical Method (Study Samples Analysis)

- Study samples were analyzed within the established stability period: ☑ Yes ☐ No
- Quality control samples range is acceptable ☑ Yes ☐ No
- Internal standard was used ☑ Yes ☐ No
- Method was validated prior to use ☑ Yes ☐ No
- Chromatograms were provided ☑ Yes ☐ No
- Overall performance is acceptable ☑ Yes ☐ No

Analyte	Vilazodone HCl	Vilazodone HCl	Vilazodone HCl	Vilazodone HCl
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Matrix	Plasma	Urine	Protein Binding Donor Cell	Protein Binding Receiver Cell
LLOQ-ULOQ (ng/mL)	0.8119 – 270.6	1 – 200	2.706 – 108.3	0.1083 – 5.413
Precision (%)	6.4 – 8.6	6.8 – 9.0	3.9 – 9.5	2.1 – 6.5
Accuracy (%)	-0.2 – 11.5	-1.4 – 8.2	-4.8 – 0.1	4.7 - 5

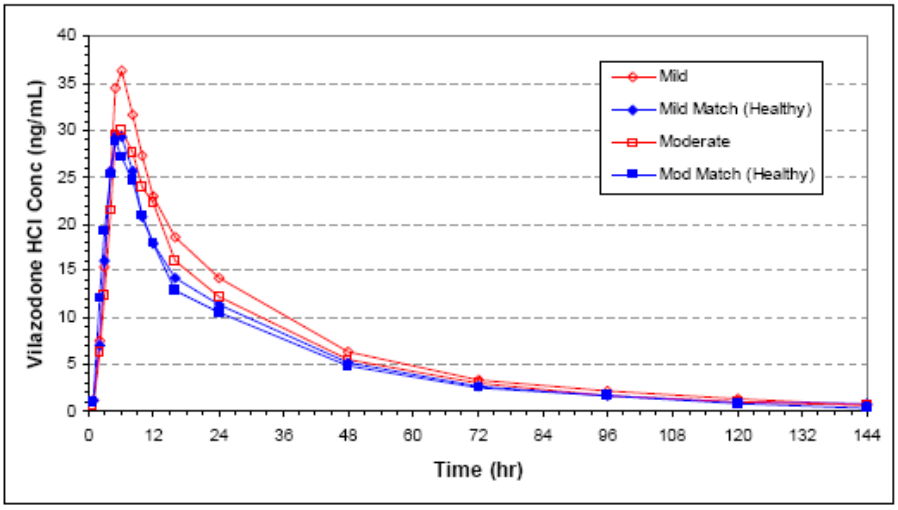
Pharmacokinetics

Renal Impairment	**Geometric Mean Ratio (Cmax) Renal Impairment/ Healthy Volunteers**	
	Point Estimate	**90% CI**
Mild	**1.08**	**0.80-1.45**
Moderate	**1.08**	**0.81-1.46**

Renal Impairment	**Geometric Mean Ratio (AUCinf) Renal Impairment/ Healthy Volunteers**	
	Point Estimate	**90% CI**
Mild	**1.26**	**0.96-1.67**

Moderate	**1.10**	**0.83-1.45**

Figure 1. Mean PlasmaVilazodone HCl Concentration Profiles (linear scale)



Safety
Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA

Conclusions
Is there is a need to adjust the dose in patients with renal impairment (mild and moderate)? ☐ Yes ☑ No

==========

Study GPP-007-CLN-CP1-2003-237 (GSK Study 008): A Single Blind, Parallel Group, Dose Rising, Randomized Placebo-Controlled, Single and Repeat Oral Dose Study to Assess the Tolerability and Describe the Pharmacokinetics of SB-659746 in Healthy Elderly and Young Volunteers

A brief overview of some essential components of the study design is given below:

Study Design	Single blind, randomized, placebo-controlled, parallel group, dosing rising single and multiple oral dose study
Objective(s)	• To evaluate the tolerability of single and multiple oral doses of SB-659746 (5mg, 10mg and 20mg od) in healthy elderly volunteers. • To characterize the single and multiple oral doses pharmacokinetics of SB-659746 (5mg, 10mg and 20mg od) in healthy elderly volunteers. • To evaluate the tolerability of single and multiple oral doses of SB-659746 20mg od in healthy young volunteers. • To characterize the single and multiple oral dose pharmacokinetics of SB-659746 20mg in healthy young volunteers.

<table>
<tr><td>Study Investigator(s)</td><td>(b) (4)</td></tr>
<tr><td>Study Site(s)</td><td>(b) (4)</td></tr>
<tr><td>Study Period</td><td>April 3, 2002 – October 30, 2002</td></tr>
<tr><td>Study Population (completed)</td><td>N=31 elderly subjects , N=12 young subjects
Age: 65-80 years (mean 68 years, elderly); 24-55 years (mean 38 years, young)
Gender: M/F = 17/14 (elderly), 4/8 (young).
Weight: 63.1-92.3 kg (mean 76.2 kg, elderly); 56.5-83.5 kg (mean 68.3 kg, young).
Race: Caucasian</td></tr>
<tr><td>Treatment Group</td><td>3 groups (5 mg, 10 mg, and 20 mg) with placebo at each group.</td></tr>
<tr><td>Dosage and Administration</td><td>Session 1: a single oral dose of placebo or study medication at 5 mg (only for elderly), 10 mg (only for elderly), or 20 mg.

Session 2: following 7-14 days wash out period, subjects received placebo or study drug (5, 10, or 20 mg), QD on Days 1-7 (the same medication that subjects received in Session 1).

Batch no: Placebo capsule U01005
5 mg capsule U01006
10 mg capsule U01007
20 mg capsule U01008
Diet:
The study medication was administered under fed conditions.</td></tr>
<tr><td>Sampling: Blood</td><td>Pre-dose, 1, 2, 3, 4, 5, 6, 8, 10, 14, 24, 32 (± 2) (not for 20 mg), 48, 72, 96, 120, 144 (only for 20 mg), 168 (only for 10 and 20 mg), and 240 (only for 10 and 20 mg) hours post-dose on Day 1 (Session1) and Day 7 (Session 2) (~ 3 mL for each time point). Pre-dose on Days 5 and 6.</td></tr>
<tr><td>Urine</td><td>None</td></tr>
<tr><td>Feces</td><td>None</td></tr>
<tr><td>Analysis</td><td>Bioanalytical Facility
(b) (4)

Analysis Dates: August 20, 2002 - October 24, 2002.

Method: LC-MS/MS with (b) (4)

<table>
<tr><th>Matrix</th><th>Analyte</th><th>LLOQ (ng/mL)</th><th>ULOQ (ng/mL)</th><th>Precision (%)*</th><th>Accuracy (%)*</th></tr>
<tr><td>Plasma</td><td>SB-659746</td><td>0.5</td><td>250</td><td>5 – 12.5</td><td>-3 – 3.2</td></tr>
</table>
*A typographical error was claimed by the sponsor, therefore the sponsor recalculated with the value assumed missing.</td></tr>
<tr><td>PK Assessment</td><td>AUC0-24, AUC inf, Cmax, Tmax, T1/2, accumulation parameters.</td></tr>
<tr><td>Safety Assessment</td><td>Vital signs, ECGs, adverse events, and laboratory tests etc</td></tr>
<tr><td>PD Assessment</td><td>None</td></tr>
</table>

PK Results:
Elderly subjects showed about a 20% lower vilazodone.exposures compared to the young.

OCP analysis:

Statistical Analysis of Major PK Parameters following Single Dose of Vilazodone at 20 mg		
Parameters	Ratio (Elderly/Young)	90% CI
Cmax	95.36	65.52-138.79
AUC0-24	86.27	61.81-120.4
Statistical Analysis of Major PK Parameters at Steady State following Multiple Doses of Vilazodone at 20 mg QD.		
Parameters	Ratio (Elderly/Young)	90% CI
Cmax	82.93	58.92-116.73
AUC0-24	81.28	62.36-105.93

Safety Result:
No death or SAE was reported. The most common adverse event across all treatment regimens was headache. Other adverse events reported more than once by subjects were diarrhoea (10mg SB-659746 repeat dose), fatigue (20mg SB-659746 single dose) and nausea (20mg SB-659746 single and repeat dose healthy young subjects only). AEs were mild (46 AEs) or moderate (28 AEs). There was no clear difference in the number of subjects reporting adverse events on placebo compared to SB-659746 at dose levels 5mg, 10mg, 20mg healthy elderly and 20mg healthy young subjects. SB-659746 5mg, 10mg and 20mg administered as single and 7 days repeat doses were generally well tolerated in the healthy elderly population.

Conclusion
Elderly subjects showed lower systemic exposure compared to young subjects by ~20%. However, this decrease is not clinical relevant. No dose adjustment is needed for elderly subjects.
==========

EXTRINSIC FACTORS

DDI with Ketoconazole

Report # ***GPP-007-CLN-CP1-2003-240***	**Study Period:** ***July 23, 2002 – December 22, 2002***	**EDR Link: NA**
Title	***A two part, randomized, double blind, crossover study to examine the pharmacokinetics of SB-659746 when coadministered with repeat doses of ketoconazole in healthy volunteers*****.**	
Study Design: This study was conducted in two parts, an open Part (Part A) followed by a double blind Part (Part B).		
Rationale: ***3A4 is a major CYP isozyme for the drug metabolism.***		

Choose from the list in each box by double clicking on the box Single-Dose Randomization Blinding Cross-Over Single-Center 2-Period Healthy Vonuteers		
Screening: 21 days	**Washout:** 7 days	
Period 1/2	Part A: 11 days, inpatient stay ☑Y ☐ N Part B: 14 days, inpatient stay ☑Y ☐ N	
Sequence	**A**	**B**
	SB-659746 s.d. at a dose of 5 mg on Day 1 (Part A) Ketoconazole 200 mg once daily for 13 days + SB-659746 s.d. 10 mg on Day 4 (Part B)	Ketoconazole 200 mg once daily for 13 days + SB-659746 s.d. 5 mg on Day 4 (Part A) Placebo once daily for 13 days + SB-659746 s.d. 10 mg on Day 4 (Part B)

Treatments: Briefly, in Part A, all eligible subjects took part in the two sessions of this single sequenced study. They were dosed with the regimens A followed by B.
A: SB-659746 s.d. at a dose of 5 mg on Day 1
B: Ketoconazole 200 mg once daily for 13 days + SB-659746 s.d. 5 mg on Day 4
None of these subjects participated in Part B of the study.

