

Fig. 17

Absorption of Desvenlafaxine versus Dissolution of Desvenlafaxine Succinate Relationships for 200 mg DVS-233 Extended Release Tablets

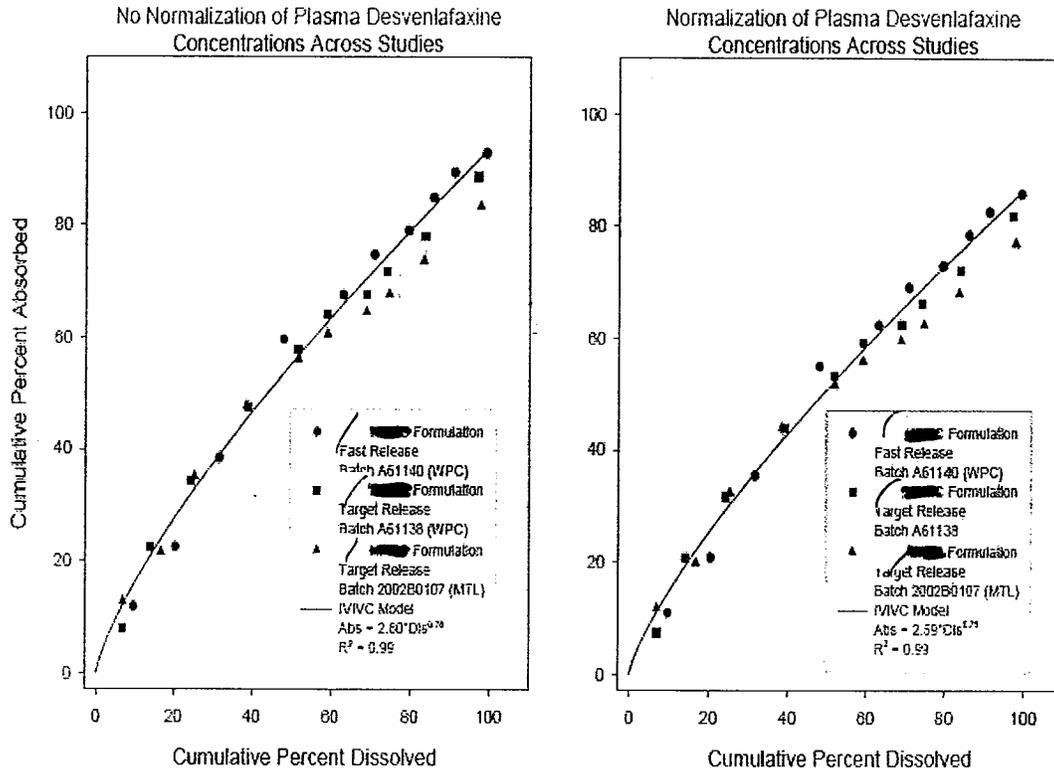


Table 8: Validation of DVS SR Tablet Level A IVIVC Model Developed and Validated Without Normalization of Plasma Desvenlafaxine Concentration-Time Data Across Studies

Internal Predictability						
Batch	Observed AUC	Predicted AUC	Absolute % PE	Observed C <sub>max</sub>	Predicted C <sub>max</sub>	Absolute % PE
1x200 mg A61140  Study 177	8870	9065	2.20	362	341	5.62
1x200 mg A61138  Study 177	8984	9178	2.16	314	307	2.01
	Average % PE		2.18	Average % PE		3.81
External Predictability						
Batch	Observed AUC	Predicted AUC	Absolute % PE	Observed C <sub>max</sub>	Predicted C <sub>max</sub>	Absolute % PE
1x200 mg 2002B0107  Study 177	8540	8722	2.13	308	309	0.36
2x50 mg 2002B0105  Study 186	4695	4843	3.16	169	155	8.00
1x100 mg 2002B0109  Study 186	4542	4677	2.96	173	157	9.53

Internal Validation Criteria: Average %PE < 10% and no individual %PE > 15%

External Validation Criteria: No individual %PE > 10%

AUC units: ng·hr/mL

C<sub>max</sub> units: ng/mL

Table 9: Validation of DVS SR Tablet Level A IVIVC Model Developed and Validated With Normalization of Plasma Desvenlafaxine Concentration-Time Data Across Studies

Internal Predictability						
Batch	Observed AUC	Predicted AUC	Absolute % PE	Observed C <sub>max</sub>	Predicted C <sub>max</sub>	Absolute % PE
1x200 mg A61140 Study 177	8870	9080	2.37	362	344	4.95
1x200 mg A61138 Study 177	8984	9192	2.32	314	307	2.07
	Average % PE		2.34	Average % PE		3.51
External Predictability						
Batch	Observed AUC	Predicted AUC	Absolute % PE	Observed C <sub>max</sub>	Predicted C <sub>max</sub>	Absolute % PE
1x200 mg 2002B0107 Study 177	8540	8772	2.72	308	309	0.36
2x50 mg 2002B0105 Study 186	4695	4888	4.10	169	170	0.59
1x100 mg 2002B0109 Study 186	4542	4723	3.98	173	171	1.50

Internal Validation Criteria: Average %PE < 10% and no individual %PE > 15%

External Validation Criteria: No individual %PE > 10%

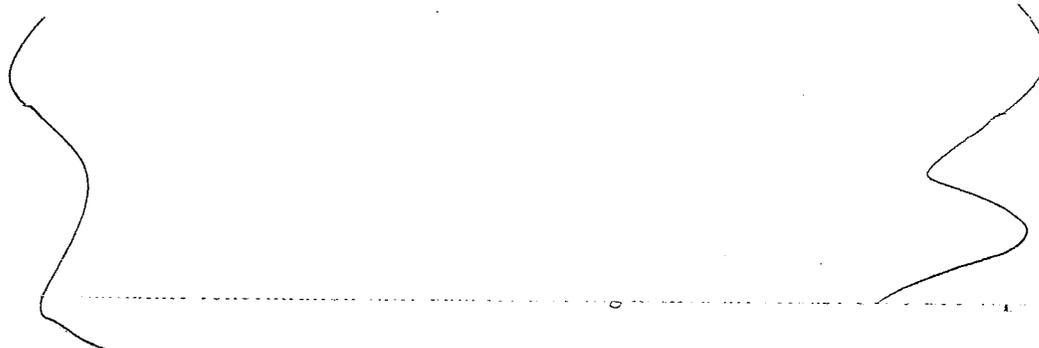
AUC units: ng·hr/mL

C<sub>max</sub> units: ng/mL

Attachment A

### LEVEL A IVIVC MODEL DEVELOPMENT

Listed below is the detailed procedure that was used for the development of the IVIVC for the 200 mg strength of Desvenlafaxine Succinate, Extended Release Tablets.



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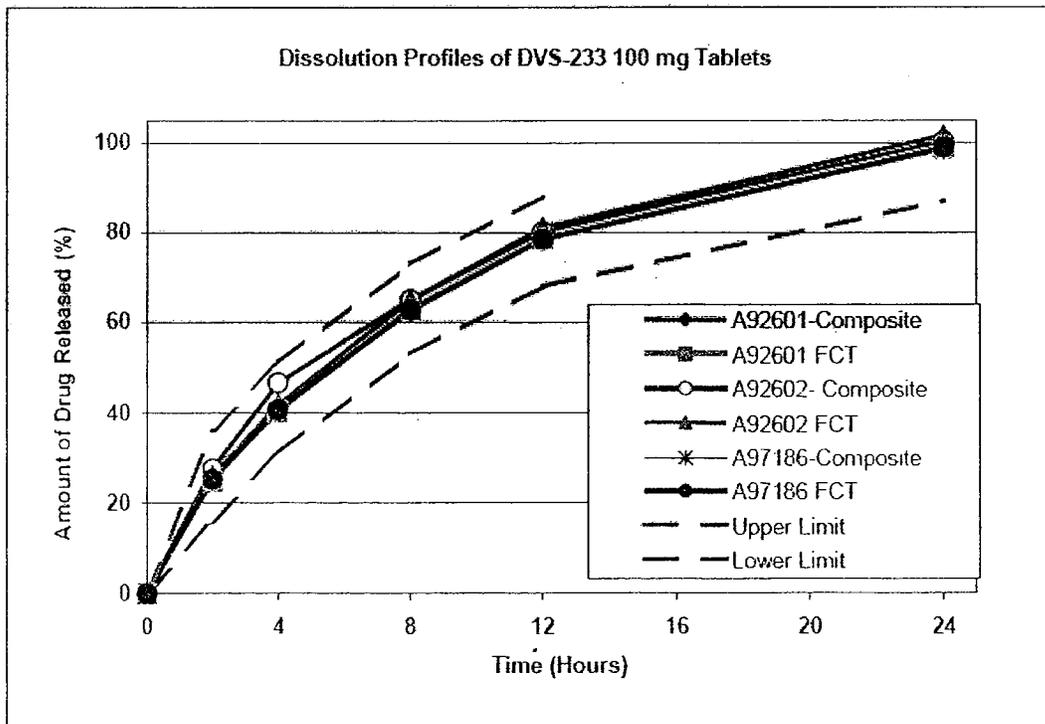


\_\_\_\_\_ in the formula. The following figure displays these dissolution profiles along with the realized dissolution results of the initial and stability results of the six 100 mg and 200 mg Desvenlafaxine Succinate tablet registration batches: these are formulated with appropriate \_\_\_\_\_ levels to meet the target drug release profile. The range of \_\_\_\_\_ content (\_\_\_\_\_) produced tablets at approximately the upper and lower bounds of the dissolution profile and supports an approximate range in dissolution profiles of \_\_\_\_\_. For the 4-, 8-, and 12-hour dissolution time points.

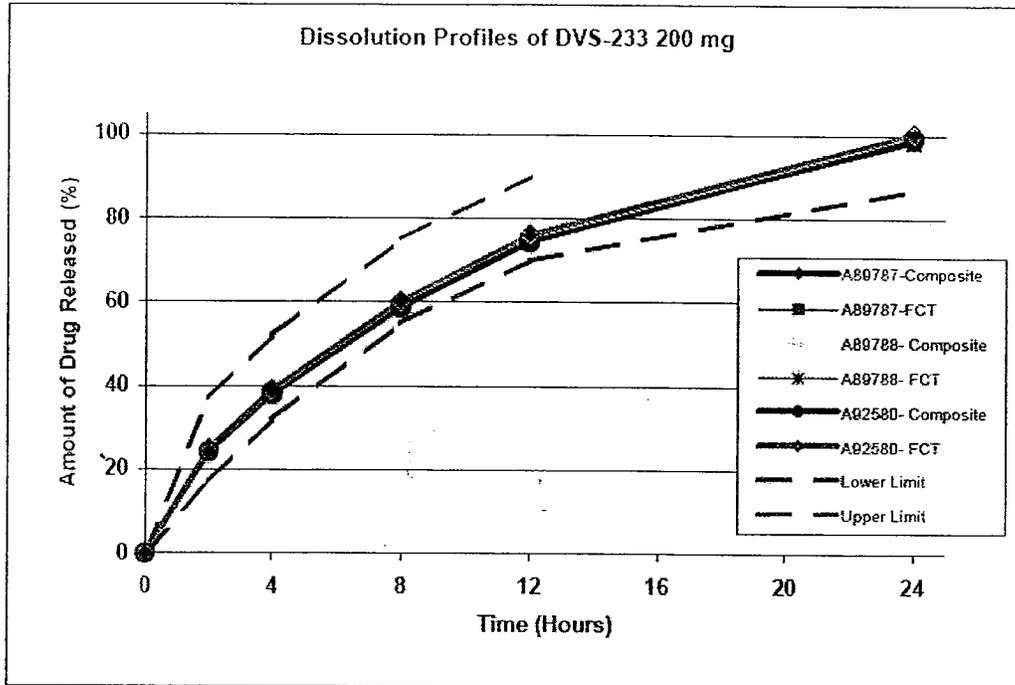
The sponsor proposed a \_\_\_\_\_

*Reviewer comments: The reviewer concludes that since level A IVIVC, which is point to point dissolution vs concentration profile, multiple point dissolution should be set for DVS SR. Therefore, the \_\_\_\_\_ point dissolution proposed by the sponsor is not acceptable by the Office of Clinical Pharmacology. The following figures illustrate the dissolution profile for the 100 mg and 200 mg registration batches.*

### 100 mg Registration Batches: Dissolution



Dissolution Profiles of DVS 200 mg (Registration Batches)



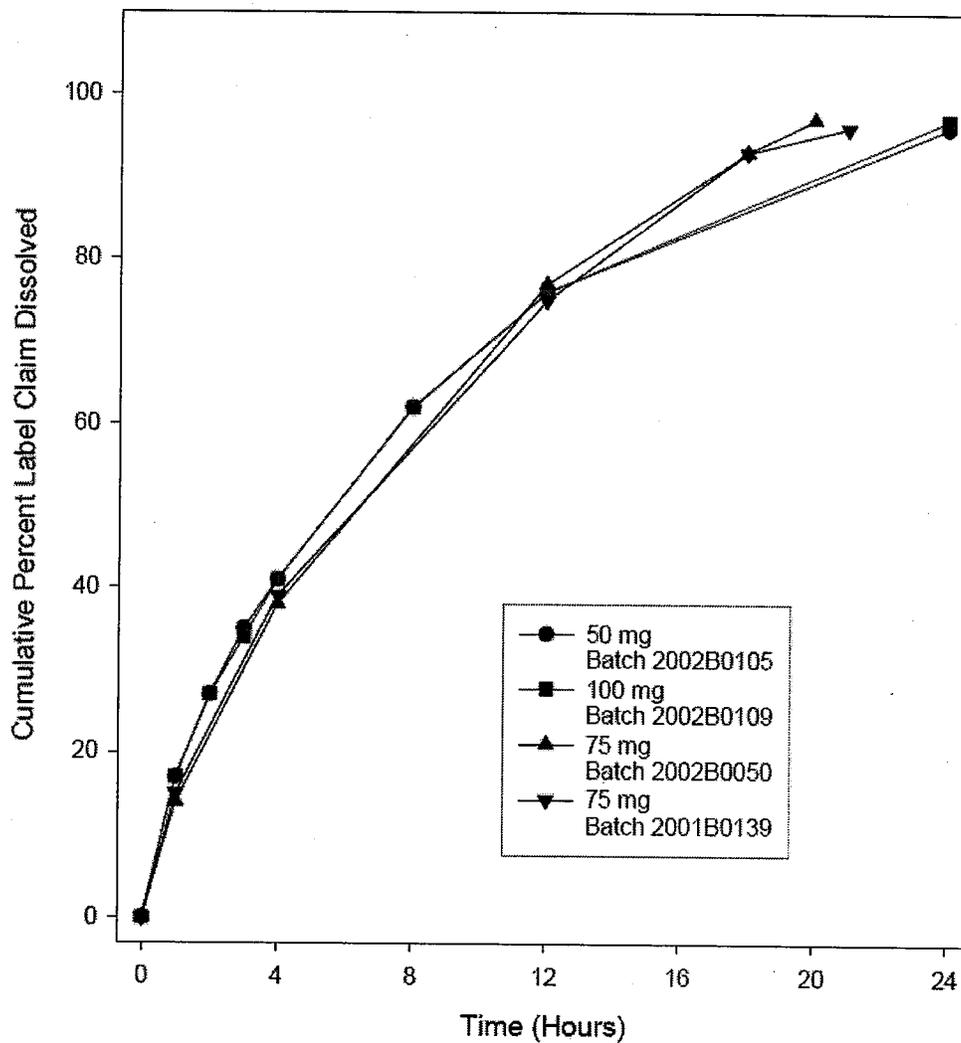
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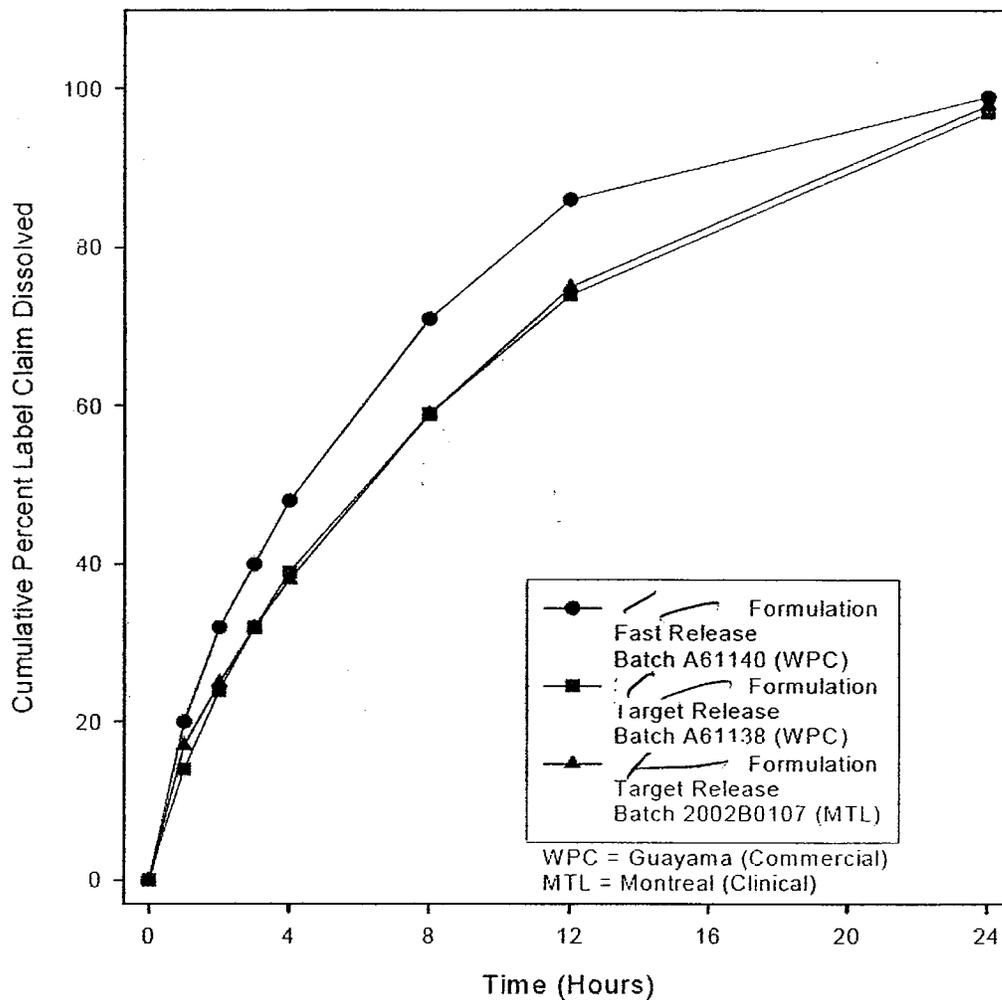
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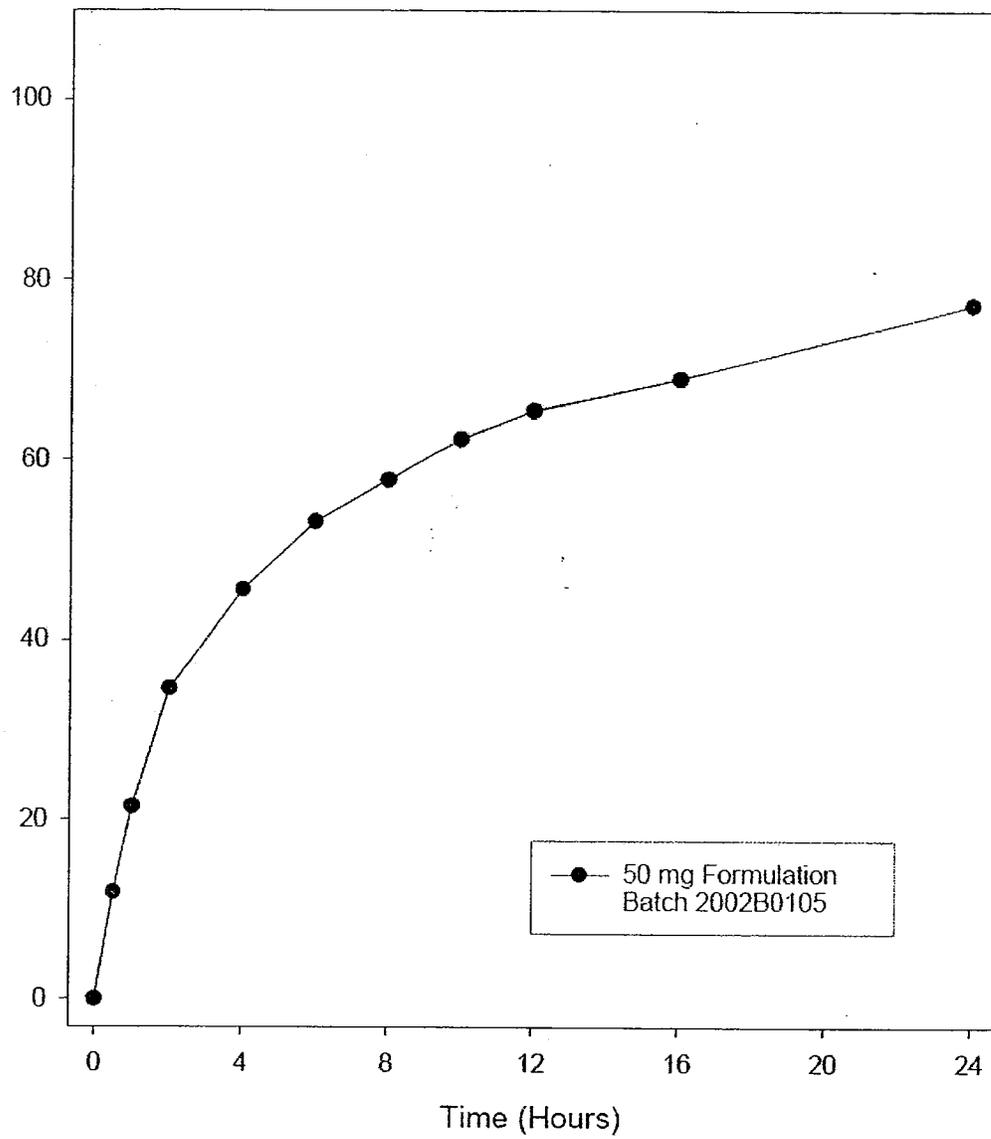
Dissolution Profiles of DVS from DVS SR 50, 75 and 100 mg Tablets Used in Bioavailability Studies (Studies 167, 186, 190)



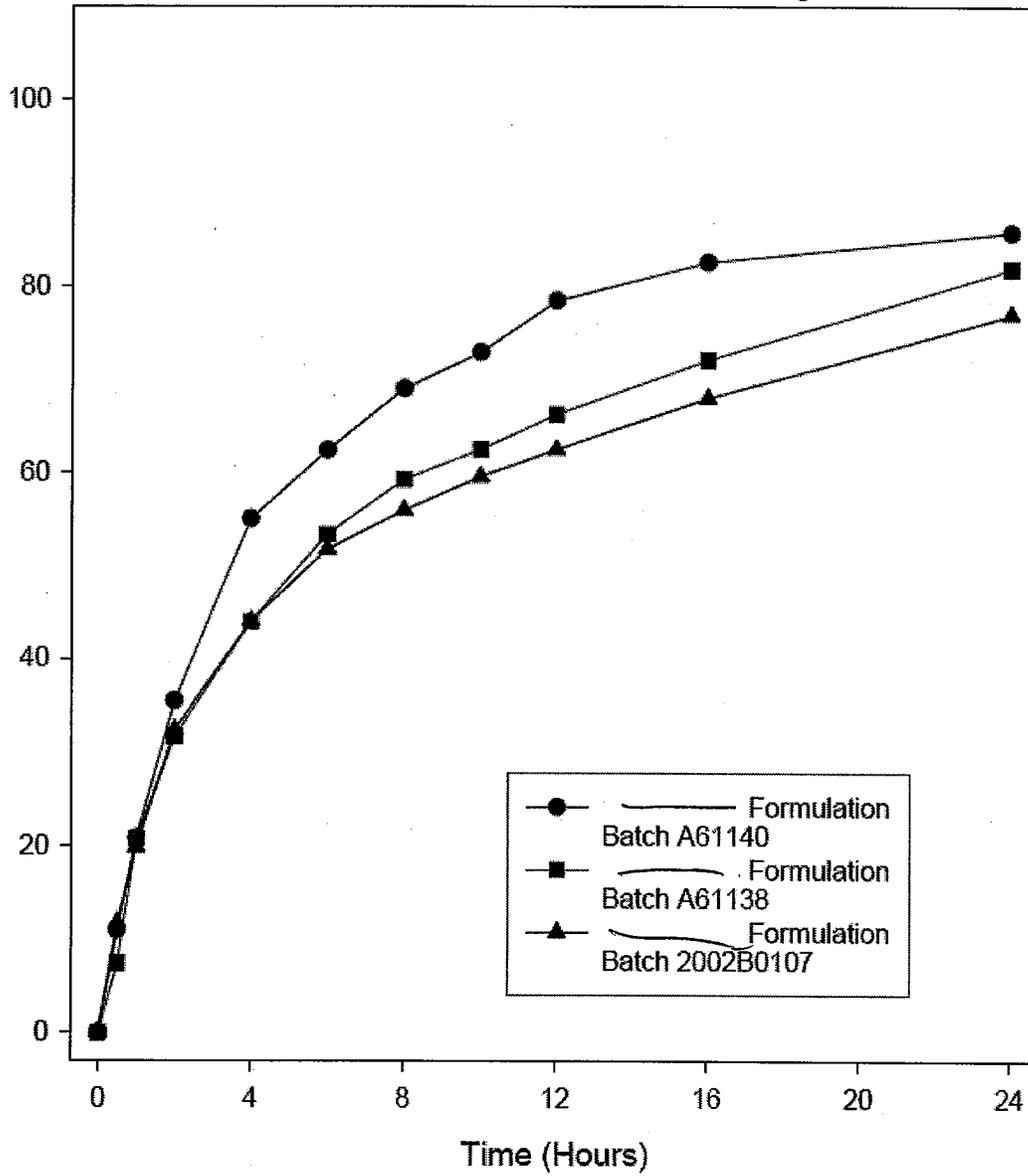
Dissolution Profiles of DVS from DVS SR 200 mg Tablets Used in Bioavailability Study  
(Study 177)