In Part B, subjects were allocated to one of the two treatment sequences CD or DC.
C: Ketoconazole 200 mg once daily for 13 days + SB-659746 s.d. 10 mg on Day 4
D: Placebo once daily for 13 days + SB-659746 s.d. 10 mg on Day 4

Diet: under fed conditions.

- **Sampling Times**

Part A: Day 1 or Day 4: prior to administration with SB-659746 (approx. –30 minutes) and at 1, 2, 3, 4, 5, 6, 8, 10, 14, 24, 32, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours post dose.

Part B: Day 4: prior to administration with SB-659746 (approx. –30 minutes) and at 1, 2, 3, 4, 5, 6, 8, 10, 14, 24, 32, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours post dose.

Analytical Method

Analyte	SB-659746
Method	LC-MS/MS with (b) (4)
Matrix	Plasma
LLOQ – ULOQ (ng/mL)	0.5 - 250
Precision (%)	4.5 – 16.9
Accuracy (%)	0.4 – 3.2

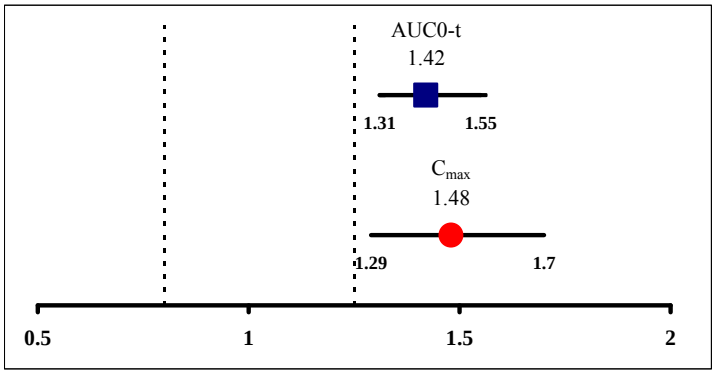
Statistical Method: ANOVA on log transformed parameters fitting for sequence, period, and treatment. LS mean and 90% CI for the difference were constructed.

Study Population :

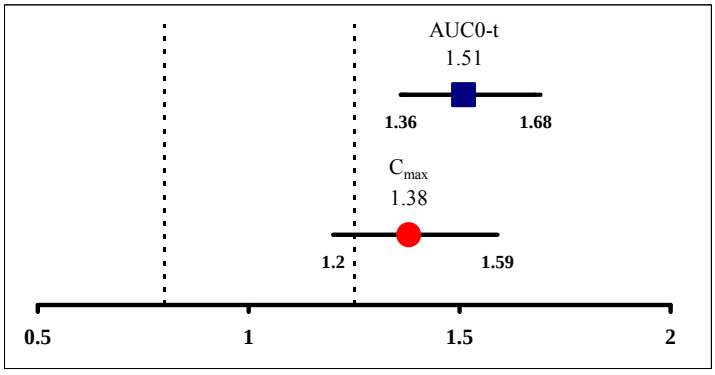
Randomized/Completed/ Discontinued Due to AE	15/15 (Part A) 22/21/1 (Part B)
Age [Mean (range)]	49 (23-60) (Part A) 49 (32-60) (Part B)
Male/Female	8/7 (Part A) 11/11 (Part B)
Race (Caucasian/Black/Asian/Hispanic)	Caucasian

Results

Part A: 5 mg Vilazodone



Part B: 10 mg Vilazodone



Safety

- Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA
- More AEs were reported in combination group compared to study drug alone.
- The severity of AEs was increased in combination group.

Conclusion

The dose should be adjusted from 40 mg to 20 mg when vilazodone is co-administered with strong CYP 3A4 inhibitor (e.g. ketoconazole).

==========

DDI with Pantoprazole (Proton Pump Inhibitor)

Report # *PGX-08-P1-03*	**Study Period:** *October 14, 2008 – October 31, 2008*	**EDR Link: NA**
Title	***Open Label, 2-Period Crossover, Multiple-Dose Study to Determine the Effect of Gastric pH on the Relative Bioavailability of the Vilazodone Oral Tablet Formulation in Healthy Volunteers***	

Study Design and Treatment:

Treatment	**Period 1**		**Period 2**	
	Inpatient	**Outpatient**	**Inpatient**	**Follow-up**
	Day 1, Day 2	**Day 3 – Day 7**	**Day 8- Day 10**	**Day 11 - Day 15**
Vilazodone (40 mg, SD)	**Day 1**		**Day 9**	
Pantoprazole (40 mg, QD)		**Day 3 - Day 7**	**Day 8, Day 9**	

Diet: Vilazodone was taken under fed conditions.

Rationale: Vilazodone solubility is greatest at acidic pH (pKa1 = 7.1). Individuals with higher than normal gastric pH because of medications (eg, antacid drugs, histamine H2-receptor antagonists, proton pump inhibitors) or because of certain disease states (eg, atrophic gastritis, pernicious anemia, chronic Helicobacter pylori infection), or after surgery (vagotomy, gastrectomy) may have slowed or decreased absorption of vilazodone because of solubility limitations encountered at the higher pH.

Choose from the list in each box by double clicking on the box
Multiple-Dose Non-Randomized Open-Label Cross-Over Single-Center 2-Period Healthy Vonuteers

Screening: 28 days	**Washout:** 7 days
Period 1/2	Period 1: 2 days, inpatient stay ☑Y ☐ N 5 days, inpatient stay ☐Y ☑ N Period 2: 3 days, inpatient stay ☑Y ☐ N

- **Sampling Times**

5 minutes pre-1st dose, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, and 144 hours post-1st dose, then 5 minutes pre-2nd dose, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, and 144 hours post-2nd dose and early termination if appropriate. (6 mL for each time point)

Analytical Method

Analyte	Vilazodone HCl
Method	LC/MS/MS with (b) (4)
Matrix	Plasma
LLOQ – ULOQ (ng/mL)	0.8119 – 270.6
Precision (%)	8.5 – 11.8
Accuracy (%)	-6 - -0.9

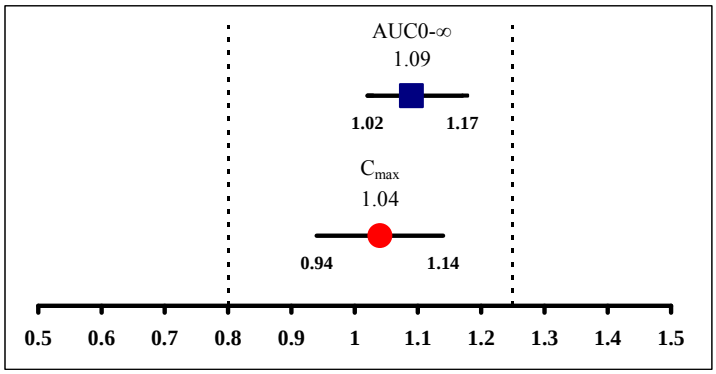
Statistical Method: ANOVA on log transformed parameters (AUC0-t, AUCinf, and Cmax) with treatment received (vilazodone alone and combination) as a fixed effect. LS mean and 90% CI for the difference were constructed.

Study Population :

Randomized/Completed/ Discontinued Due to AE	37/23/14
Age [Mean (range)]	36.1 (20-69)* 38.1 (21-69)**
Male/Female	16/21* 14/9**
Race (Caucasian/Black/Asian/Hispanic)	Caucasian (36) /Black (1)* Caucasian (22)/ Black (1)**

* Safety population
** PK Evaluable Population

Results



Safety

- Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA
- More AEs were reported in combination group (59.5%) compared to study drug alone (41.7%).

Conclusion

The systemic exposure is similar between vilazodone alone and vilazodone combined with proton pump inhibitor (pantoprazole). No dose adjustment is need when vilazodone administered along with the proton pump inhibitor.

==========

DDI with Ethanol

Report # ***PGX-08-P1-04***	**Study Period:** ***October 20, 2008 –November 30, 2008***	**EDR Link: NA**
Title	***Open Label, 2-Period Cross-Over, Multiple-Dose Study to Determine the Effect of Ethanol on the Relative Bioavailability of the Vilazodone Oral***	

	Tablet Formulation in Healthy Volunteers

Study Design and Treatment:

The subjects were randomized to one of the two following treatment sequences:

- Vilazodone 40 mg (two 20 mg tablets) alone followind 10 days later by vilazodone 40 mg (two 20 mg tablets) and 1.0 oz (30 mL) ethanol
- Vilazodone 40 mg (two 30 mg tablets) and 1.0 oz (30 mL) ethanol followed 10 days later by vilazodone 40 mg (two 20 mg tablets) alone.

The hydroalcoholic solution was prepared by adding water to 30 mL (1 oz) of alcohol (95% ethanol and 5% water by volume) for a total volume of 240 mL. This amount of ethanol is approximately equivalent to the amount consumed with two shots of 80-proof liquor.

Diet: under fed conditions.

Rationale: Since vilazodone is only sparingly soluble in alcohol, alcohol may affect the dissolution of vilazodone.

Choose from the list in each box by double clicking on the box
Single-Dose Randomized Open-Label Cross-Over Single-Center 2-Period Healthy Vonuteers

Screening: 28 days	**Washout:** 10 days	
Period 1/2	Period 1: 4 days, inpatient stay ☑Y ☐ N Period 2: 4 days, inpatient stay ☑Y ☐ N	
Sequence	**A** Vilazodone 40 mg (two 20 mg tablets) alone	**B** Vilazodone 40 mg (two 20 mg tablets) and 1.0 oz (30 mL) of ethanol

- **Sampling Times**

5 minutes pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, and 144 hours post-dose and, if appropriate, at early termination. (6 mL blood sample for each time point).

Analytical Method

Analyte	Vilazodone HCl
Method	LC/MS/MS with (b) (4)
Matrix	Plasma
LLOQ – ULOQ (ng/mL)	0.8119 – 270.6
Precision (%)	5.9 – 8.1
Accuracy (%)	-1.6 – 3.1

Statistical Method: ANOVA on log transformed parameters (AUC0-t, AUCinf, and Cmax) with treatment received (vilazodone alone and combination) as a fixed effect. LS mean and 90% CI for the difference were constructed.

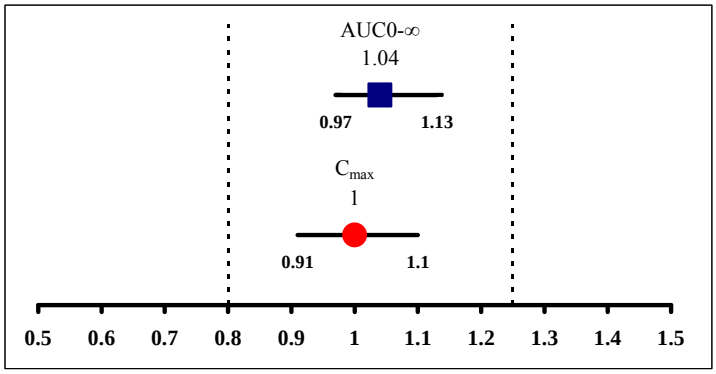
Study Population :

Randomized/Completed/ Discontinued Due to AE	37/26/10
Age [Mean (range)]	39.5 (22-56)*

	40.3 (22-56)**
Male/Female	27/10* 21/5**
Race (Caucasian/Black/Asian/Hispanic)	Caucasian (34) /Black (3)* Caucasian (24)/ Black (2)**

* Safety population
** PK Evaluable Population

Results



Safety

- Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA
- The incidences of AEs between two treatment groups were comparable (52% v.s., 56%).

Conclusion

The co-administration of 30 mL of ethanol had no significant effect on the absorption of vilazodone. No dose adjustment is need when vilazodone administered along with alcohol.

==========

DDI with Five CYP Enzyme Substrates (Cocktail Study)

Report # *GPP-007-CLN-CP1-2003-234 (GSK Study 005)*	**Study Period:** *February 21, 2002 –May 24, 2002*	**EDR Link: NA**
Title	***An open label, single sequence, two session study to investigate the pharmacokinetics of nifedipine and multiple cytochrome P450 substrates administered as single doses before and after dosing with oral SB-659746 (20 mg od) for up to 10 days in healthy volunteers***	

Study Design and Treatment:

Session	Study Days	Study Drug		Target Enzyme
1	Day 1	Nifedipine	20 mg	CYP3A4
	Day 3 (CYP substrate cocktail)	Debrisoquine Mephenytoin Flurbiprofen Caffeine	10 mg 100 mg 50 mg 100 mg	CYP2D6 CYP2C19 CYP2C9 CYP1A2
2	Days 1-7	SB-659746	20 mg	
	Day 8	SB-659746 Nifedipine	20 mg + 20 mg	CYP3A4
	Day 9	SB-659746	20 mg	
	Day 10 (CYP substrate cocktail)	SB-659746 Debrisoquine Mephenytoin Flurbiprofen Caffeine	20 mg + 10 mg 100 mg 50 mg 100 mg	CYP2D6 CYP2C19 CYP2C9 CYP1A2

Diet: under fed conditions.

Primary PK Endpoints:

CYP Enzyme	Substrate	Parameter of CYP Enzyme Activity	Sample Collection
CYP3A4	Nifedipine	Nifedipine (plasma)	$AUC_{(0-\infty)}$
CYP1A2	Caffeine	Paraxanthine and caffeine (plasma)	8 h plasma ratio
CYP2D6	Debrisoquine	4-Hydroxydebrisoquine / debrisoquine	0-8 h urine ratio
CYP2C9	Flurbiprofen	4'-Hydroxyflurbiprofen / Flurbiprofen (plasma)	0-8 h urine $AUC_{(0-8)}$
CYP2C19	Mephenytoin	4'-Hydroxymephenytoin recovery	0-8 h urine

h = hours

Secondary PK Endpoints:

CYP Enzyme	Substrate	Parameter of CYP Enzyme Activity	Sample Collection
CYP3A4	Nifedipine	Nifedipine (plasma)	C_{max}, t_{max}, $t_{1/2}$
CYP2C9	Flurbiprofen	Flurbiprofen (plasma)	$AUC_{(0-\infty)}$ [1]
CYP2C19	Mephenytoin	S- / R-mephenytoin	Urine ratio, 8 h
	SB-659746	Pre-dose plasma SB-659746 concentrations	Days 6, 7, 8 and 9

Rationale: In vitro data showed that vilazodone might inhibit a number of CYP enzymes:

CYP Enzyme	Substrate	IC50 (μM)
CYP2C8	Paclitaxel	1.8
CYP2D6	5-Bufuralol	2.7
CYP2C9	Tolbutamide	8.1
CYP2C19	S-mephenytoin	11
CYP1A2	Caffeine	21
CYP3A4	Nifedipine	20
	Lovastatin	22
	Testosterone	29
	Midazolam	40

Choose from the list in each box by double clicking on the box
Multiple-Dose Non-Randomized Open-Label Sequential Single-Center 2-Session Healthy Vonuteers

Screening: 28 days	**Washout:** ≥ 3 days
Session 1/2	Session 1: 3 days, inpatient stay ☑Y ☐ N Session 2: 4 days, inpatient stay ☑Y ☐ N

- **Sampling Times**

Plasma Sample:
Nifedipine: Day 1 and Day 8: pre-dose, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 18, and 24 hours post-dosing (2.6 mL blood sample for each time point).
Flurbiprofen: Day 3 and Day 10: pre-dose, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-dosing (~3.4 mL blood sample at each time point).
Caffeine and Paraxanthine: Day 3 and Day 10: pre-dose, and 8 hours post-dosing (~2.5 mL blood sample at each time point).
SB-659746: Days 6-10: pre-dose (4 mL blood sample at each time point).

Urine Sample:
Day 3 and Day 10: 0-24 hours

Analytical Method
Caffeine/Paraxanthine: Caffeine and paraxanthine plasma concentrations were determined by protein precipitation followed by HPLC with UV detection. The lower limit of quantification

(LLQ) for caffeine and paraxanthine was 100 ng/mL and 50 ng/mL, respectively.

Flurbiprofen plasma concentrations were determined using a method based on protein precipitation followed by HPLC with fluorescence detection. The LLQ for flurbiprofen was 100 ng/mL. Urine samples were assayed for 4'-hydroxyflurbiprofen using a method involving enzymatic deconjugation and (b) (4) followed by LC/MS/MS analysis employing positive-ion electrospray ionization. The LLQ for 4'-hydroxyflurbiprofen was 1 µg/mL.

Urine samples were assayed for 4'-hydroxymephenytoin using a method involving enzymatic deconjugation and (b) (4) followed by LC/MS/MS analysis employing positive-ion electrospray ionization. The LLQ for 4'-hydroxymephenytoin was 1 µg/mL. The S/R-ratio of mephenytoin was determined using a method involving (b) (4) followed by gas chromatography with NP detection from a 0.5-mL aliquot of human urine.

Plasma samples were assayed for nifedipine using a method based on protein precipitation followed by LC/MS/MS analysis employing negative-ion electrospray ionization. The LLQ for nifedipine was 0.5 ng/mL.

Statistical Method: Following log-transformation, primary pharmacokinetic endpoints were analyzed by analysis of variance (ANOVA) appropriate to the study design, fitting terms for subject and session. Point estimates and corresponding 90% confidence intervals (CI) were computed for the difference, Session 2 – Session 1, using the residual error from the ANOVA (MSE).

Study Population: All subjects were CYP2D6 EM.