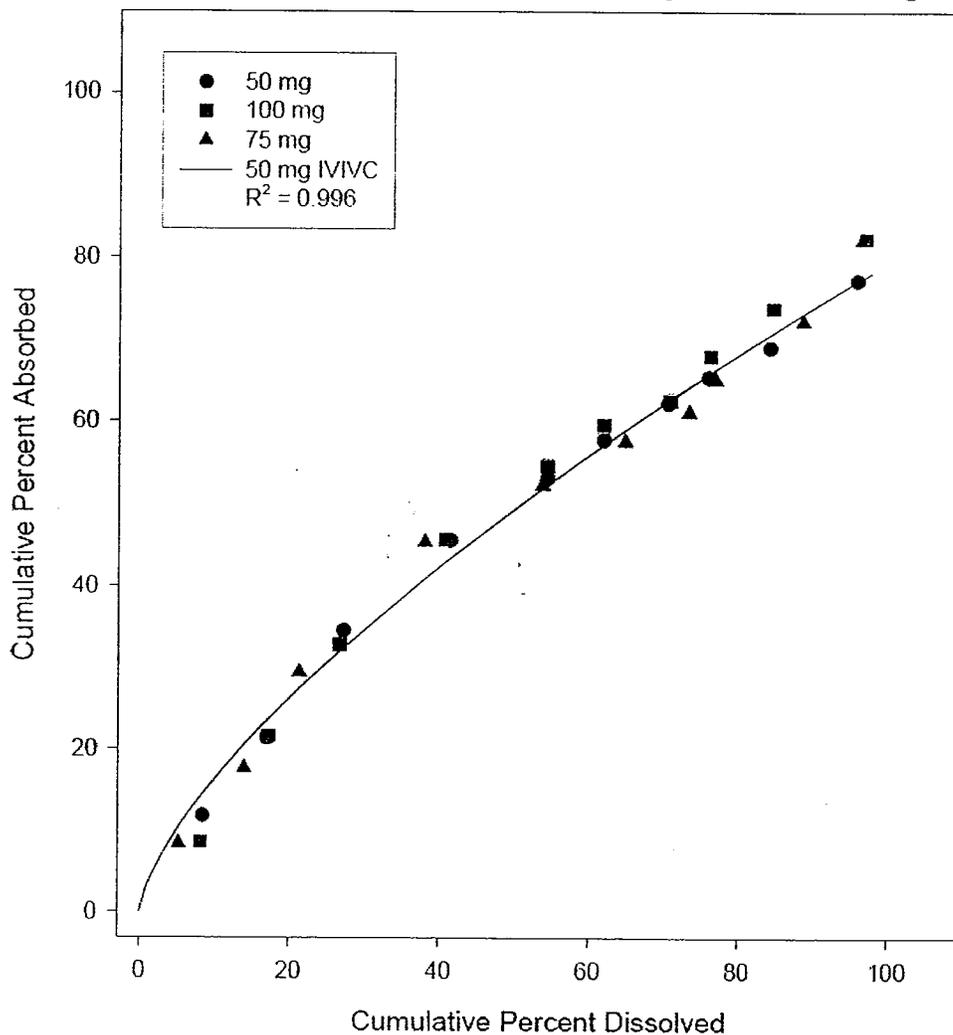
Absorption Profile of DVS from DVS-233 SR 50 mg Tablets



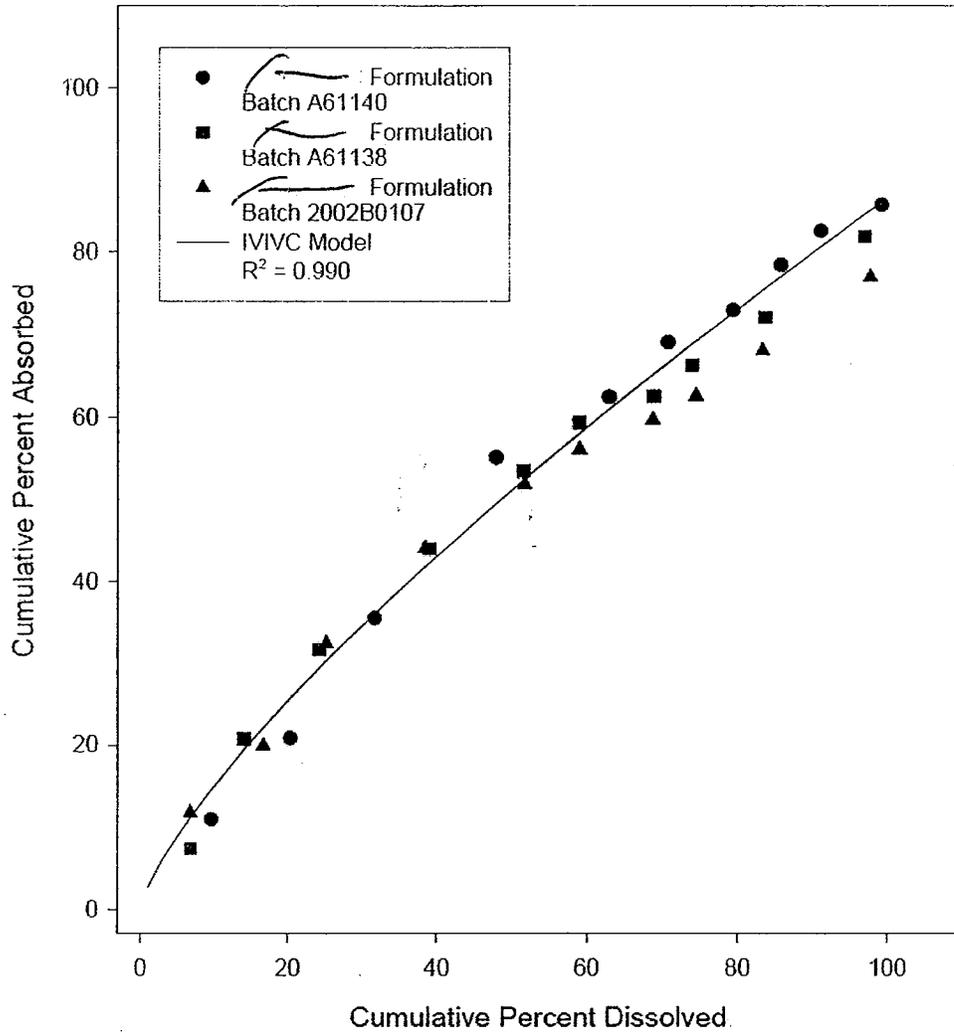
Absorption Profiles of DVS from DVS SR 200 mg Tablets



Absorption of DVS versus Dissolution of DVS Relationship for DVS SR 50 mg Tablets



Absorption of DVS versus Dissolution of DVS Relationship for DVS SR 200 mg Tablets



Dissolution of DVSSR 50 mg Tablets: Evaluation of pH

6.3 Dissolution of DVS-233 SR 50 mg Tablets: Evaluation of pH			
Media	Time (hr)	Batch A35065	
		AVG (%)	Range (%)
Water			
0.1N HCl			
pH 4.5 Buffer			
pH 6.8 Buffer			
0.1N HCl/pH 6.8 Buffer 2-stage			
0.9% NaCl	$t_2$		
	2	26	
	4	42	
	8	63	
	12	77	
	24	97	

Dissolution conditions: USP apparatus 1 (baskets), 100 rpm, 0.9 L of media, 37°C  
Reference media: 0.9% NaCl.

Dissolution of DVS SR 200 mg Tablets: Evaluation of pH

6.4 Dissolution of DVS-233 SR 200 mg Tablets: Evaluation of pH							
Media	Time (hr)	Batch A61140		Batch A61138		Batch A43079	
		AVG (%)	Range (%)	AVG (%)	Range (%)	AVG (%)	Range (%)
Water							
0.1N HCl							
pH 4.5 Buffer							
pH 6.8 Buffer							
0.1N HCl/pH 6.8 Buffer 2-stage							
0.9% NaCl	2	32		24		25	
	4	48		39		39	
	8	71		59		60	
	12	86		74		76	
	24	99		97		97	

Dissolution conditions: USP apparatus 1 (baskets), 100 rpm, 0.9L of media, 37°C  
 Reference media: 0.9% NaCl within each batch

Bioavailability Assessment of Dissolution Specifications for DVS SR Tablets Based on Mean Dissolution Profile

<b>6.8 Bioavailability Assessment of Dissolution Specifications for DVS-233 SR Tablets Based on Mean Dissolution Profile</b>			
Dissolution of 50 mg SR Tablets – Cumulative Percent Released			
Time (Hr)	Reference Profile	Lower Limit (-10%)	Upper Limit (+10%)
2	27	17	37
4	41	31	51
8	62	51	72
12	76	66	86
24	96	86	106
Bioavailability of 2x50 mg DVS-233 SR Tablet			
Parameter	Reference Tablet	Lower Limit Tablet	Upper Limit Tablet
AUC (ng-hr/mL)	4798	4462	5108
% Difference <sup>a</sup>	--	7.0	6.5
% Difference <sup>b</sup>	--	13	
C <sub>max</sub> (ng/mL)	157	143	173
% Difference <sup>a</sup>	--	9.1	10
% Difference <sup>b</sup>	--	18	
t <sub>max</sub> (hr)	6	10	6
Dissolution of 200 mg SR Tablets – Cumulative Percent Released			
Time (Hr)	Reference Profile	Lower Limit (-10%)	Upper Limit (+10%)
2	25	15	35
4	38	28	48
8	59	49	69
12	75	65	85
24	98	88	108
Bioavailability of 1x200 mg DVS-233 SR Tablet			
Parameter	Reference Tablet	Lower Limit Tablet	Upper Limit Tablet
AUC (ng-hr/mL)	9558	8802	10286
% Difference <sup>a</sup>	--	7.9	7.6
% Difference <sup>b</sup>	--	14	
C <sub>max</sub> (ng/mL)	308	282	340
% Difference <sup>a</sup>	--	8.4	10
% Difference <sup>b</sup>	--	17	
t <sub>max</sub> (hr)	8	10	8
a. Difference from reference			
b. Difference from lower limit to upper limit			

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**Title (GTR 17425):** Protein Binding of Wy-45,233 in Rat, Dog and Human Plasma

**Objective:** To determine the plasma protein binding of Wy-45,233 in Rat, Dog and Human Plasma.

**Materials and Methods:** Wy-45233 was obtained from the sponsor. The amount of total protein in each plasma sample was determined before and after dialysis, using \_\_\_\_\_ Reagent (\_\_\_\_\_\_). The values obtained were used to correct for volume shifts during dialysis.

The plasma protein binding of Wy-45,233 was determined by equilibrium dialysis using a multi-chamber stainless steel dialysis block with a \_\_\_\_\_ dialysis membrane separating each pair of cells. Three pre-dialysis concentrations of Wy-45,233 were tested for each species: for humans 0.1, 0.25 and 0.5 µg/mL. Wy-45,233 was dissolved in deionized water to make a stock solution of 1000 µg/mL. Serial dilutions were prepared with deionized water to obtain 0.05 to 5.0 µg/mL solutions for standard curves. Appropriate concentrations of Wy-233 were added to plasma to achieve pre-dialysis concentrations stated above for humans. One ml of plasma was then dialyzed against an equal volume of 0.067M sodium phosphate buffer (pH 7.4). The dialysis block was placed in an \_\_\_\_\_ mechanical shaker and incubated at 37°C for 8 hours. After completion of dialysis, plasma and buffer were removed and 700 µl of each fraction was extracted and analyzed by HPLC. The time to achieve equilibrium was determined in a pilot study in which four 1.0 mL aliquots of human plasma containing 0.25 µg/mL Wy-45,233 were dialyzed against 1.0 mL aliquots of sodium phosphate buffer (pH 7.4) for 3, 4, 5, 6, 7, 8, 9 and 10 hours.

Calculation:

The percent of Wy-45,233 bound to plasma protein was calculated as follows (1):

$$\frac{(C_P - C_B)DF}{(C_P - C_B)DF + C_B} \times 100$$

Where  $C_P$  = plasma Wy-45,233 concentration  
 $C_B$  = buffer Wy-45,233 concentration  
 $DF$  = Dilution factor, i.e.

$$DF = \frac{\text{Total Protein Concentration Pre-dialysis}}{\text{Total Protein Concentration Post-dialysis}}$$

The dilution factor corrects the bound drug concentration for dilution of protein. This mathematical correction is based upon the assumption that binding parameters are not altered by small changes in protein concentration.

Results: The following table contain the summary of WY-45.233 bound to proteins.

The Protein Binding of Wy-45,233 in the Plasma of Rats, Dogs and Human

Species	Post-incubation plasma Wy-45,233 concentration ( $\mu\text{g/ml}$ )	N	Percent bound to plasma proteins <sup>a</sup>
Rat	1.11	5	40.8 $\pm$ 6.7
	2.68	5	37.8 $\pm$ 4.3
	5.94	4	40.5 $\pm$ 9.2
	All concentrations	14	39.6 $\pm$ 6.4
Dog	0.34	4	25.6 $\pm$ 9.4
	0.82	5	25.7 $\pm$ 10.9
	1.67	5	26.5 $\pm$ 3.9
	All concentrations	14	26.0 $\pm$ 7.9
Human	0.035	2	24.6
	0.124	5	32.1 $\pm$ 11.9
	0.200	5	29.7 $\pm$ 13.8
	All concentrations	12	29.9 $\pm$ 12.1

<sup>a</sup>Mean $\pm$  SD

The degrees of binding were not concentration dependent in any of the species. The protein binding of Wy-45.233 was significantly lower in dog and human plasma than in the plasma of rats. The degree of protein of is low, therefore, changes in protein binding are not expected to influence the pharmacokinetics of Wy-45,233 in these species.

*Reviewer's comments: The binding of WY-45,233 to human plasma was low (about 30%) and is not expected to have clinical significance.*

Attachment

Table I. The Protein Binding of Wy-45,233 in Rat Plasma

Pre-incubation plasma Wy-45,233 concentration( $\mu\text{g/ml}$ )	Post-incubation concentration of Wy-45,233 ( $\mu\text{g/ml}$ )		Percent bound to plasma proteins
	plasma	buffer	
3.2	1.19	0.62	49.4
	1.03	0.69	34.8
	1.17	0.71	40.6
	1.13	0.64	45.1
	1.04	0.70	33.8
Mean $\pm$ SD	1.11 $\pm$ 0.07		
8.0	2.62	1.48	44.3
	2.57	1.67	36.1
	2.85	1.79	38.1
	2.36	1.62	32.4
	2.99	1.88	37.8
Mean $\pm$ SD	2.68 $\pm$ 0.25		
16.0	5.33	3.32	39.2
	6.19	3.42	46.3
	6.68	3.55	48.4
	5.54	4.06	28.1
	3.96	3.97	-0.4 <sup>a</sup>
Mean $\pm$ SD	5.94 $\pm$ 0.62 <sup>b</sup>		

<sup>a</sup>Spurious value, not included in calculations.

<sup>b</sup>N = 4

Table II. The Protein Binding of Wy-45,233 in Dog Plasma

Pre-incubation plasma Wy-45,233 concentration ( $\mu\text{g/ml}$ )	Post-incubation concentration of Wy-45,233 ( $\mu\text{g/ml}$ )		Percent bound to plasma proteins
	plasma	buffer	
1.0	0.38	0.29	24.1
	0.41	0.25	39.3
	0.29	0.24	19.2
	0.29	0.23	19.7
	0.26	0.26	1.8 <sup>a</sup>
Mean $\pm$ SD	0.34 $\pm$ 0.06 <sup>b</sup>		
2.5	0.86	0.63	25.6
	0.90	0.73	18.9
	0.67	0.56	15.5
	0.72	0.53	24.5
	0.96	0.52	43.8
Mean $\pm$ SD	0.82 $\pm$ 0.12		
5.0	1.80	1.22	31.8
	1.80	1.28	28.3
	1.84	1.38	24.3
	1.39	1.08	21.7
	1.52	1.11	26.6
Mean $\pm$ SD	1.67 $\pm$ 0.20		

<sup>a</sup>Spurious value, not included in calculations.