Randomized/Completed/ Discontinued Due to AE	45/44/1
Age [Mean (range)]	38 (20-59)
Male/Female	22/23
Race (Caucasian/Black/Asian/Hispanic)	Caucasian

Results

1. Effect of SB-659746 on CYP3A4 Activity (Plasma PK of Nifedipine):

Nifedipine Pharmacokinetics (Mean (SD))

Parameter [Units]	Endpoint	Nifedipine Alone (Session 1)	Nifedipine + SB-659746 (Session 2)
$AUC_{(0-\infty)}$ [ng·h/mL]	Primary	338 (111)	339 (126)
C_{max} [ng/mL]	Secondary	91.7 (51.9)	104 (52)
t_{max} [1] [hours]	Secondary	1.50 (0.25-4.00)	1.50 (0.25-4.00)
$t_{1/2}$ [hours]	--	4.80 (1.14)	4.88 (1.30)

2. Effect of SB-659746 on CYP1A2 Activity (Paraxanthine/Caffeine Plasma Ratio at 8 Hours Post-Dose):

Mean (SD) of Paraxanthine/Caffeine Plasma Ratios

Caffeine Alone (Session 1)	Caffeine + SB-659746 (Session 2)
1.08 (0.48)	0.98 (0.38)

3. Effect of SB-659746 on CYP2D6 Activity (Total Urinary 0-8 Hours Recovery Ratio of 4'-Hydroxydebrisoquine / Debrisoquine):

Mean Ratio of Urinary Recovery of 4'-Hydroxydebrisoquine / Debrisoquine (Mean (SD))

Debrisoquine Alone (Session 1)	Debrisoquine + SB-659746 (Session 2)
1.99 (1.30)	2.18 (1.64)

4. Effect of SB-659746 on CYP2C9 Activity (CLform Values for 4'-Hydroxyflurbiprofen and Flurbiprofen Plasma AUC(0-Infinity))

Flurbiprofen Phamacokinetics (Mean (SD))

Parameter [Units]	Endpoint	Flurbiprofen Alone (Session 1)	Flurbiprofen + SB-659746 (Session 2)
CL_{form} of 4-hydroxy-flurbiprofen [L/h]	Primary	0.300 (0.185)	0.289 (0.153)
$AUC_{(0-\infty)}$ [µg·h/mL]	Secondary	34.8 (10.0)	35.0 (9.7)

Point Estimate and 90% Confidence Interval for CYP2C9 Activity (Urine 0-8 Hours Recovery 4'-Hydroxyflurbiprofen / AUC(0-8) of Flurbiprofen) (L/h)

Comparison	Point Estimate	90% CI
Session 2: Session 1	1.00	(0.79, 1.25)

5. Effect of SB-659746 on CYP2C19 Activity (4'-Hydroxymephenytoin Recovery and S/R Mephenytoin Ratio in 0-8 Hours Urine)

Mephenytoin Phamacokinetics (Mean (SD))

Parameter [units]	Endpoint	Mephenytoin Alone (Session 1)	Mephenytoin + SB-659746 (Session 2)
4'-hydroxymephenytoin urinary recovery [µmol/mmol creatinine]	Primary	27.6 (11.5)	30.3 (12.3)
S/R-mephenytoin ratio	Secondary	0.173 (0.230)	0.150 (0.211)

Point Estimate and 90% Confidence Interval for CYP2C19 Activity (4'-Hydroxymephenytoin Urinary Recovery) (µmol/mmol Creatinine)

Comparison	Point Estimate	90% CI
Session 2: Session 1	1.11	(0.97, 1.28)

Safety

- Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA
- The incidences of AEs between two treatment groups were comparable (52% v.s., 56%).

Conclusion

- Steady-state dosing of SB-659746 at 20 mg QD had no effect on the in-vivo activities of CYP1A2, 2D6, 2C9, and 3A4. Following repeat administration of SB-659746 (20

mg od) there was a small increase (on average 11%; 90%-CI: 0.97 to 1.28) in 4'-hydroxymephenytoin urinary recovery, indicating a slight increase in the rate or extent of metabolism of S-mephenytoin to 4-hydroxymepheytoin via CYP2C19. This was supported by a slight decrease of approximately 13% in S/R-mephenytoin ratio after co-administration of SB-659746.
Comments
Based on the in vitro study, inhibition effect of vilazodone on CYP2C8 was more potent than on the other CYP enzymes (IC50=1.8 uM). However, the sponsor did not conduct an in vivo DDI study to evaluate the vilazodone inhibition effect on 2C8.

==========

ANALYTICAL SECTION

During the development of vilazodone, there were three assays used for determination of vilazodone concentrations in human biomatrix samples:

- HPLC assay with fluorescence detection (developed by Merck, as part of the original development program).
- LC-MS/MS assay (developed by GSK in support of their development program).
- LC-MS/MS assay (developed for PGxHealth by (b) (4)

HPLC with Fluorescence Detection Methodology

Analytical Lab	Sample Matrix	Analyte	Assay Range (ng/mL)	LLOQ (ng/mL)	Accuracy (%)	Precision (%)
Merk	Plasma	Vilazodone	0.4-100	0.4	Intrabatch ≤ 15% at 0.4 ng/mL; ≤ 6% at higher concentrations Interbatch 3% at 0.4 ng/mL; 3% at higher concentrations	Intrabatch ≤ 15% at 0.4 ng/mL; ≤ 7.5% at higher concentrations Interbatch 12.2% at 0.4 ng/mL; ≤ 3.7% at higher concentrations
	Urine	Vilazodone	10-1000	10	-5.3% - -18%	1.5% - 12.2%
		M10	10-1000	10	-4.9% - 14%	0.5% - 5%
		M17	10-1000	10	-12.3% - 13%	0.6% - 12.5%
(b) (4)	Plasma	Vilazodone	0.4-200	0.4	9.4% at 0.4 ng/mL	14.2% at 0.4 ng/mL

LC-MS/MS Methodology (Measuring Vilazodone in Plasma)

Analytical Lab	Sample Matrix	Assay Range (ng/mL)	LLOQ (ng/mL)	Accuracy (%)	Precision (%)
GSK	Plasma	0.5-250	0.5	3.32% to 6.68% at 0.5 – 250 ng/mL	Within run 5.37% to 1.88%; Between run 5.41% to 1.96% at 0.5 to 250 ng/mL
(b) (4)	Plasma	0.5 - 250	0.5	Average bias ranged from 3.0% at 0.5 ng/mL to 8.2% at 250 ng/mL	Intrabatch 11.4% at 0.5 ng/mL; 5.9% at 250 ng/mL Interbatch 6.8% at 0.5 ng/mL; 3.7% at 250 ng/mL

LC-MS/MS Methodology (developed by (b) (4) for PGxHealth)

Validation Parameter		Result
LLOQ (0.75 ng/mL)		11.59% CV 102.1% Accuracy
Between-Run Statistics		4.582 – 11.59% CV 100.1 – 102.9% Accuracy
Carryover		LLOQ % Accuracy = 116.9%
Autosampler Stability		72 hours at Room Temperature
Within-Run Statistics	LLOQ Low QC Mid QC High QC	4/4 runs acceptable 4/4 runs acceptable 4/4 runs acceptable 4/4 runs acceptable
Analyte/IS Selectivity	Analyte IS	19.35% Interference 0.2149% Interference
Selectivity at LLOQ	K_2EDTA	6/6 lots acceptable
Cross-Analyte Interference	Analyte IS	14.31% 0.3115%
Dilution Effect	(20–fold)	3.832% CV 104.0% Accuracy
Recovery	Analyte IS	75.14 Average % Recovery 6.646% CV 36.52 Average % Recovery 4.566% CV
Matrix Effect	Analyte IS	-49.40% consistent across range -39.41% consistent across range
Standard Curve Regression Parameters	Quantification Range Correlation Coefficient	0.75 – 250 ng/mL 0.9998
Stock Solution Stability (6 hr at room temperature)	10 ng/mL Vilazodone 10 ng/mL d4-Vilazodone	-1.036 % Difference -0.04184 % Difference
Freeze-Thaw Stability	4 cycles	0.8344 – 3.446% CV 93.13 – 95.57% Accuracy
Short-Term Stability in Matrix	5 hours	0.5590 – 2.828% CV 98.40 – 100.0% Accuracy
Whole Blood Stability	1.5 ng/mL 1 hr RT 200 ng/mL 1 hr RT	-7.284% Difference -0.2633% Difference
Stock Solution Stability 120 days at -70 ± 10 °C	1 mg/mL Vilazodone	-5.389% Difference
Long-Term Stability in Human Plasma 43 Days at -20 ± 10 °C 98 Days at -70 ± 10 °C	Vilazodone	1.530-6.050% CV 109.1-113.5% Accuracy 3.134 – 6.008% CV 103.6-103.8% Accuracy

Validation Parameter	Requirement	Result	Pass/Fail
Analyte/IS Selectivity			
Vilazodone	≤ 20%	5.6%	Pass
D_4-Vilazodone (IS)	≤ 5%	1.6%	Pass
Cross-Analyte Interference			
Vilazodone	≤ 20%	5.3%	Pass
IS	≤ 5%	0.65%	Pass
Selectivity at LLOQ	≤ 20% CV	0.3860 – 9.910% CV	Pass
	80 – 120% Accuracy for 5/6 lots	89.22 – 108.6% Accuracy in 5/6 lots of human urine	Pass
	80 – 120% Accuracy for mean of 6 lots	95.23% Accuracy	Pass
	≤ 20% CV	20.04% CV	Pass [1]
[1] Overall % CV considered acceptable as major contribution to variability exhibited by one lot of urine.			

(continued)

Within-Run Statistics for Low, Mid, and High QCs	≤ 15% CV 85 – 115% Accuracy	1.172 – 33.80% CV 90.06 – 110.2% Accuracy	Pass [2] Pass
Within-Run Statistics for LLOQ QC	≤ 20% CV 80 – 120% Accuracy	5.764 – 11.80% CV 89.95 –114.4% Accuracy	Pass Pass
Between-Run Statistics	≤ 15% (≤ 20% at LLOQ) CV	3.240 – 13.01% CV	Pass
	85 – 115% (80 – 120% at LLOQ) Accuracy	100.0-106.0% Accuracy	Pass
Carryover Vilazodone	80-120% Accuracy	111.4% Accuracy	Pass [3]
Dilution Effect (10-fold dilution)	≤ 15% CV 85 – 115% Accuracy	4.463% CV 105.5% Accuracy	Pass Pass
Calibration Curve Regression Parameters Quantification Range Correlation Coefficient	 1.0 – 200 ng/mL R ≥ 0.990	 1.0 – 200 ng/mL R = 0.9996	 Pass Pass
Freeze-Thaw Stability (3 cycles)	≤ 15% CV 85 – 115% Accuracy	2.366 – 7.191% CV 96.58 – 102.7% Accuracy	Pass Pass
4-Hour 10-Minute Room Temperature Stability in Human Urine	≤ 15% CV 85 – 115% Accuracy	2.367 – 6.405% CV 101.0 – 102.9% Accuracy	Pass Pass
68-Hour Autosampler Stability	≤ 15% CV 85 – 115% Accuracy	3.414 – 5.019% CV 102.8 – 110.6% Accuracy	Pass Pass

[2] The % CV does not pass for one QC level in only one analytical run; within-run statistics pass.
[3] The API 3000™ instrument will be used routinely for sample analysis. The API 4000™ instrument should only be used if the API 3000™ instrument is not available, and in that case, the study samples will be injected in order of increasing concentration and the results will be examined to determine if any samples require reanalysis due to potential carryover interference.

Validation Parameter	Requirement	Result	Pass/Fail
Analyte/IS Matrix Interference Vilazodone D_4-Vilazodone (IS)	% Interference: ≤ 20% of LLOQ ≤ 10% of IS in blanks	 10.95% 5 of 6 sample values 5.390% 6 of 6 sample values	 Pass Pass
Cross-Analyte Interference Vilazodone IS	% Interference: ≤ 20% ≤ 5%	 18.87% 1.383%	 Pass Pass
			(continued)
Selectivity at LLOQ (2500 pg Vilazodone/g)	75 – 125% Accuracy for 5/6 lots of fecal homogenate 75 – 125% Accuracy for mean of 5/6 lots of fecal homogenate ≤ 25% CV	94.89 – 117.1% Accuracy in 6/6 lots 105.4% for 6/6 lots 8.847%	Pass Pass Pass
Within-Run Statistics for Low, Mid, and High QCs	≤ 20% CV 80 – 120% Accuracy	0.9504 – 12.97% CV 89.94 – 110.3% Accuracy	Pass Pass
Within-Run Statistics for LLOQ QC (2500 pg/g)	≤ 25% CV 75 – 125% Accuracy	4.519 – 9.247% CV 89.59 – 122.6% Accuracy	Pass Pass
Between-Run Statistics	≤ 20% (≤ 25% at LLOQ) CV 80 – 120% (75-125% at LLOQ) Accuracy	2.187 – 14.83% CV 98.09 – 102.4% Accuracy	Pass Pass
Carryover Vilazodone IS	≤ 30% of LLOQ ≤ 5% of IS in blanks	9.665% 1.369%	Pass Pass
Dilution Effect (5-Fold Dilution)	80 – 120% Accuracy for 4/6 replicates 80 – 120% Accuracy Overall ≤ 20% CV Overall	Acceptable % Accuracy for 5/6 replicates 111.3% Accuracy 7.251% CV	Pass Pass Pass
Extraction Recovery Vilazodone IS	≤ 20% CV	72.96% with 1.893% CV 70.94% with 5.750% CV	Pass Pass
Matrix Effect Vilazodone IS	Consistent across concentrations	-79.44% with -4.780% CV -78.98%	Acceptable
Calibration Curve Regression Parameters Quantification Range Correlation Coefficient	R ≥ 0.99	 2,500 – 80,000 pg/g (2.5 – 80 ng/g) R = 0.9996	 Pass Pass
5.42-Hour Room Temperature Stability in Human Fecal Homogenate	80 – 120% Accuracy for at least 2 of 3 replicates ≤ 20% CV 80 – 120% Accuracy for mean of at least 2 of 3 replicates	Acceptable % Accuracy 2.416 – 3.567% CV 98.34 – 99.22% Accuracy	Pass Pass Pass
30.5-Hour Autosampler Stability	80 – 120% Accuracy for 2/3 replicates 80 – 120% mean Accuracy ≤ 20% CV	Acceptable % Accuracy for 3/3 replicates for High QC and 6/6 replicates for Low QC 93.47 – 104.1% Accuracy 1.685 – 8.516% CV	Pass Pass
Run Length	None Assigned	96 Samples	Acceptable

OCP Reviewer's Comments: The analytical method developed for the analysis of vilazodone was adequately validated and acceptable.

==========

3.2 Pharmacometric Review

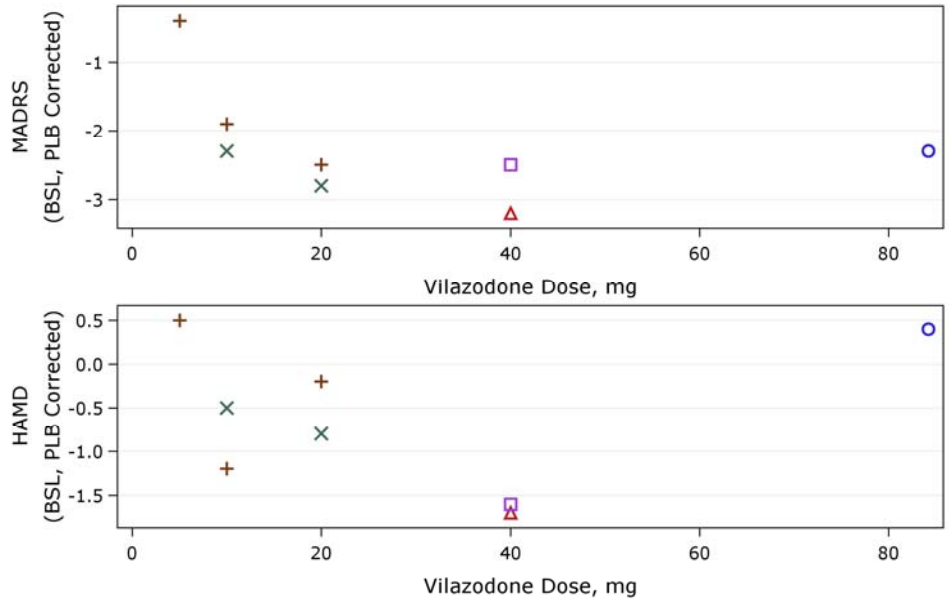
Summary of Findings

Key Review Questions
The purpose of this review is to address the following key questions.

What are the characteristics of the exposure-response relationships (dose-response, concentration-response) with regards to efficacy?

Error! Reference source not found. shows the relationship between dose and baseline, placebo subracted HAMD-total, MADRS score in early dose finding (Phase-II) and registration trials (Phase-III). From early dose finding studies, the sponsor identified that doses in the range of 20-40 mg would provide optimal benefit/risk ratio. The sponsor conducted 2 registration trials in which patients were administered 40 mg dose with food. Sponsor has shown benefit of the drug after 40 mg dose in registration trials.

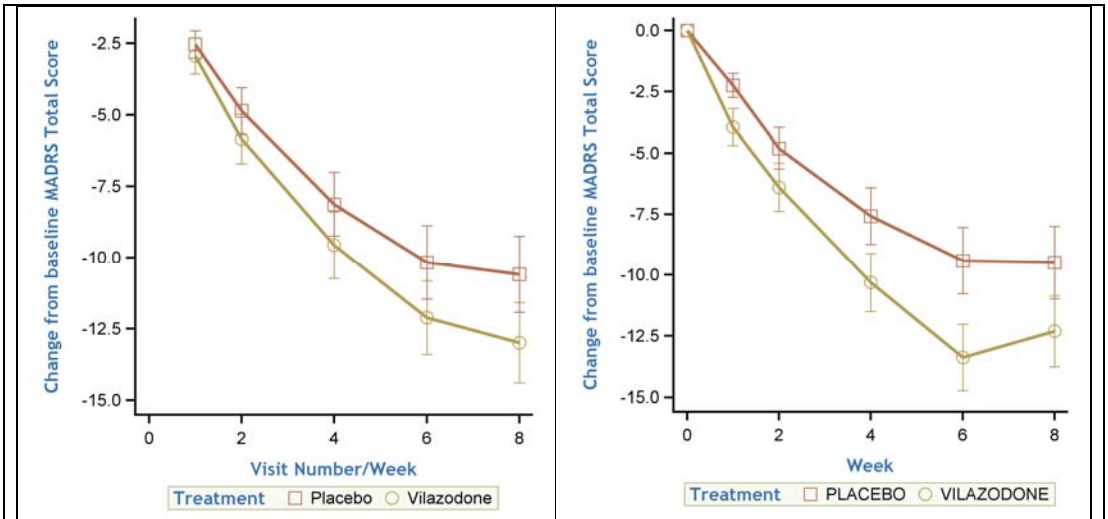
Figure 1. Relationship between Vilazodone dose, mg and baseline, placebo corrected MADRS, HAMD (Total) scores. Data from early dose finding studies (Phase-II) and registration trials (Phase-III) are shown.



BSL: Baseline; PLB: Placebo;
The change in MADRS score in control groups (Fluoxetine, Citalopram) in Phase-II studies were notsignificantly different from placebo. The data shown for 40 mg is from Phase-III trials.

Error! Reference source not found. shows the time course of change from baseline MADRS scores in placebo and Vilazodone 40 mg dose in registration trials.