<sup>b</sup>N = 4

Table III. The Protein Binding of Wy-45,233 in Human Plasma

Pre-incubation plasma Wy-45,233 concentration ( $\mu\text{g/ml}$ )	Post-incubation concentration of <u>Wy-45,233 (<math>\mu\text{g/ml}</math>)</u>		Percent bound to plasma proteins
	plasma	buffer	
0.10	0.034	0.029	14.9
	0.035	0.024	34.2
	0.048	0.070	-95.9 <sup>a</sup>
	0.020	0.032	-67.8 <sup>a</sup>
	0.020	0.021	-5.0 <sup>a</sup>
Mean $\pm$ SD	0.035 $\pm$ 0.001 <sup>b</sup>		
0.25	0.098	0.059	40.2
	0.136	0.095	31.0
	0.110	0.063	43.9
	0.161	0.110	32.2
	0.116	0.101	13.1
Mean $\pm$ SD	0.124 $\pm$ 0.025		
0.50	0.270	0.203	25.4
	0.196	0.124	37.8
	0.209	0.187	11.3
	0.175	0.093	47.9
	0.146	0.109	26.2
Mean $\pm$ SD	0.200 $\pm$ 0.046		

<sup>a</sup>Spurious value, not included in calculations.

<sup>b</sup>N=2

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**Title (Protocol No. 01\_0457): DVS-233 (Wy-45233): P450 Isozyme Identification Study Using cDNA Expressed P450 Isozymes and Chemical Inhibition Studies in Human Microsomes**

**Background:** DVS-233 (WY-45233) is the succinate salt of the O-desmethyl metabolite of venlafaxine (WY-45030). Previous studies have shown that venlafaxine is metabolized primarily to O-desmethyl venlafaxine in humans by CYP2D6. It has been proposed that the N-desmethylation of venlafaxine may be catalyzed by CYP3A4, since CYP3A4 specific inhibitors decreased the formation of N-desmethyl venlafaxine metabolites in human liver microsomes. In the current study, the CYP450 isozymes involved in the metabolism of DVS-233 to two of its major microsomal metabolites were investigated. The approaches utilized were chemical inhibition studies in human liver microsomes and metabolism by recombinant expressed human CYP450 isozymes.

**Materials and Methods:** WY-45233, 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol (DVS-233, lot P4656-234-10) as the succinate salt, WY-46689, 4-[2-(methylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol and WY-46965, 4-[2-amino-1-(1-hydroxycyclohexyl)ethyl]phenol) were obtained from Wyeth-Ayerst Research, Princeton, NJ. The cDNA-expressed human cytochrome CYP450 in E. coli membranes was prepared in house from constructs obtained from the \_\_\_\_\_

\_\_\_\_\_ The human liver microsomes used in these studies were from either a characterized microsome bank, prepared and analyzed by \_\_\_\_\_, or were prepared in-house from human livers obtained from \_\_\_\_\_ NADPH, \_\_\_\_\_ naphthoflavone, coumarin, quinidine, diethylthiocarbamate, ketoconazole and benzylimidazole were obtained from \_\_\_\_\_

Sulfaphenazole, S(+) mephénytoin and dextrorphan were obtained from \_\_\_\_\_

\_\_\_\_\_ HPLC grade water and acetonitrile were obtained from \_\_\_\_\_

\_\_\_\_\_ All other reagents were AR grade or better.

**Microsome Preparation:** Human liver microsomes were from two sources. Samples 3, 6, 15, 16, 17 and 19 were from a bank of microsomes prepared and characterized by Dr. \_\_\_\_\_ Microsomes 99-1, 99-2, 99-3, 99-4, 99-5 and 99-6 were prepared in house from livers obtained from \_\_\_\_\_. These microsomes were prepared by \_\_\_\_\_ described by \_\_\_\_\_ with slight modifications as described in Princeton SOP 55-006.01. Microsomal protein and cytochrome P450 content were determined by the methods of \_\_\_\_\_ respectively, and detailed in Princeton SOPs 55-001.02 and 55-016.00, respectively. Microsomes were stored at -80°C in aliquots of 500-1000 µL until use.

**Optimization Experiments:**



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**Analytical Method:** DVS-233, its metabolites, and ~~the~~ the internal standard used, were detected by LC/MS/MS analysis in the selected reaction monitoring mode (LC/SRM)..

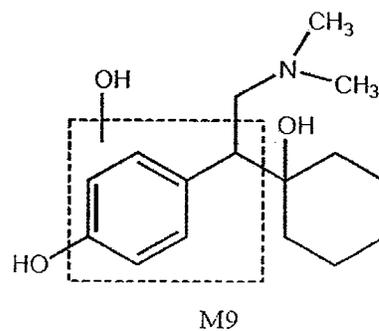
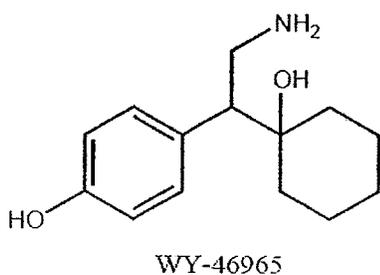
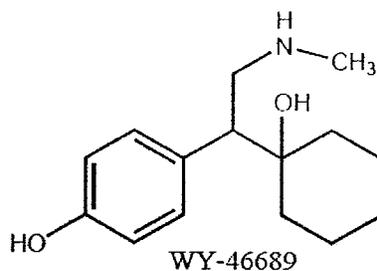
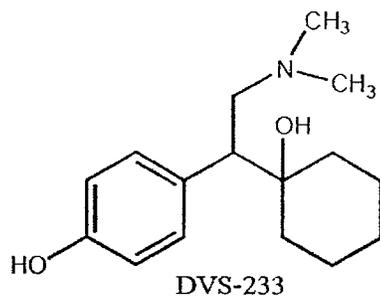
**Calculations:** Optimum conditions were defined as those where metabolite formation was linear with respect to time or protein concentration. The  $K_m$  for DVS-233 metabolism to WY-46689 was determined using a Lineweaver-Burk plot of the rate of WY-46689 formation at various DVS-233 concentrations. The amount of WY-46689 formed was determined using the ratio between the area of the peak for WY-46689 and the area of the peak for dextrorphan. This calibration curve was linear between 2.5 and 500 nM WY-46689. The  $K_m$  value for M9 formation was estimated using a similar plot but the absolute rates could not be determined because no synthetic M9 was available. Values for DVS-233 metabolism by the various cDNAs were expressed relative to the metabolism observed in human liver microsomes using the formula:  $(ac - bc) / (am - bm)$ , where  $ac$  is the peak area of metabolite in the cDNA incubations with NADPH and  $bc$  is the peak area of metabolite in the cDNA incubations with no NADPH,  $am$  and  $bm$  are the corresponding values for incubations of DVS-233 using human liver microsomes.

The percent inhibition in reactions with the chemical inhibitors was based on the amount of metabolite detected. Values were expressed as percent inhibition relative to a control reaction using the formula: % inhibition =  $((a - b) / a) * 100\%$ , where  $a$  = peak area of metabolite with no inhibitor present and  $b$  = peak area of metabolite with inhibitor present.

**Results:** Two major metabolites, WY-46689 and a metabolite designated M9, were identified as the most abundant metabolites of DVS-233 in human liver microsomes incubated with NADPH. The following figure contains the structures of the metabolites.

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Structures of DVS-233, WY-46689, WY-46965 and M9



Linear rate conditions were determined using the formation of WY-46689 as an indicator of metabolism. It was determined that the reaction was linear through 30 min and 1 mg/mL microsomal protein using 10  $\mu$ M DVS-233. It was not possible to determine if M9 formation was linear under these conditions because M9 levels were too low to be accurately determined at 10  $\mu$ M DVS-233. The  $K_m$  for WY-46689 formation was found to be approximately 290  $\mu$ M DVS-233. Linear rate conditions (300  $\mu$ M DVS-233, 1 mg/mL protein, 30 min incubation) were used in the inhibition studies. Similar conditions were used for the cDNA studies, but the CYP450 content was kept constant at 500 nM.

Several of the cDNA isoforms investigated were capable of metabolizing DVS-233 to varying extents. Incubations with CYP2C19 produced the highest amounts of both WY-46689 and M9, with the levels of each metabolite produced being approximately 4.7 times higher than the levels seen using human liver microsomes. Incubations of

DVS-233 with CYP3A4 and CYP2C8 also led to the formation of both WY-46689 and M9, with CYP3A4 producing approximately twice as much of each metabolite as CYP2C8. CYP2C9 was capable of metabolizing DVS-233 to WY-46689, but not M9. No metabolism was detected with CYP1A1, CYP1A2, CYP2A6 or CYP2D6.

Metabolism of DVS-233 (300  $\mu$ M) During Incubations with Membranes from E. Coli Transfected With Various cDNAs

	Amount of each metabolite formed relative to the amount formed using human liver microsomes <sup>a</sup>	
	WY-46689	M9
Microsomes <sup>b</sup>	1.0	1.0
CYP1A1	ND <sup>c</sup>	ND
CYP 1A2	ND	ND
CYP 2A6	ND	ND
CYP 2C8	1.03	0.49
CYP 2C9	0.33	ND
CYP 2C19	4.74	4.66
CYP 2D6	ND	ND
CYP 3A4	1.91	0.88

a: Based on amount detected by LC/MS

b: Pool of 12 individual human liver microsome samples

c: ND indicates metabolite was not detected

Results of the current studies, summarized in TABLES X and Y, indicate that more than one human liver CYP450 isozymes may be involved in the metabolism of DVS-233. The metabolites measured were WY-46689, the N-desmethyl product of DVS-233, and M9, the benzyl group hydroxylation product of DVS-233. The most significant CYP450 isozyme involved in the formation of WY-46689 and M9, as determined by chemical inhibition studies, is CYP3A4. However, since 50  $\mu$ M ketoconazole did not completely inhibit metabolism. Inhibitors of CYP2C9 and CYP2E1 also exhibited some mild effects on DVS-233 metabolism. Many of the enzymes were capable of metabolizing DVS-233 to WY-46689 and M9; from highest to lowest activity they were CYP2C19, CYP3A4, and CYP2C8. WY-46689, but not M9, was also detected following incubations of DVS-233 with CYP2C9.

Inhibition of Metabolism of DVS-233 (300  $\mu$ M) By CYP450 Isozyme Specific Inhibitors in Human Liver Microsomes

Inhibitor	$\mu$ M	Enzyme Inhibited	Percent Inhibition of WY-46689 formation <sup>a</sup>	Percent Inhibition of M9 formation <sup>a</sup>
$\alpha$ -Naphthoflavone	1	CYP1A2	0	0
Coumarin	100	CYP2A6	0	0
Sulfaphenazole	10	CYP2C9	21	0
S(+)-Mephenytoin	100	CYP2C19	0	0
Quinidine	10	CYP2D6	4	0
Diethyldithiocarbamate	100	CYP2E1	15	19
Ketoconazole	50	CYP3A4	76	58
"	5	"	65	29
"	1	"	29	0
Benzylimidazole	1000	All CYPs	87	99
Heat Inactivation	1 min at 50°C	FMO	0	0

a: Values are expressed as percent inhibition relative to a control reaction.

**Conclusions:** The sponsor concluded that in this study, CYP2C8, CYP2C9, CYP2C19 and CYP2E1 may play some role in the metabolism of DVS-233 but the major CYP450 isozyme involved in the metabolism of DVS-233 to WY-46689 and M9 is CYP3A4. Co-administration of drugs that affect CYP3A4 activity may affect the CYP450-mediated metabolism of the DVS-233.

*Reviewer's comments:* The reviewer agrees with the general sponsor's conclusions. However, 300  $\mu$ M concentration might be too high a concentration to be have been used in the study. Hence, inference from this study should be done with cautionu

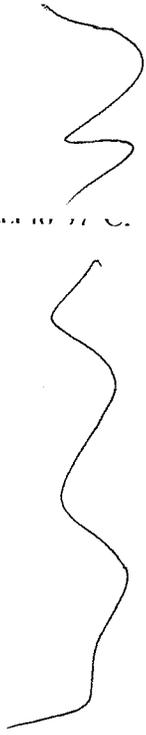
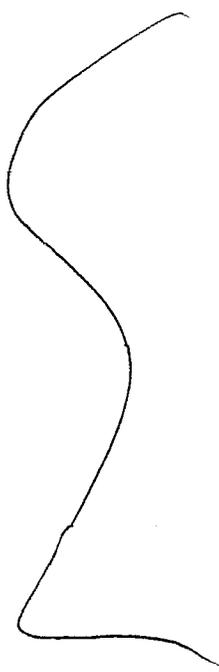
**Title (Protocol No.: 01\_0430): DVS-233 (Wy-45233): In Vitro Metabolism In Cryopreserved Human Hepatocytes And Liver Microsomes Of Sprague/Dawley Rats, Beagle Dogs And Humans.**

**Objective:** In the current study, the in vitro metabolism of DVS-233 was investigated using cryopreserved human hepatocytes and liver microsomes from rats, dogs and humans.