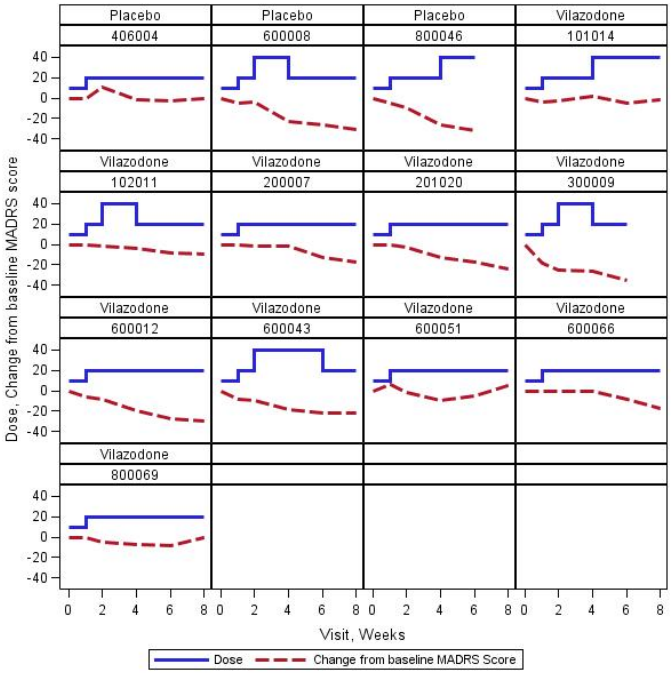
Figure 2. Time course of change from baseline MADRS scores (+/- SE) in placebo and Vilazodone 40 mg dose in registration trials.



CLDA-07-DO-02 GNSC-04-DP-02

Figure 3 shows the dose titration pattern, and time course of change from baseline MADRS score in 10 patients (Vilazodone group) who required 20 mg dose during the study (GNSC-04-DP-02).

Figure 3. Dose titration pattern, and time course of change from baseline MADRS score in patients who required 20 mg dose during the study (GNSC-04-DP-02).



Overall, based on the data from registration trials and early dose finding studies it appears that a lower dose of 20 mg will also have similar benefit as 40 mg.

What are the characteristics of the exposure-response relationships (dose-response, concentration-response) with regards to safety?

Error! Reference source not found. shows the relationship between Vilazodone dose and safety events such as nausea, dizziness, insomnia, abnormal/excessive dreams, somnolence.

Figure 4. Relationship between percentage of patients with adverse events and Vilazodone dose, mg.

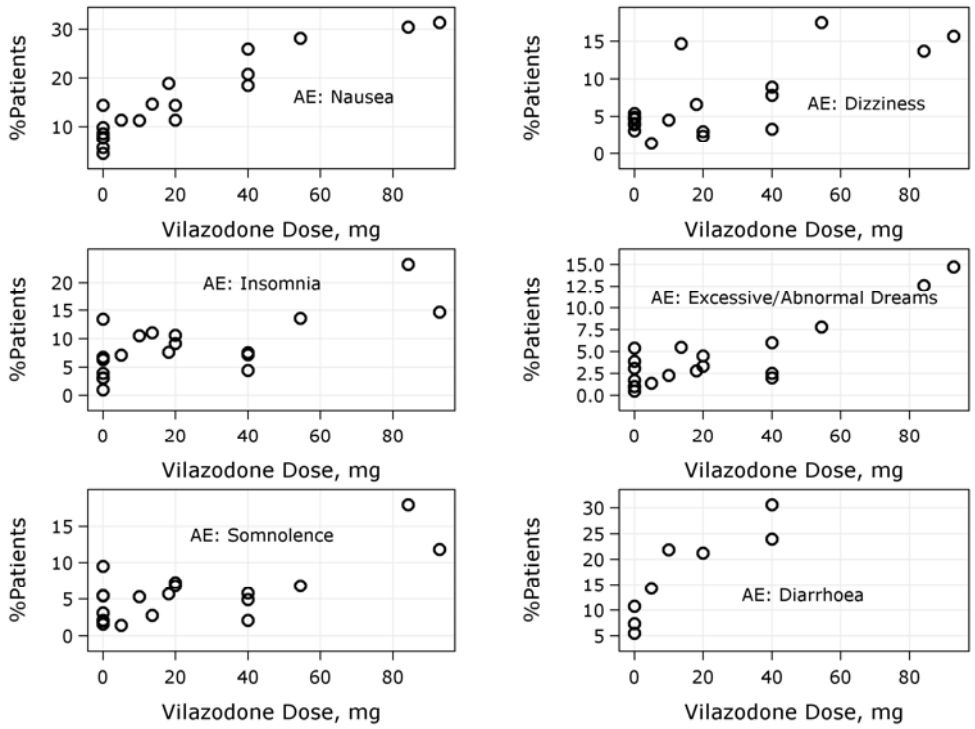
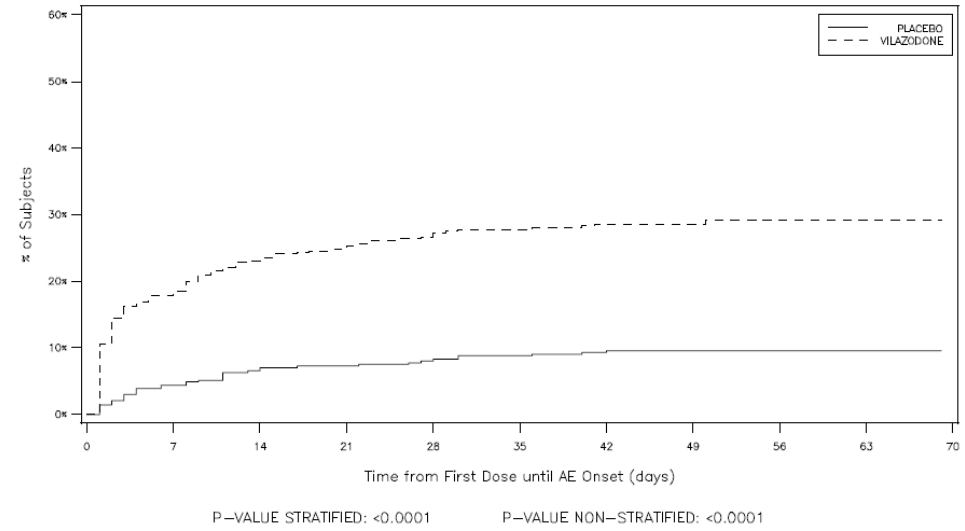


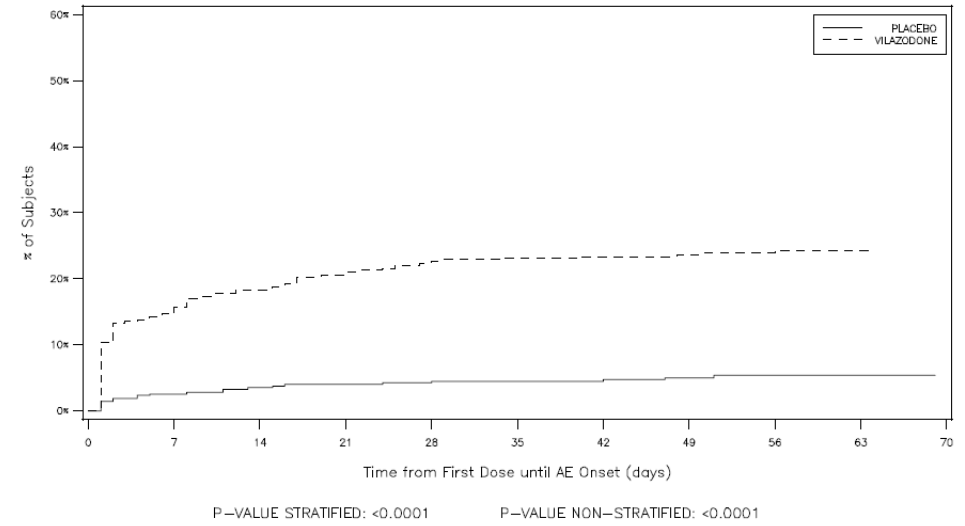
Figure 5 shows the time of onset of safety events such as Diarrhea, Nausea, Insomnia (Placebo-controlled Phase 3 Database Safety Population). Figure 5 indicates that the most of the adverse events occur during the initial titration phase (10 mg/day for 7 days (Days 1-7), 20 mg/day for 7 days (Days 8-14) in comparison to maintanence phase (40 mg/day for 42 days (Days 15-56)).

Figure 5. Time to Onset of Commonly Occurring Adverse Event: Diarrhea, Nausea, Insomnia (Placebo-controlled Phase 3 Database Safety Population).



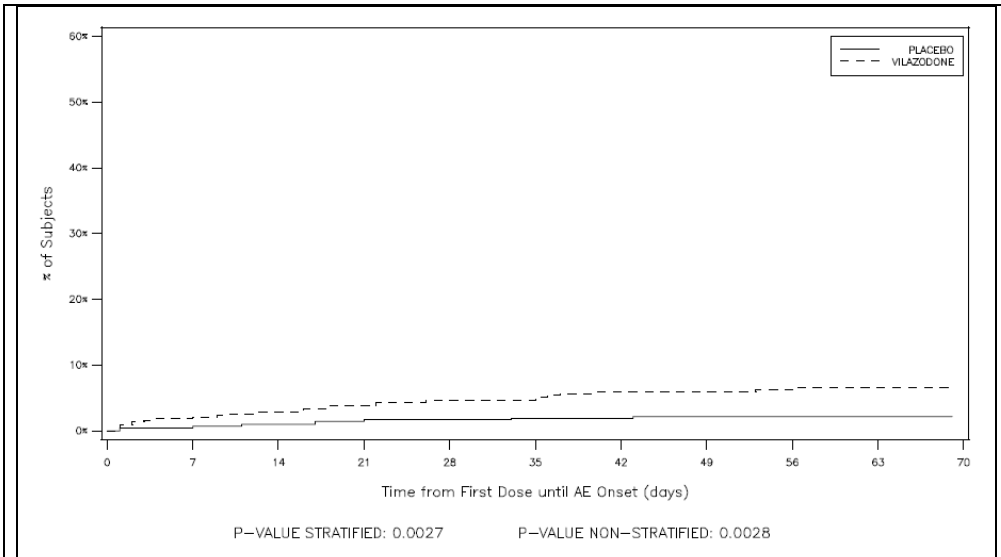
Note: Time to onset is calculated as (Onset Date) – (First Dose Date) + 1. Subjects with a missing or partial onset date are assigned 1 day as time to onset. Subjects who did not experience the event are censored with a time to onset calculated as (End of Study Date) – (First Dose Date) + 1.

Diarrhoea



Note: Time to onset is calculated as (Onset Date) – (First Dose Date) + 1. Subjects with a missing or partial onset date are assigned 1 day as time to onset. Subjects who did not experience the event are censored with a time to onset calculated as (End of Study Date) – (First Dose Date) + 1.

Nausea



Insomnia

Recommendations

NA

Label Statements

Labeling statements to be removed are shown in ~~red strikethrough font~~ and suggested labeling to be included is shown in underline blue font.

Pertinent regulatory background

Refer to review by Dr Bei Yu

Results of Sponsor's Analysis

NA

Reviewer's Analysis

NA

Introduction

NA

Objectives

Analysis objectives are:

1. Add objectives

Methods

Data Sets

Data sets used are summarized in Table 1.

Table 1. Analysis Data Sets

Study Number	Name	Link to EDR

Software

NA

Models

Results

Listing of Analyses Codes and Output Files

File Name	Description	Location in \\cdsnas\pharmacometrics\

==========

3.3 Pharmacogenomic Review

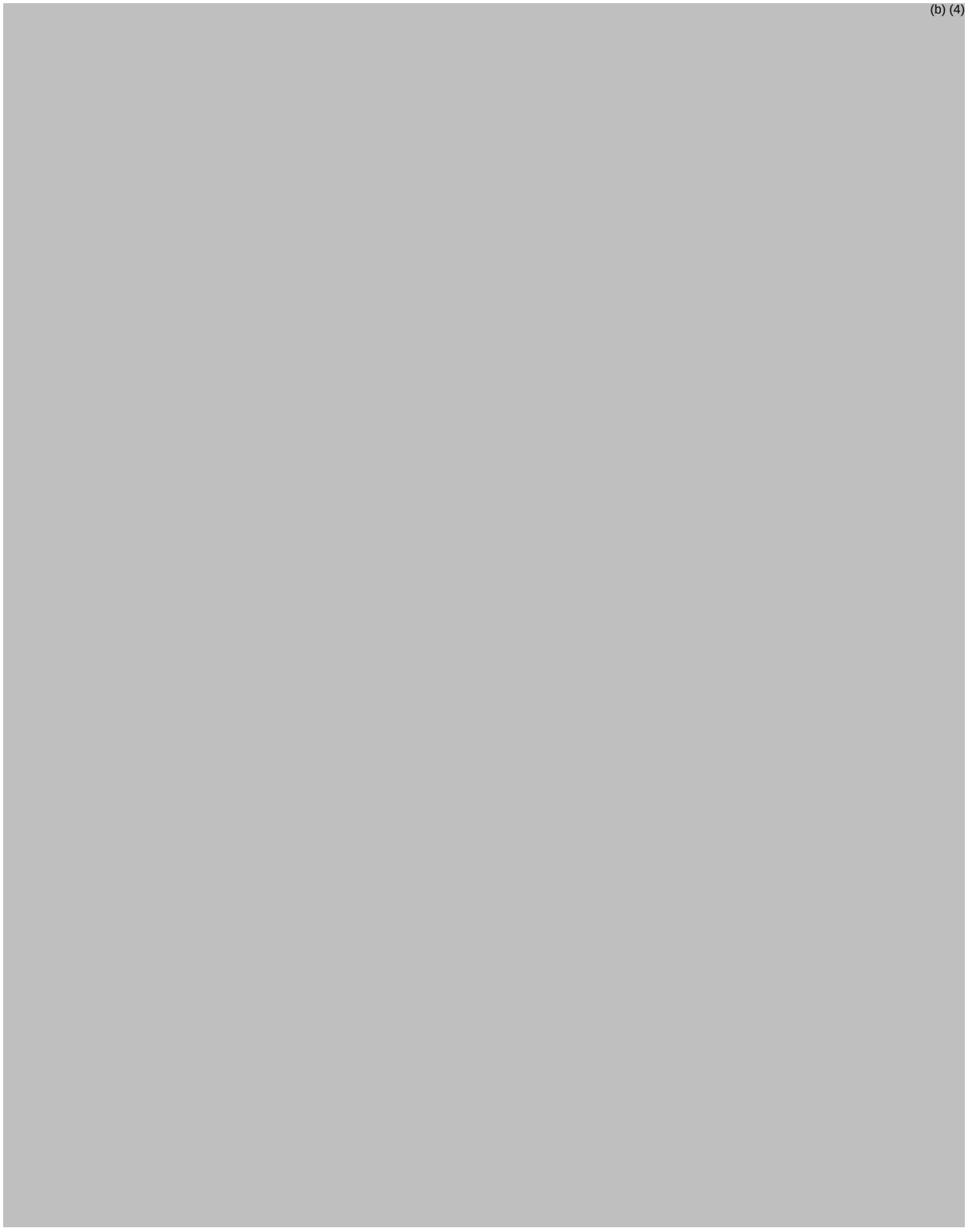
CLINICAL PHARMACOLOGY GENOMICS GROUP REVIEW

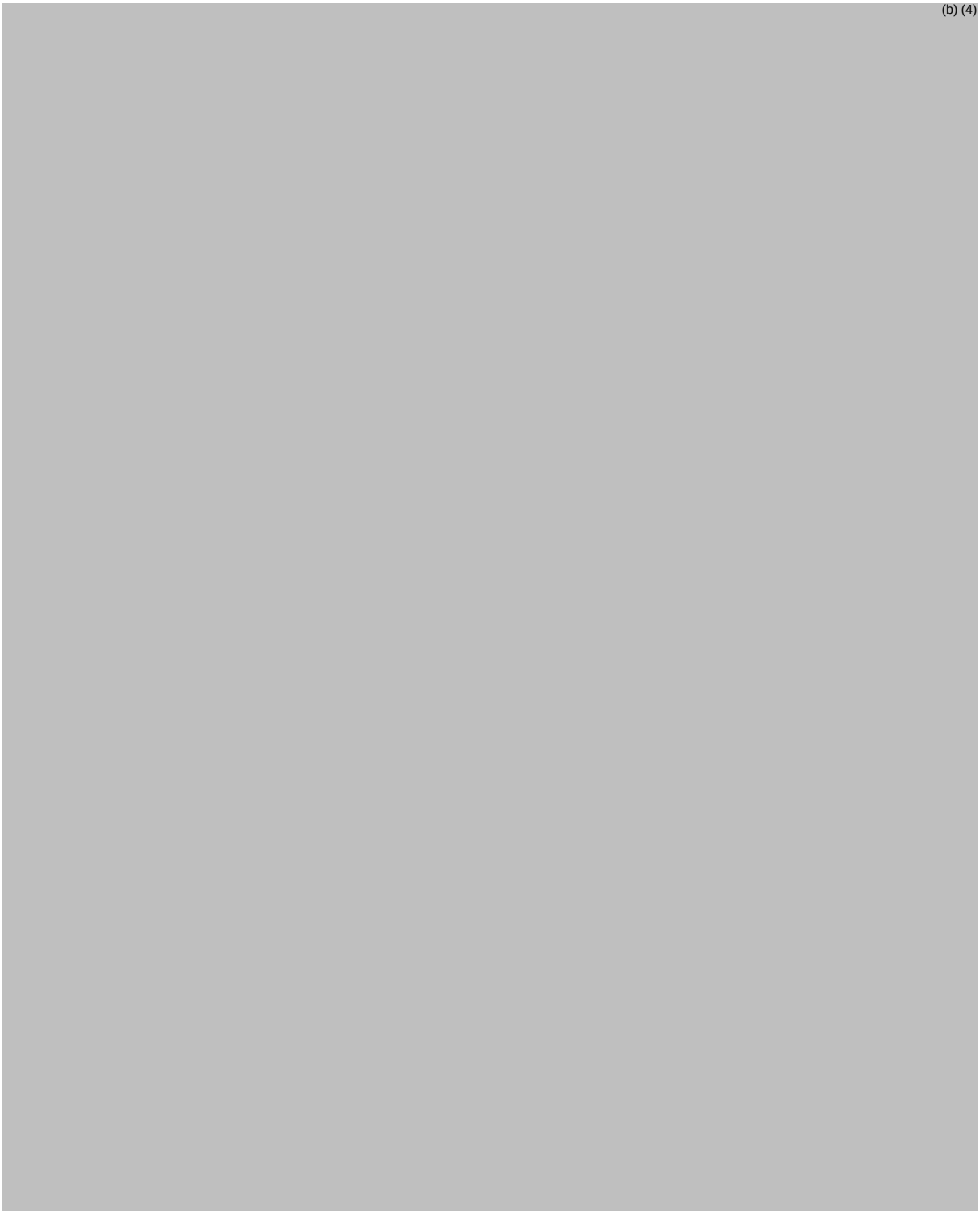
NDA Number	022567
Submission Date	22 March 2010
Applicant Name	PGxHealth, LLC
Generic Name	Vilazodone
Proposed Indication	Major depressive disorder (MDD)
Primary Reviewer	Issam Zineh, Pharm.D., M.P.H.
Secondary Reviewer	Li Zhang, Ph.D.