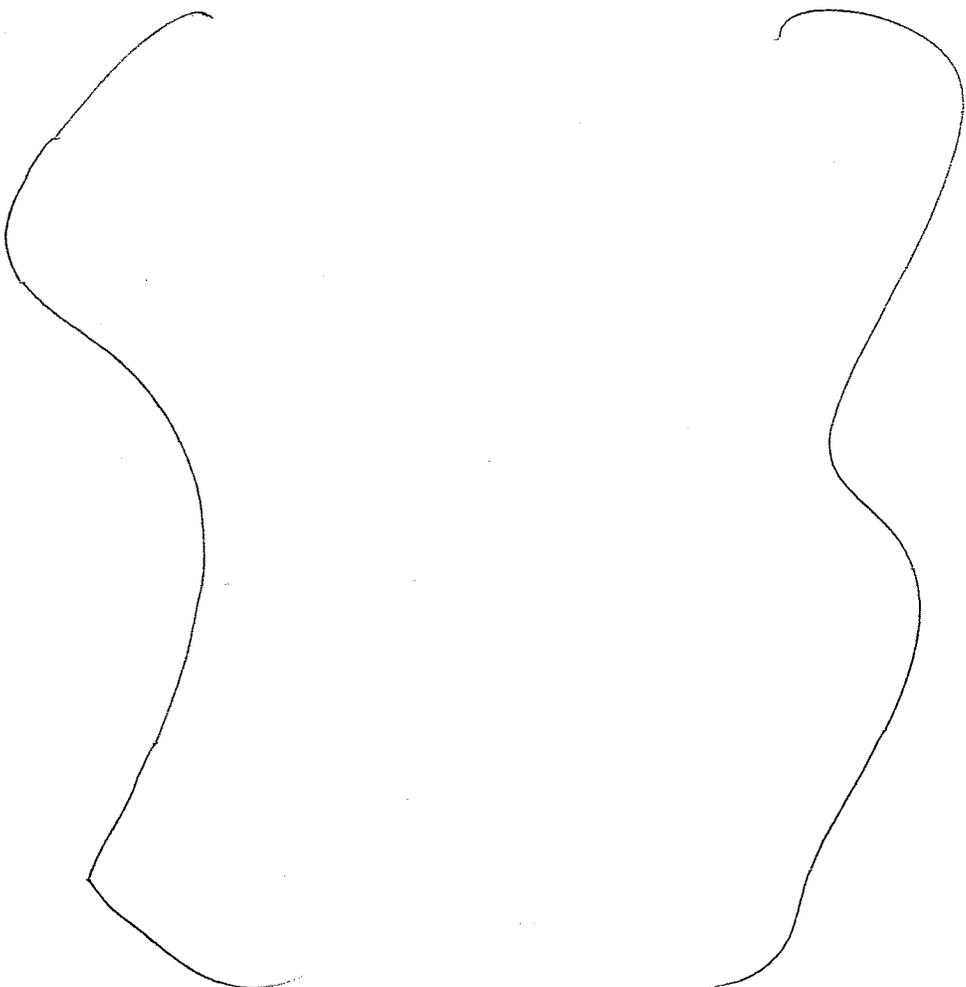
**Materials and Methods:** DVS-233 (Wy-45233) (4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol succinate) (Lot P4656-234-10), WY-46689 (4-[2-(methylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol) (Lot P3882-165) and WY-46965 (4-[2-amino-1-(1-hydroxycyclohexyl)ethyl]phenol) (Lot I-07970-272-1) were obtained from Wyeth Research, Princeton, NJ. Cryopreserved human hepatocytes from three individuals (Lot #s 70, 94 and 133), hepatocyte suspension media, hepatocyte culture media and liver microsomes from male Sprague Dawley rats (Lot # LMO, N=68, 17 mg/mL, 0.30 nmol P450/mg protein), and male beagle dogs (Lot # M100006, N=5, 24 mg/mL, 0.57 nmol P450/mg protein) were obtained from \_\_\_\_\_ . Uridine 5'-diphosphoglucuronic acid triammonium salt (UDPGA) was obtained from \_\_\_\_\_. Ammonium acetate and magnesium chloride was obtained from \_\_\_\_\_. All other reagents were analytical grade or better.

Human liver microsomes were from two sources. Microsome samples 3, 6, 15, 16, 17 and 19 were prepared from livers received from \_\_\_\_\_. Microsomes 99-1, 99-2, 99-3, 99-4, 99-5 and 99-6 were prepared in-house from livers obtained from \_\_\_\_\_ .

\_\_\_\_\_. Microsomes



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**Results:** LC/MS analysis of samples incubated with DVS-233 using rat, dog and human liver microsomes yielded 10, 9 and 7 DVS-233 related metabolites, respectively. Rat liver microsomes produced eight hydroxy DVS-233 metabolites; six cyclohexane ring hydroxy metabolites, one ethyl group hydroxy metabolite and one benzyl group hydroxy metabolite. Dog liver microsomes produced all of these hydroxy metabolites, except the ethyl group hydroxy metabolite. Human liver microsomes produced three of the cyclohexane ring hydroxy metabolites and the benzyl group hydroxy metabolite. All species generated both N-desmethyl DVS-233 and the O-glucuronide of DVS-233. In addition to these metabolites, an N-oxide product of DVS-233 was detected in all samples, including DVS-233 incubated in the absence of microsomes. Following incubations of DVS-233 with cryopreserved human hepatocytes one cyclohexane ring hydroxy DVS-233 metabolite, N-desmethyl DVS-233 and the O-glucuronide of DVS-233 were detected by LC/MS.

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The proposed metabolic pathways for DVS-233 metabolism in cryopreserved human hepatocytes and liver microsomes from rats, dogs and humans are shown in the following figure. In all the in vitro systems used, the turnover of DVS-233 was low and metabolites generally represented less than 5% of the DVS-233-related peaks detected. Ten metabolites and one degradation product of DVS-233 were detected and characterized by LC/MS in the various metabolic systems. The glucuronide of WY-45233 had previously been observed as a major metabolite in dogs and humans, and as a minor metabolite in rats, following administration of venlafaxine. Glucuronidation of DVS-233 appeared to represent a prominent metabolic pathway for all species studied. WY-46689, the N-demethyl metabolite of DVS-233, and WY-46965, the N-didemethyl metabolite of DVS-233, have both been detected in rats, dogs, and humans following administration of venlafaxine. In the current study, WY-46689, the N-demethyl metabolite of DVS-233, was detected in microsomal samples from each species and in human hepatocyte samples. No WY-46965, the N-didemethyl metabolite of DVS-233, was observed in hepatocyte or microsomal samples from any species. Metabolites M1-M6 were characterized as hydroxylation products of the cyclohexanol ring.

In summary, a total of 10 metabolites of DVS-233 were characterized by LC/MS. The major metabolic pathways observed for DVS-233 were similar to those previously observed for venlafaxine. Oxidation, N-demethylation and glucuronidation were the metabolic pathways observed in each species. Oxidation appeared to be most significant in liver microsomes from rats and least significant in human liver microsomes.

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Summary Of In Vitro Metabolites Of DVS-233 Characterized By LC/MS

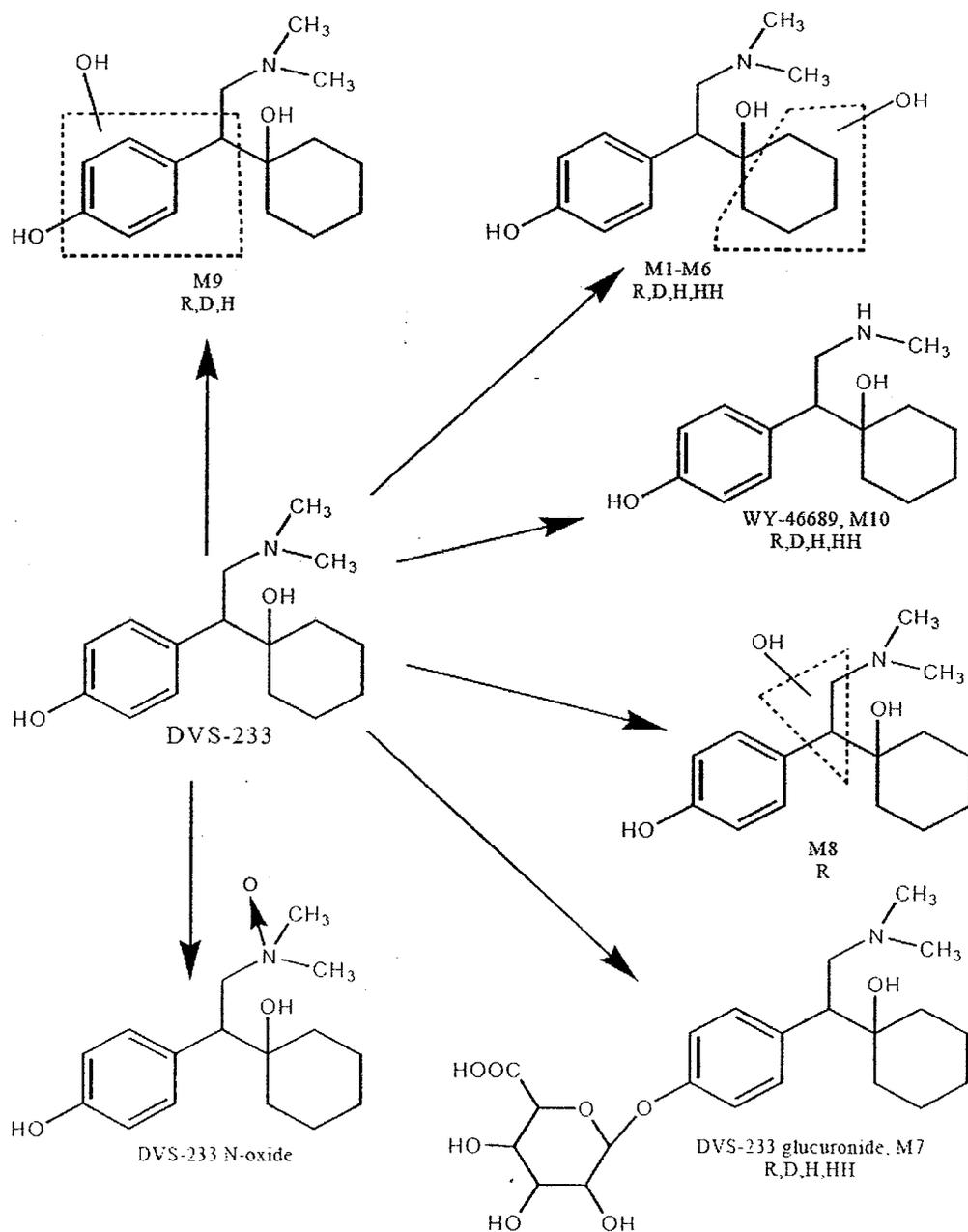
Metabolite	Ret. Time (min) <sup>a</sup>	[M+H] <sup>+</sup>	Site of Metabolism	Proposed Structure	Species and Matrix <sup>b</sup>
M1	3.9	280	Cyclohexane ring	Hydroxy DVS-233	R, D, H
M2	4.2	280	Cyclohexane ring	Hydroxy DVS-233	R, D, H
M3	6.5	280	Cyclohexane ring	Hydroxy DVS-233	R, D
M4	7.9	280	Cyclohexane ring	Hydroxy DVS-233	R, D
M5	11.8	280	Cyclohexane ring	Hydroxy DVS-233	R, D, H
M6	13.5	280	Cyclohexane ring	Hydroxy DVS-233	R, D, H, HH
M7	14.8	440	Phenol -OH group	O-Glucuronide of DVS-233	R, D, H, HH
M8	22.4	280	Ethyl group	Hydroxy DVS-233	R
M9	28.4	280	Benzyl group	Hydroxy DVS-233	R, D, H
M10	31.9	250	Dimethylamino group	N-Desmethyl DVS-233	R, D, H, HH
DVS-233	32.5	264	Unchanged DVS-233	Unchanged DVS-233	R, D, H, HH

a: LC/MS retention times taken from or normalized to data file DVS23MH011010.

b: R = Rat liver microsomes, D = Dog liver microsomes, H = Human liver microsomes, and HH = Human hepatocytes

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Primary Dvs-233 Metabolites And Reaction Products Detected Using Cryopreserved Human Hepatocytes And Liver Microsomes From Rat, Dog And Human



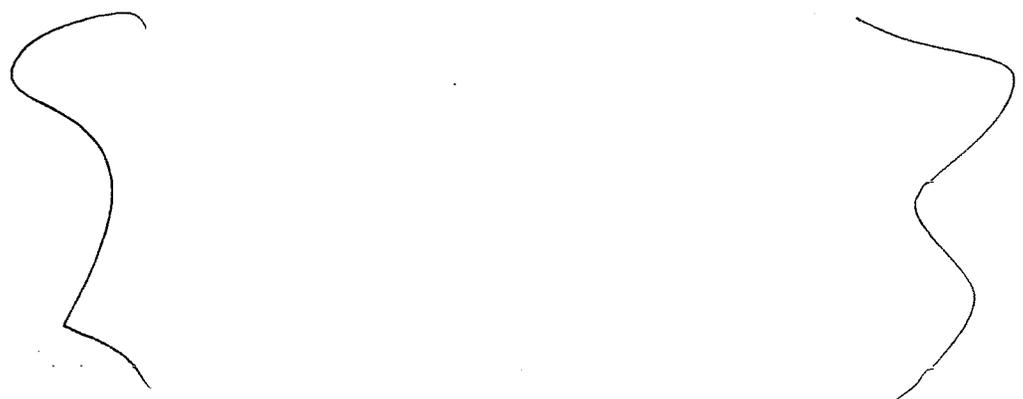
**TITLE:** Inhibition of Cytochrome P450 Enzymes in Human Liver Microsomes: Comparator Study (Protocol 04\_1793)

**Objective:** To determine and compare the inhibitory effect of DVS-233 and five currently used SSRI/SNRIs, venlafaxine (Effexor), S, S duloxetine (Cymbalta), paroxetine (Paxil), sertraline (Zoloft) and bupropion (Wellbutrin), on the catalytic activity of cytochrome P-450 (CYP) enzymes CYP1A2, 2A6, 2C19, 2C8, 2C9, 2D6 and 3A in human liver microsomes.

IC<sub>50</sub> values for the inhibition of the different CYP enzymes by the SSRI/SNRIs were determined using CYP-isoform specific probe substrates at concentrations near their respective K<sub>m</sub> values. In addition, K<sub>i</sub> values for the inhibition of CYP2D6 activity were determined for all the drugs studied, and K<sub>i</sub> values for the inhibition of CYP2C19 and CYP3A (midazolam 1'-hydroxylation and testosterone 6β-hydroxylation) activity were determined for selected substrates based on the magnitude of their IC<sub>50</sub> values.

**Materials and Methods:** DVS-233 succinate monohydrate (WY-45233), Lot RB1626, purity \_\_\_\_\_ was synthesized by Wyeth Research. Venlafaxine hydrochloride (WY-45030), Lot P4656-256-4, purity \_\_\_\_\_; S, S duloxetine (WAY-209473-A-2), Lot P6979-128-AR, purity \_\_\_\_\_; and paroxetine hydrochloride (WAY-209472-A-1), Lot P6839-167-4, purity \_\_\_\_\_ were synthesized by Wyeth Research, Princeton, NJ. Sertraline hydrochloride, CAS# 79559-97-0, purity \_\_\_\_\_ and Bupropion hydrochloride, CAS# 31677-93-7, purity \_\_\_\_\_ were purchased from \_\_\_\_\_. Resorufin, ethoxyresorufin, and dextropran were purchased from \_\_\_\_\_. *o*-Mephenytoin, 4'-hydroxy-S-mephenytoin, bufuralol hydrochloride, 1'-hydroxybufuralol, 1'-hydroxymidazolam, 6α-hydroxypaclitaxel, 4'-hydroxydiclofenac, 7-hydroxycoumarin and paclitaxel were purchased from \_\_\_\_\_. Diclofenac, midazolam, testosterone, 6β-hydroxytestosterone, coumarin, α-naphthoflavone, quercetin, sulfaphenazole, tranilcypromine, quinidine and ketoconazole were purchased from \_\_\_\_\_. HPLC grade water, methanol, and acetonitrile were obtained from \_\_\_\_\_. All other chemicals were reagent grade or better and were purchased from \_\_\_\_\_ unless indicated otherwise.

Liver microsomes consisted of a pool of human liver microsomes from 50 donors (30 males, ages 6 to 77 and 20 females, ages 30 to 78, medical histories available) and were purchased from \_\_\_\_\_. Catalytic activities of various cytochrome P450 enzymes, including CYPs tested in this study, were provided by the vendor.

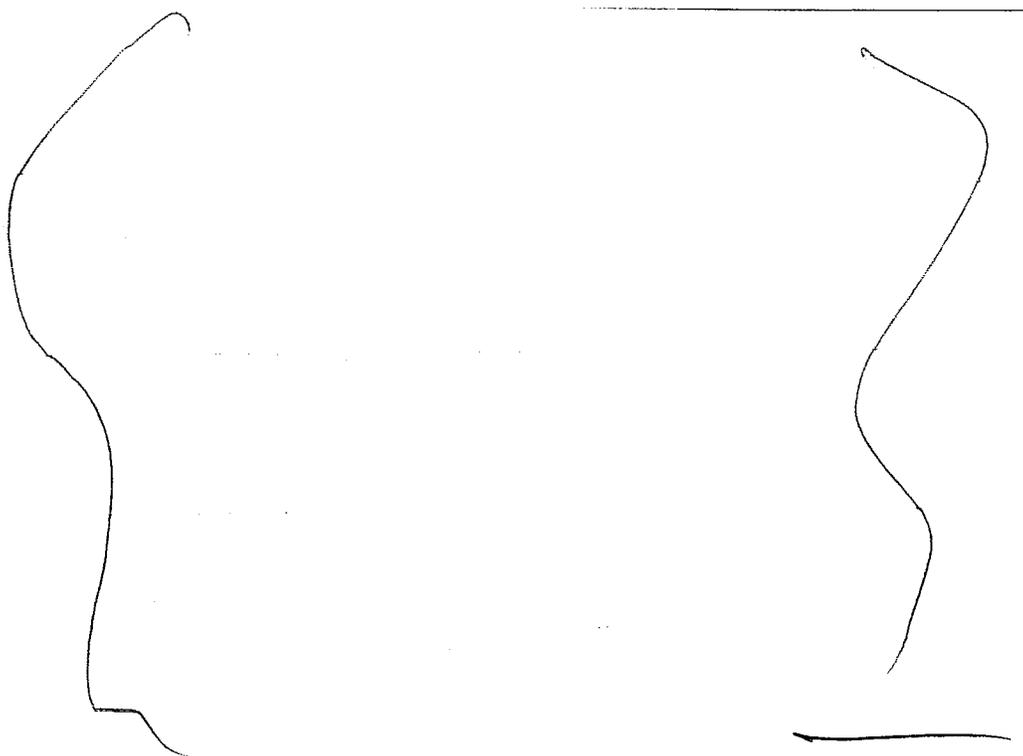


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X § 552(b)(4) Trade Secret / Confidential

       § 552(b)(4) Draft Labeling

       § 552(b)(5) Deliberative Process



**Data Calculations:** For the determination of IC<sub>50</sub> values, calibration curves of resorufin (0.0004 to 0.2 μM), 7-hydroxycoumarin (0.005 to 2.5 μM), 6α-hydroxypaclitaxel (0.002 to 1 μM), 4'-hydroxydiclofenac (0.01 to 5 μM), 4'-hydroxy-*S*-mephenytoin (0.001 to 0.5 μM), 1'-hydroxybufuralol (0.002 to 1 μM), 1'-hydroxymidazolam (0.005 to 2.5 μM) and 6β-hydroxytestosterone (0.025 μM to 10 μM) were used for quantitation. Standard solutions were extracted as previously described for sample solutions. Concentrations of all metabolites were determined by extrapolating the response calculated from the peak area ratio to the peak area of internal standard to that of the standard curve. Values for sample wells containing no DVS-233 or comparators were averaged and these averages were used as the control values (ie, 100% enzyme activity). Values for samples containing DVS-233 or comparators were expressed as a percentage of this 100% activity value. IC<sub>50</sub> values were calculated from plots generated from the inhibitory effect E<sub>max</sub> model (model 103) utilizing WinNonlin Professional, version 4.1, with the exception of testosterone-6β-hydroxylation, where the  $\frac{v}{v_0} = \frac{v_{max}}{v_{max} + K_m(1 + \frac{[I]}{K_i})}$  equation was used.

For the determination of K<sub>i</sub> values, 8-point calibration curves of 1'-hydroxy bufuralol (0.0005 to 1 μM), 4'-hydroxy *S*-mephenytoin (0.001 to 1 μM), 1'-hydroxy midazolam (0.001 to 2 μM) and 6β-hydroxy testosterone (0.01 to 10 μM) were used for quantitation. The apparent K<sub>m</sub> values and kinetics of inhibition were determined by non-linear regression plots using simple E<sub>max</sub> model (model 101) utilizing WinNonlin Professional, version 4.1, Lineweaver-Burk plots and Eadie-Hofstee plots were used with K<sub>i</sub> values determined by  $\frac{v}{v_0}$  plots.

**Results:** The IC<sub>50</sub> values of DVS-233 and comparators on the catalytic activity of different CYP enzymes in human liver microsomes are summarized in the following table. DVS-233 did not inhibit CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6β-hydroxylation activity at the highest concentration tested (100

$\mu\text{M}$ ) and weakly inhibited CYP1A2 activity (extrapolated  $\text{IC}_{50}$  value of  $130 \mu\text{M}$ ). Venlafaxine did not inhibit CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6 $\beta$ -hydroxylation activity at the highest concentration tested ( $100 \mu\text{M}$ ), and inhibited CYP2D6 activity ( $\text{IC}_{50}$  value of  $69 \mu\text{M}$ ). S, S Duloxetine did not inhibit CYP1A2 and CYP2C9 activity at the highest concentration tested ( $100 \mu\text{M}$ ), weakly inhibited CYP2A6 and CYP2C8 activity (extrapolated  $\text{IC}_{50}$  values of  $270$  and  $180 \mu\text{M}$ , respectively), and inhibited CYP2C19, CYP2D6 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6 $\beta$ -hydroxylation activity ( $\text{IC}_{50}$  values of  $49$ ,  $6.0$ ,  $89$  and  $54 \mu\text{M}$ , respectively). Paroxetine did not inhibit CYP2C8 activity at the highest concentration tested ( $100 \mu\text{M}$ ), weakly inhibited CYP2A6 activity (extrapolated  $\text{IC}_{50}$  value of  $210 \mu\text{M}$ ), and inhibited CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6 $\beta$ -hydroxylation activity ( $\text{IC}_{50}$  values of  $80$ ,  $63$ ,  $70$ ,  $2.0$ ,  $32$  and  $59 \mu\text{M}$ , respectively). Sertraline weakly inhibited CYP2C8 and CYP2C9 activity (extrapolated  $\text{IC}_{50}$  values of  $350$  and  $120 \mu\text{M}$ , respectively) and inhibited CYP1A2, CYP2A6, CYP2C19, CYP2D6 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6 $\beta$ -hydroxylation activity ( $\text{IC}_{50}$  values of  $61$ ,  $51$ ,  $27$ ,  $5.0$ ,  $26$  and  $41 \mu\text{M}$ , respectively). Bupropion did not inhibit CYP2A6, CYP2C8 and CYP2C9 activity at the highest concentration tested ( $100 \mu\text{M}$ ), weakly inhibited CYP1A2 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6 $\beta$ -hydroxylation activity (extrapolated  $\text{IC}_{50}$  values of  $160$ ,  $120$  and  $270 \mu\text{M}$ , respectively) and inhibited CYP2C19 and CYP2D6 activity ( $\text{IC}_{50}$  values of  $43$  and  $28 \mu\text{M}$ , respectively).  $\alpha$ -naphthoflavone, tranilcypromine ( $10 \mu\text{M}$ ), quercetin, sulfaphenazole, tranilcypromine ( $50 \mu\text{M}$ ), quinidine and ketoconazole inhibited CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A activity, respectively.

Cytochrome P450 Probe Substrates Used in the Cocktail Method for the Determination of  $\text{IC}_{50}$  Values

Human CYP450 Isozyme	Probe Substrate	Substrate Concentration ( $\mu\text{M}$ ) <sup>a</sup>	Metabolite Monitored
CYP1A2	Ethoxyresorufin	1	Resorufin
CYP2A6	Coumarin	2.5	7-Hydroxycoumarin
CYP2C8	Paclitaxel	10	6 $\alpha$ -Hydroxypaclitaxel
CYP2C9	Diclofenac	10	4'-Hydroxydiclofenac
CYP2C19	S-mephenytoin	40	4'-Hydroxy-S-mephenytoin
CYP2D6	Bufuralol	5	1'-Hydroxybufuralol
CYP3A	Midazolam	2.5	1'-Hydroxymidazolam
CYP3A <sup>b</sup>	Testosterone	50	6 $\beta$ -Hydroxytestosterone

a. Approximate  $K_m$  value

b. CYP3A activity was determined separately using testosterone as an additional probe substrate.

Estimated IC<sub>50</sub> Values (μM) for the inhibition of CYP Enzymes by DVS-233 and Comparators in Human Liver Microsomes

P450	DVS-233	Venlafaxine	S,S Duloxetine	Paroxetine	Sertraline	Bupropion
CYP1A2	130 ± 88	NC	NC	80 ± 10	61 ± 7	160 ± 29
CYP2A6	NC	NC	270 ± 78	210 ± 29	51 ± 2	NC
CYP2C19	NC	NC	49 ± 8	70 ± 14	27 ± 3	43 ± 9
CYP2C8	NC	NC	180 ± 91	NC	350 ± 150	NC
CYP2C9	NC	NC	NC	63 ± 5	120 ± 19	NC
CYP2D6	NC	69 ± 3	6.0 ± 0.6	2.0 ± 1	5.0 ± 0.6	28 ± 5
CYP3A (mdz)	NC	NC	89 ± 13	32 ± 9	26 ± 3	120 ± 22
CYP3A (test)	NC	NC	54 ± 4	59 ± 14	41 ± 7	270 ± 90

Values are mean ± SD of three separate determinations each performed in triplicate

NC. Not calculated due to lack of inhibition at 100 μM

mdz. Midazolam

test. Testosterone

Effect of Isoform Selective Inhibitors on the Catalytic Activities of Different Cytochrome P450 Enzymes in Human Liver Microsomes

CYP Enzyme	Inhibitor	Conc (μM)	% Control
1A2	α-Naphthoflavone	20	20 ± 2.8
2A6	Tranlycypromine	10	2.5 ± 0.5
2C8	Quercetin	20	20 ± 6.0
2C9	Sulfaphenazole	10	13 ± 1.4
2C19	Tranlycypromine	50	12 ± 1.7
2D6	Quinidine	10	5.3 ± 1.0
3A	Ketoconazole	1	2.0 ± 1.5

Values are mean ± SD of six separate determinations each performed in triplicate

K<sub>i</sub> values for the inhibition of CYP2D6-mediated bufuralol-1'-hydroxylation were > 300 μM for DVS-233, 93 μM for venlafaxine, 4.5 μM for S, S duloxetine, 3.8 μM for paroxetine, 4.5 μM for sertraline and 28 μM for bupropion. The K<sub>m</sub> and V<sub>max</sub> values for CYP2D6-mediated bufuralol-1'-hydroxylation were 20 ± 3.6 μM and 97.4 ± 19.7 pmol min<sup>-1</sup> mg<sup>-1</sup>, respectively (mean ± SD, n = 12).

K<sub>i</sub> Values and Mode of Inhibition for the Inhibition of CYP Enzymes by DVS-233 and Comparators in Human Microsomes

	CYP2D6	CYP2C19	CYP3A <sup>a</sup>	CYP3A <sup>b</sup>
DVS-233	> 300	ND	ND	ND
Venlafaxine	93 (competitive)	ND	ND	ND
S, S Duloxetine	4.5 (competitive)	24 (competitive)	28 (mixed)	26 <sup>c</sup>
Paroxetine	4.5 (competitive)	78 (mixed)	25 (non-competitive)	46 <sup>c</sup>
Sertraline	3.8 (competitive)	28 (mixed)	43 (non-competitive)	23 <sup>c</sup>
Bupropion	28 (competitive)	13 (competitive)	ND	ND

Values are mean of two separate determinations each performed in duplicate

ND. Not determined due to minimal or no inhibition in IC<sub>50</sub> studies

a. Midazolam-1'-hydroxylation

b. Testosterone-6β-hydroxylation

c. K<sub>i</sub> was only an estimate due to autoactivation (homotropic cooperativity) kinetics for testosterone 6β-hydroxylation. Mode of inhibition was not determined.