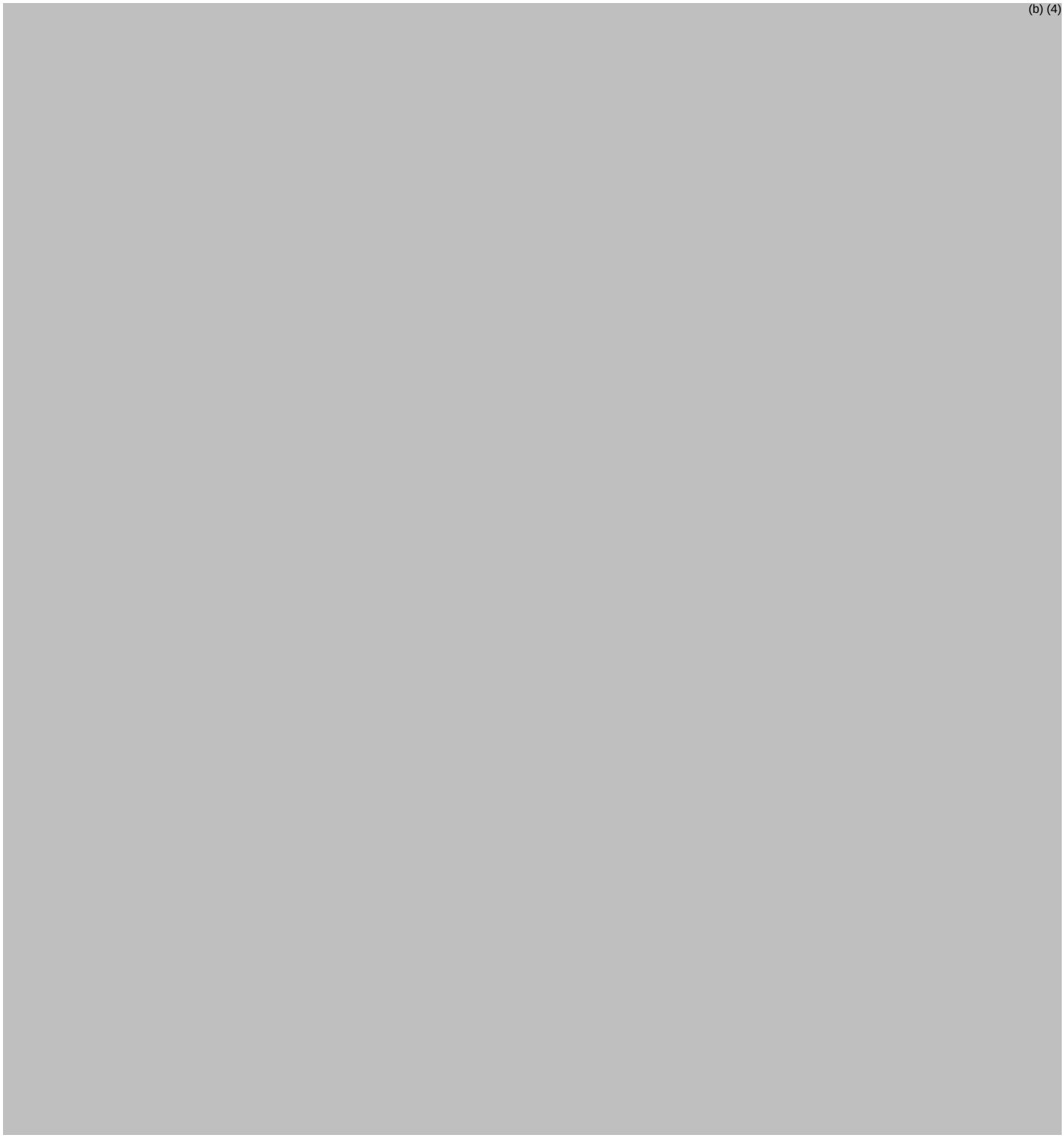
1 Background

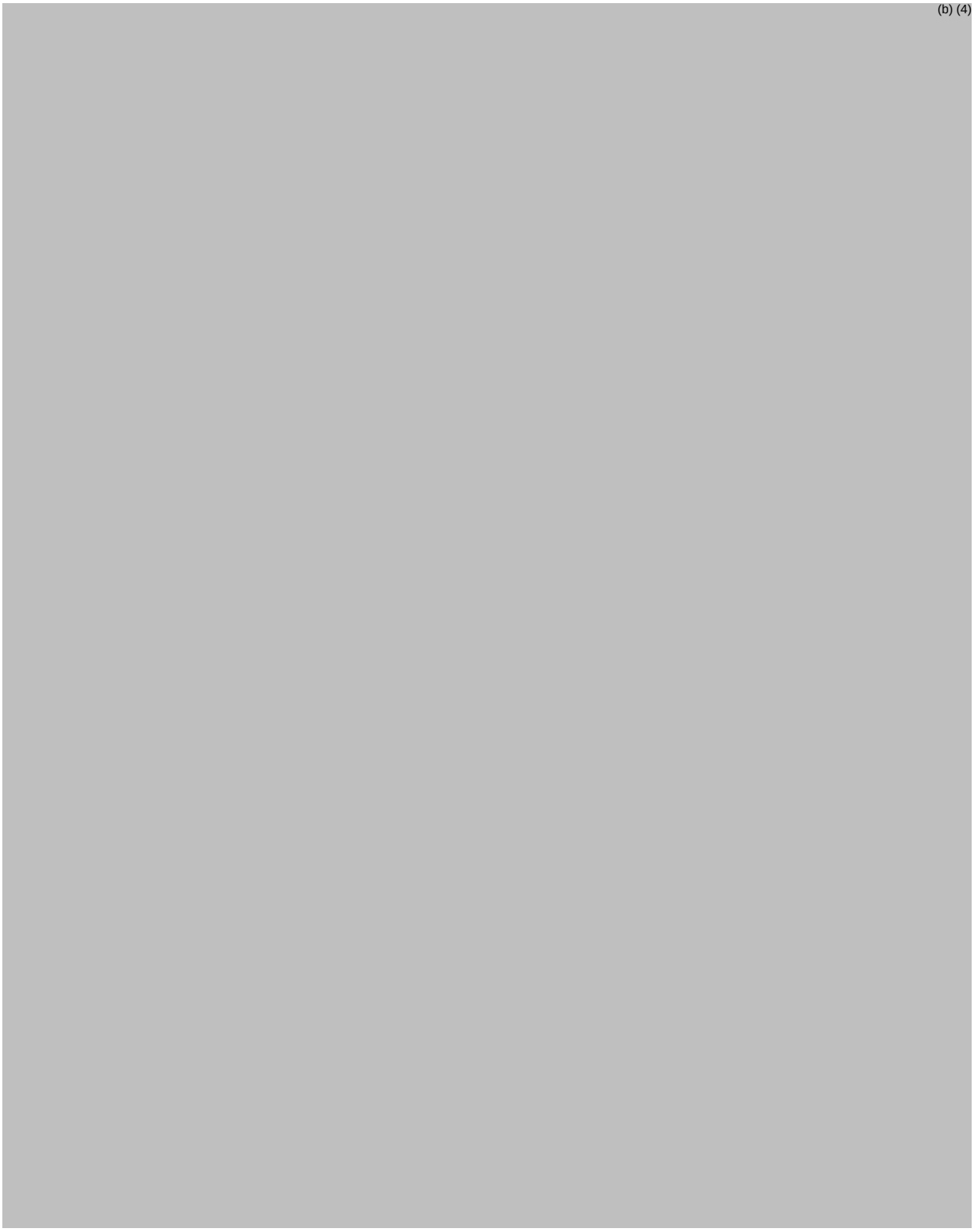
Vilazodone is a dual SSRI/partial 5-HT1A agonist under review for the treatment of MDD. There is an abundance of literature on the pharmacogenomics (Pgx) of anti-depressants [e.g., PMID 15111987, PMID 19558256]. DNA samples for candidate pharmacogenetic analyses were collected as part of the vilazodone clinical development program. In addition, the short-term effect of vilazodone on metabolic and stress hormones (e.g., growth hormone, prolactin, adrenocorticotropic hormone [ACTH], and cortisol) was evaluated in several early phase studies.

(b) (4)


(b) (4)


(b) (4)


(b) (4)


(b) (4)

4 Comments

Genotype data were available for assessment of the relationships between genetic variations in CYP2C19, CYP2D6, and ACE with vilazodone response. There were no meaningful associations with MADRS by responder analyses or change at 8 weeks. There were also no robust associations with discontinuation rates or failure to reach target dose that could explain the similar responses in CYP2C19 UMs and PMs. Genotype data were not available for all pivotal studies in which DNA was collected. Given the complicated vilazodone metabolic pathway, it is uncertain whether genetic variation on pharmacokinetic-related genes would likely to result in clinically meaningful differences in either response or adverse events.

The transient elevations in growth hormone and cortisol in early phase clinical studies did not seem to translate into excess incidence of treatment-emergent adverse events such as hypertension, dysglycemia/diabetes, and musculoskeletal pain in the short term pivotal studies. Of note, palpitations (but not hypokalemia) occurred at a significantly higher rate in vilazodone-treated patients.

5 Recommendations

The Office of Clinical Pharmacology Genomics Group has reviewed NDA 022567 for vilazodone in the treatment of MDD. The application is acceptable from our perspective.

5.1 Postmarketing commitments/requirements

The sponsor should include their safety pharmacogenetic assessment in a separate report.

5.2 Label recommendations

Should vilazodone be approved, the label should reflect the increased incidence of palpitations in vilazodone-treated patients.

_____

Li Zhang, PhD
Reviewer, Genomics Group, OCP

_____

Issam Zineh, PharmD, MPH
Associate Director, Genomics Group, OCP

1 Page of Draft Labeling has been Withheld in Full as b4 (CCI/TS) immediately following this page.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

BEI YU
12/08/2010

HUIXIA ZHANG
12/08/2010

JEE E LEE
12/08/2010

JOGARAO V GOBBURU
12/08/2010

VENKATESH A BHATTARAM
12/08/2010

YANING WANG
12/08/2010

ISSAM ZINEH
12/08/2010
Concur

MEHUL U MEHTA
12/08/2010