DVS showed virtually no inhibition of CYP2D6 activity at concentrations up to 300 μM. The K<sub>i</sub> value could not be accurately determined due to the lack of inhibition, and was considered > 300 μM. Venlafaxine inhibited CYP2D6 activity in a concentration dependent manner. S, S Duloxetine, Paroxetine, Sertraline and bupropion inhibited CYP2D6 activity in a concentration dependent manner. The K<sub>i</sub> values are provided in the table above.

K<sub>i</sub> values for the inhibition of CYP2C19-mediated *S*-mephenytoin-4'-hydroxylation were 24 μM for S, S duloxetine, 78 μM for paroxetine, 28 μM for sertraline and 13 μM for bupropion. DVS-233 and venlafaxine were not evaluated due to their lack of inhibition of CYP2C19 activity at the highest concentration used (100 μM) in the IC<sub>50</sub> studies. The K<sub>m</sub> and V<sub>max</sub> values for CYP2C19-mediated *S*-mephenytoin-4'-hydroxylation were 40 ± 6.0 μM and 50.0 ± 5.18 pmol min<sup>-1</sup> mg<sup>-1</sup>, respectively (mean ± SD, n = 8).

K<sub>i</sub> values for the inhibition of CYP3A-mediated midazolam-1'-hydroxylation were 28 μM for S, S duloxetine, 25 μM for paroxetine and 43 μM for sertraline. DVS-233, venlafaxine and bupropion were not evaluated due to their lack of inhibition at the highest concentration used (100 μM) or an IC<sub>50</sub> value > 100 μM. The K<sub>m</sub> and V<sub>max</sub> values for CYP3A-mediated midazolam-1'-hydroxylation were 4.9 ± 2.0 μM and 1580 ± 135 pmol min<sup>-1</sup> mg<sup>-1</sup>, respectively (mean ± SD, n = 6).

**Summary:** DVS-233 showed no inhibition of any of the CYP enzymes studied at the highest drug concentration evaluated (100 μM) with the exception of CYP1A2 (IC<sub>50</sub> = 130 μM). Venlafaxine showed minimal inhibition of CYP2D6 (K<sub>i</sub> = 93 μM) and no inhibition of the other CYP enzymes studied. In contrast, S, S duloxetine showed inhibition of CYP2D6 (K<sub>i</sub> = 4.5 μM), 2C19 (K<sub>i</sub> = 24 μM), 3A (K<sub>i</sub> = 28 μM for midazolam-1'-hydroxylation and 26 μM for testosterone-6β-hydroxylation), 2A6 (IC<sub>50</sub> = 270 μM) and 2C8 (IC<sub>50</sub> = 180 μM), and no inhibition of CYP1A2 or 2C9 activity at the highest drug concentration used (100 μM).

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*Reviewer's comments: In vitro studies indicate that DVS is not an inhibitor of the CYP P450 isozymes except a possible inhibition of CYP1A2.*

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Attachment

Km and Vmax Values for the Formation of Probe Substrate Metabolites of CYP Enzymes tested in Ki studies Using Human Liver Microsomes

Human CYP450 Isozyme	Probe Substrate	Metabolite Monitored	K <sub>m</sub> (μM)	V <sub>max</sub> (pmol min <sup>-1</sup> mg <sup>-1</sup> )
CYP2C19	<i>S</i> -mephenytoin	4'-Hydroxy- <i>S</i> -mephenytoin	40 ± 6.0 <sup>a</sup>	50.0 ± 5.18 <sup>a</sup>
CYP2D6	Bufuralol	1'-Hydroxybufuralol	20 ± 3.6 <sup>b</sup>	97.4 ± 19.7 <sup>b</sup>
CYP3A	Midazolam	1'-Hydroxymidazolam	4.9 ± 2.0 <sup>c</sup>	1580 ± 135 <sup>c</sup>
CYP3A	Testosterone	6β-Hydroxytestosterone	67 ± 25 <sup>c,d</sup>	2770 ± 456 <sup>c</sup>

- Values are mean ± SD of eight separate determinations each performed in duplicate
- Values are mean ± SD of twelve separate determinations each performed in duplicate
- Values are mean ± SD of six separate determinations each performed in duplicate
- Represents S<sub>50</sub> value, gamma or n = 1.23 ± 0.09

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Figure 2. Representative Direct and Lineweaver-Burk Plots of the Inhibition of CYP2D6 Activity (Bufuralol-1'-Hydroxylation) by DVS-233 in Human Liver Microsomes

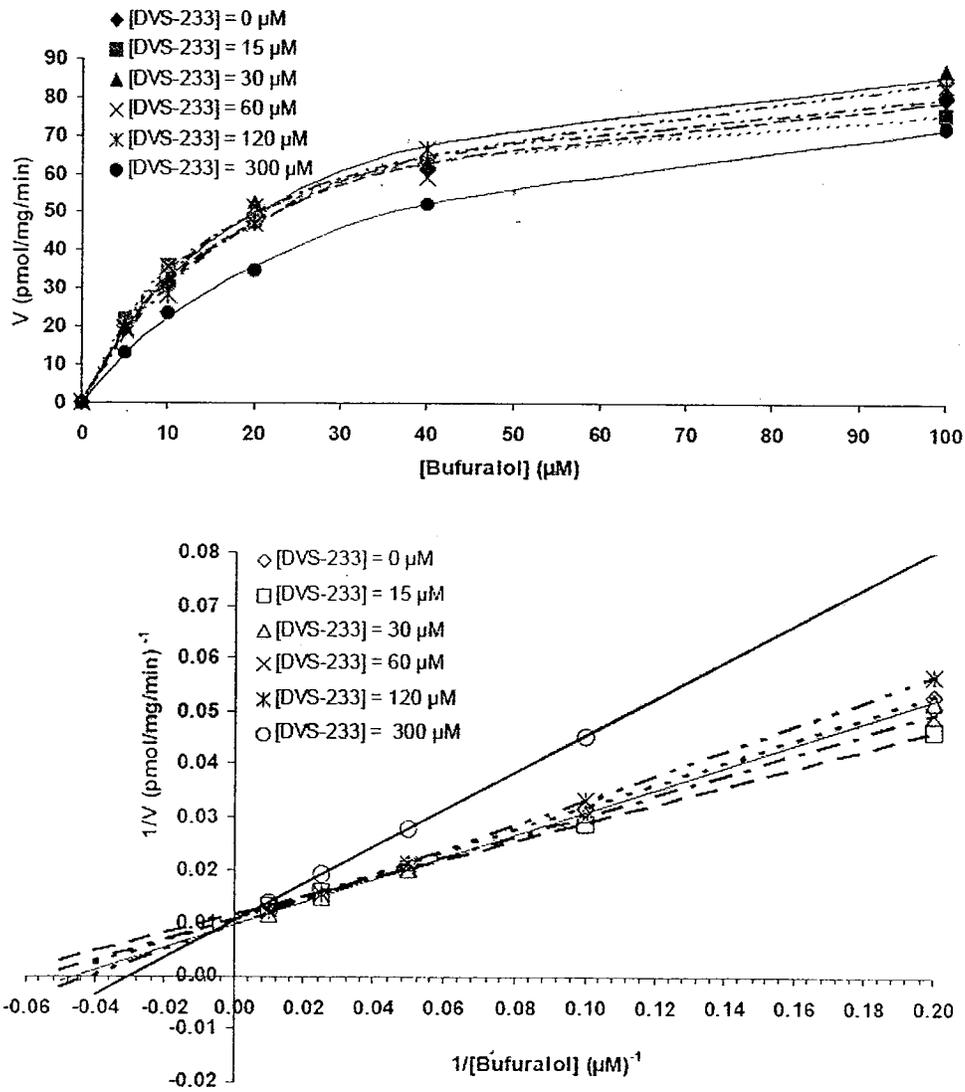
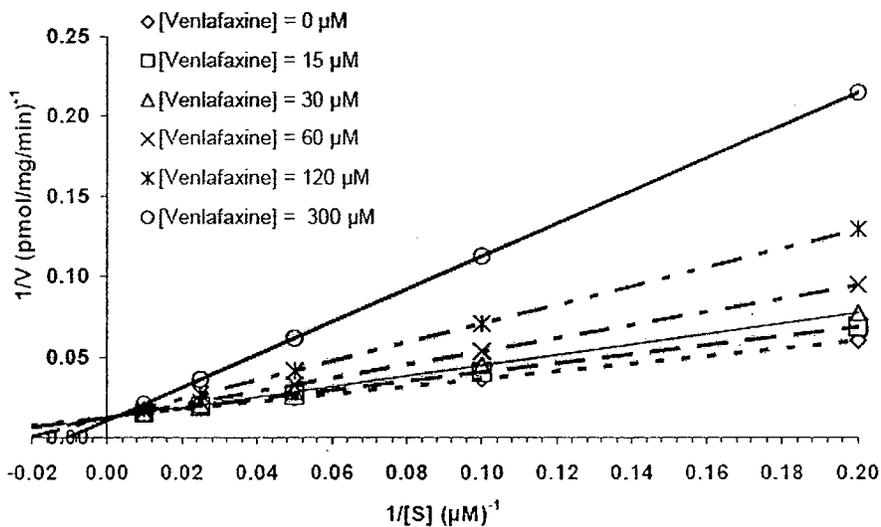
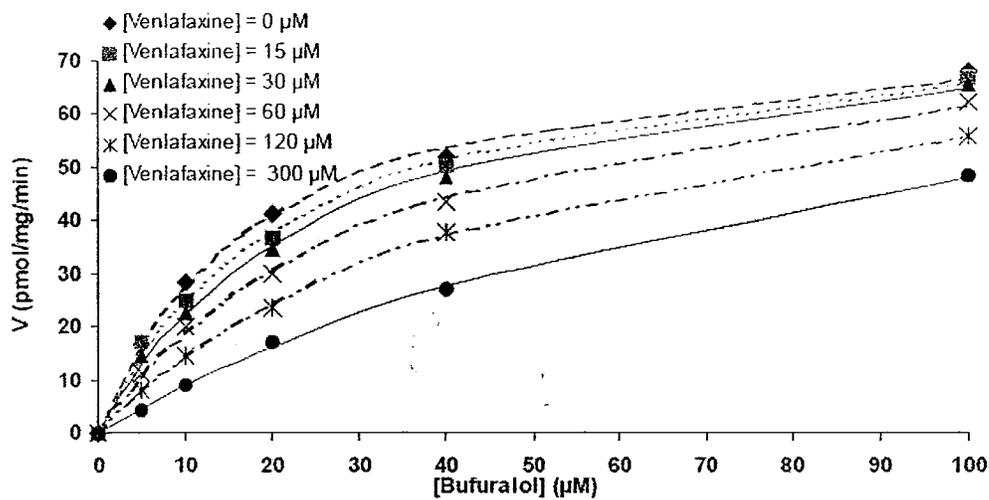


Figure 3. Representative Direct and Lineweaver-Burk Plots of the Inhibition of CYP2D6 Activity (Bufuralol-1'-Hydroxylation) by Venlafaxine in Human Liver Microsomes



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**Title (RPT 59746):** DVS-233: Inhibition of P-glycoprotein Activity in Caco-2 Monolayers: Comparator Study (Protocol 04\_2291).

**Objective:** To determine IC<sub>50</sub> values for the inhibition of P-glycoprotein (P-gp) activity by DVS-233 and six other antidepressants, venlafaxine (Effexor), S, S duloxetine (Cymbalta), paroxetine (paxil), sertraline (Zoloft), bupropion (Wellbutrin) and amitriptyline in Caco-2 monolayers.

**Materials and Methods:** DVS-233 succinate monohydrate (WY-45233), Lot RB1626, purity \_\_\_\_\_, was synthesized by Wyeth Research. Venlafaxine hydrochloride (WY-45030), Lot P4656-256-4, purity \_\_\_\_\_, S, S-duloxetine (WAY-209473-A-2), Lot P6979-128-AR, purity \_\_\_\_\_, paroxetine hydrochloride (WAY-209472-A-1), Lot P6839-167-4, purity \_\_\_\_\_, were synthesized by Wyeth Research. Sertraline hydrochloride, CAS# 79559-97-0, purity \_\_\_\_\_ and bupropion hydrochloride, CAS# 31677-93-7, purity \_\_\_\_\_ were purchased from \_\_\_\_\_. Amitriptyline hydrochloride, Lot 033K1077, purity \_\_\_\_\_ was purchased from \_\_\_\_\_. Verapamil and digoxin were purchased from \_\_\_\_\_. [3H]-Digoxin (37 ci/mmol) was purchased from \_\_\_\_\_. All cell culture media supplies were purchased from \_\_\_\_\_. The \_\_\_\_\_ transwell plates for growing the cell cultures and \_\_\_\_\_ for coating insert membranes were received from \_\_\_\_\_.

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**Analytical Method:** To an aliquot of medium from the apical (100  $\mu$ L) or basolateral (250-300  $\mu$ L) compartment \_\_\_\_\_ was added (5 mL) and mixtures were counted for 10 min or until %  $\sigma$  of 2 was reached to determine radioactivity using a \_\_\_\_\_ Model \_\_\_\_\_ liquid scintillation counter

**Data Analysis:** The transport of digoxin across Caco-2 monolayers was determined by the amount of drug permeated (pmol), the rate of permeation (pmol/sec) and the apparent permeability coefficient ( $P_{app}$ ).  $P_{app}$  (cm/sec) was calculated by  $P_{app} = (dQ/dt) \times 1 / (A \times C_0)$ , where  $dQ/dt$  is the rate of drug appearance in the receiver compartment ( $\mu$ mole/sec),  $C_0$  is the initial drug concentration in the donor compartment ( $\mu$ M), and  $A$  is the surface area of the monolayer ( $cm^2$ ). These values (mean  $\pm$  SD of  $n=3$ ) were calculated in both A $\rightarrow$ B (apical to basolateral) and B $\rightarrow$ A (basolateral to apical) directions, and in the absence or presence of putative inhibitors. Mean and standard deviation values and Student's t-test for significance ( $p < 0.05$ ) were calculated. Degree of inhibition was calculated using the following relationship: % Inhibition =  $(1 - (i_{B \rightarrow A} - i_{A \rightarrow B}) / (a_{B \rightarrow A} - a_{A \rightarrow B})) \times 100\%$ , where  $i$  and  $a$  are the flux of digoxin in the presence and absence of the putative inhibitor, respectively. Values for inserts containing no DVS-233 or comparators were averaged and these averages were used as the control values (i.e., 100% P-gp activity). Percent inhibition values for samples containing DVS-233 or comparators were expressed as a percentage of the control activity.  $IC_{50}$  values were calculated from plots generated from the inhibitory effect  $E_{max}$  model.

Results: Inhibition of P-gp activity in the presence of increasing concentrations of DVS-233, venlafaxine, S,S-duloxetine, paroxetine, bupropion, sertraline, amitriptyline and verapamil is shown in the following tables. Corresponding  $IC_{50}$  values and representative plots are summarized in are provided in the following tables and figures. DVS-233 and venlafaxine showed minimal inhibition (less than 20% control activity) of P-gp-mediated

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digoxin efflux at the highest concentration used (250  $\mu\text{M}$ ), and  $\text{IC}_{50}$  values could not be defined due to lack of concentration dependency. The  $\text{IC}_{50}$  values for the inhibition of P-gp-mediated digoxin efflux by amitriptyline was 129  $\mu\text{M}$ , and by S,S-duloxetine, paroxetine, sertraline, bupropion were extrapolated to be greater than 250  $\mu\text{M}$  (highest concentration tested). The corresponding % inhibition at a putative inhibitor concentration of 250  $\mu\text{M}$  were approximately 70, 40, 47, 45 and 36%, respectively. Verapamil, a known P-gp inhibitor, had an  $\text{IC}_{50}$  value of  $12.2 \pm 1.5$   $\mu\text{M}$  under the same conditions used and showed 90% inhibition at an inhibitor concentration of 250  $\mu\text{M}$ .

**Summary:** With the exception of verapamil which is a relatively potent inhibitor of P-gp activity ( $\text{IC}_{50}$  value of 12.2  $\mu\text{M}$ ), all the other drugs evaluated in this study showed low inhibition of P-gp activity (extrapolated  $\text{IC}_{50}$  values of  $>100$   $\mu\text{M}$  or not definable). Using a fluorimetric assay with calcein-acetoxymethylester as a P-gp substrate and two different cell systems (L-MDR1 cells and primary porcine brain endothelial cells), verapamil ( $\text{IC}_{50}$  values of approximately 2-19  $\mu\text{M}$ ), paroxetine and sertraline ( $\text{IC}_{50}$  values not definable to approximately 30  $\mu\text{M}$ ) showed higher inhibition of P-gp activity than O-desmethyl-venlafaxine and venlafaxine ( $\text{IC}_{50}$  values not definable). The sponsor stated that the relatively high  $\text{IC}_{50}$  values for the inhibition of P-gp activity by the antidepressants evaluated in this study suggest a low potential of clinical drug-drug interactions for these drugs via inhibition of P-gp activity. Additionally, due to the lowest in vitro inhibition of P-gp activity, DVS-233 and venlafaxine may have the least likelihood to engage in clinical drug-drug interactions via inhibition of P-gp.

*Reviewer's comments: DVS does not appear to be an inhibitor of Pgp. The reviewer agrees with the sponsor's conclusion that DVS may have the least likelihood to engage in clinical drug-drug interaction via inhibition of P-gp.*

**APPEARS THIS WAY  
ON ORIGINAL**

Table 1. Inhibition of P-gp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of DVS-233 and Venlafaxine

DVS-233 ( $\mu\text{M}$ )	$P_{\text{app}}$ (A to B), $\times 10^{-6}$ cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A), $\times 10^{-6}$ cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A)/ $P_{\text{app}}$ (A to B) Ratio	% Control
0	0.290 $\pm$ 0.014	8.39 $\pm$ 0.78	28.9 $\pm$ 1.5	100
1	0.287 $\pm$ 0.048	7.73 $\pm$ 0.50	27.3 $\pm$ 2.7	92.1 $\pm$ 4.8
2.5	0.264 $\pm$ 0.011	7.69 $\pm$ 0.26	29.1 $\pm$ 2.1	92.2 $\pm$ 10
10	0.272 $\pm$ 0.013	7.49 $\pm$ 0.26	27.6 $\pm$ 1.9	89.6 $\pm$ 7.1
25	0.273 $\pm$ 0.009	7.49 $\pm$ 0.21	27.5 $\pm$ 1.4	89.5 $\pm$ 7.9
50	0.264 $\pm$ 0.006	7.16 $\pm$ 0.91	27.2 $\pm$ 3.9	85.5 $\pm$ 13
100	0.288 $\pm$ 0.032	8.00 $\pm$ 0.66	28.1 $\pm$ 4.7	96.3 $\pm$ 18
250	0.276 $\pm$ 0.013	7.55 $\pm$ 0.39	27.4 $\pm$ 0.9	90.1 $\pm$ 5.7

Venlafaxine ( $\mu\text{M}$ )	$P_{\text{app}}$ (A to B), $\times 10^{-6}$ cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A), $\times 10^{-6}$ cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A)/ $P_{\text{app}}$ (A to B) Ratio	% Control
0	0.218 $\pm$ 0.069	7.78 $\pm$ 1.76	36.5 $\pm$ 4.2	100
1	0.213 $\pm$ 0.068	7.65 $\pm$ 2.30	36.5 $\pm$ 5.7	97.5 $\pm$ 12
2.5	0.214 $\pm$ 0.064	7.32 $\pm$ 1.74	34.8 $\pm$ 2.7	93.8 $\pm$ 5.3
10	0.218 $\pm$ 0.071	6.62 $\pm$ 1.62	31.1 $\pm$ 3.2	84.3 $\pm$ 2.5
25	0.216 $\pm$ 0.069	7.15 $\pm$ 1.78	33.8 $\pm$ 3.6	91.4 $\pm$ 2.8
50	0.321 $\pm$ 0.201	6.30 $\pm$ 1.31	23.6 $\pm$ 9.8	79.9 $\pm$ 7.7
100	0.255 $\pm$ 0.041	6.84 $\pm$ 1.28	26.8 $\pm$ 1.9	87.6 $\pm$ 4.5
250	0.293 $\pm$ 0.072	6.51 $\pm$ 1.05	22.6 $\pm$ 2.4	83.2 $\pm$ 6.9

Results are mean  $\pm$  SD of three determinations each conducted on a separate day

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Table 2. Inhibition of P-gp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of S,S-Duloxetine and Paroxetine

S,S-Duloxetine ( $\mu\text{M}$ )	$P_{\text{app}}$ (A to B), x 10 <sup>-6</sup> cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A), x 10 <sup>-6</sup> cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A)/ $P_{\text{app}}$ (A to B) Ratio	% Control
0	0.218 $\pm$ 0.069	7.78 $\pm$ 1.76	36.5 $\pm$ 4.2	100
1	0.213 $\pm$ 0.071	7.23 $\pm$ 1.64	35.0 $\pm$ 4.9	92.9 $\pm$ 0.8
2.5	0.216 $\pm$ 0.077	6.90 $\pm$ 1.45	33.5 $\pm$ 6.5	88.7 $\pm$ 2.1
10	0.221 $\pm$ 0.071	6.87 $\pm$ 1.64	31.9 $\pm$ 3.7	87.8 $\pm$ 4.0
25	0.257 $\pm$ 0.078	6.35 $\pm$ 1.28	25.3 $\pm$ 3.2	81.0 $\pm$ 2.7
50	0.319 $\pm$ 0.115	6.06 $\pm$ 1.41	19.8 $\pm$ 3.1	76.0 $\pm$ 3.0
100	0.398 $\pm$ 0.116	5.33 $\pm$ 1.17	13.7 $\pm$ 1.6	65.5 $\pm$ 5.1
250	1.49 $\pm$ 0.415	6.05 $\pm$ 1.41	4.1 $\pm$ 0.2	60.3 $\pm$ 1.9

Paroxetine ( $\mu\text{M}$ )	$P_{\text{app}}$ (A to B), x 10 <sup>-6</sup> cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A), x 10 <sup>-6</sup> cm/sec (Mean $\pm$ SD)	$P_{\text{app}}$ (B to A)/ $P_{\text{app}}$ (A to B) Ratio	% Control
0	0.320 $\pm$ 0.017	8.65 $\pm$ 0.08	27.1 $\pm$ 1.4	100
1	0.291 $\pm$ 0.008	7.59 $\pm$ 0.41	26.1 $\pm$ 2.1	87.6 $\pm$ 4.6
2.5	0.295 $\pm$ 0.007	7.30 $\pm$ 0.13	24.7 $\pm$ 1.0	84.0 $\pm$ 0.9
10	0.321 $\pm$ 0.023	7.13 $\pm$ 0.32	22.3 $\pm$ 2.6	81.7 $\pm$ 3.8
25	0.398 $\pm$ 0.002	6.78 $\pm$ 0.31	17.0 $\pm$ 0.8	76.6 $\pm$ 3.2
50	0.477 $\pm$ 0.018	6.39 $\pm$ 0.47	13.4 $\pm$ 1.4	71.0 $\pm$ 5.9
100	0.745 $\pm$ 0.027	5.48 $\pm$ 0.36	7.4 $\pm$ 0.7	56.9 $\pm$ 4.8
250	2.63 $\pm$ 0.332	7.08 $\pm$ 0.06	2.7 $\pm$ 0.3	53.3 $\pm$ 3.8

Results are mean  $\pm$  SD of three determinations each conducted on a separate day

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Table 3. Inhibition of P-gp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of Sertraline and Bupropion

Sertraline (uM)	$P_{app}$ (A to B), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}$ (B to A), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}$ (B to A)/ $P_{app}$ (A to B) Ratio	% Control
0	0.357 ± 0.020	8.64 ± 0.31	24.3 ± 2.2	100
1	0.334 ± 0.097	7.62 ± 0.13	22.9 ± 1.0	88.1 ± 2.2
2.5	0.381 ± 0.076	7.48 ± 0.36	20.2 ± 4.3	85.7 ± 3.5
10	0.372 ± 0.061	7.53 ± 0.13	20.6 ± 3.4	86.4 ± 1.9
25	0.381 ± 0.043	6.77 ± 0.18	17.9 ± 1.9	77.2 ± 2.8
50	0.448 ± 0.042	6.83 ± 0.37	15.3 ± 1.1	77.1 ± 3.3
100	0.582 ± 0.071	5.80 ± 0.18	10.0 ± 0.9	63.1 ± 3.7
250	2.32 ± 1.59	6.93 ± 0.31	4.0 ± 2.3	55.4 ± 14

Bupropion (uM)	$P_{app}$ (A to B), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}$ (B to A), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}$ (B to A)/ $P_{app}$ (A to B) Ratio	% Control
0	0.320 ± 0.017	8.65 ± 0.08	27.1 ± 1.4	100
1	0.296 ± 0.018	7.37 ± 0.39	25.0 ± 2.8	86.6 ± 6.0
2.5	0.291 ± 0.019	8.07 ± 0.20	27.8 ± 2.5	90.9 ± 5.8
10	0.327 ± 0.043	7.20 ± 0.14	22.3 ± 3.6	83.4 ± 2.2
25	0.331 ± 0.057	7.63 ± 0.43	23.7 ± 5.5	85.8 ± 8.1
50	0.333 ± 0.051	6.86 ± 0.21	20.9 ± 2.8	75.8 ± 5.2
100	0.341 ± 0.034	7.04 ± 0.26	20.8 ± 2.9	72.3 ± 16
250	0.623 ± 0.108	6.36 ± 0.55	10.4 ± 1.8	63.9 ± 12

Results are mean ± SD of three determinations each conducted on a separate day

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Table 4. Inhibition of P-gp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of Amitriptyline and Verapamil

Amitriptyline (uM)	$P_{app}$ (A to B), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}$ (B to A), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}(B\ to\ A)/P_{app}(A\ to\ B)$ Ratio	% Control
0	0.357 ± 0.020	8.64 ± 0.31	24.3 ± 2.2	100
1	0.390 ± 0.003	7.62 ± 0.34	19.5 ± 0.7	91.8 ± 7.1
2.5	0.342 ± 0.030	7.98 ± 0.35	23.5 ± 2.7	92.2 ± 0.9
10	0.354 ± 0.028	6.86 ± 0.49	19.5 ± 2.2	78.6 ± 3.9
25	0.434 ± 0.037	7.23 ± 0.14	16.7 ± 1.3	82.1 ± 2.4
50	0.586 ± 0.036	5.83 ± 0.26	10.0 ± 1.0	63.3 ± 1.1
100	0.830 ± 0.106	5.44 ± 0.10	6.6 ± 0.8	55.7 ± 2.5
250	1.81 ± 0.078	4.26 ± 0.02	2.4 ± 0.1	29.6 ± 0.6

Verapamil (uM)	$P_{app}$ (A to B), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}$ (B to A), x 10 <sup>-6</sup> cm/sec (Mean ± SD)	$P_{app}(B\ to\ A)/P_{app}(A\ to\ B)$ Ratio	% Control
0	0.290 ± 0.014	8.39 ± 0.78	28.9 ± 1.5	100
1	0.331 ± 0.006	7.21 ± 0.26	21.8 ± 0.8	85.6 ± 11
2.5	0.430 ± 0.004	7.59 ± 0.33	17.6 ± 0.7	89.2 ± 12
10	0.813 ± 0.025	4.84 ± 0.13	6.0 ± 0.2	50.0 ± 5.6
25	1.25 ± 0.121	4.11 ± 0.11	3.3 ± 0.2	35.5 ± 3.0
50	1.62 ± 0.088	3.00 ± 0.11	1.9 ± 0.0	17.1 ± 2.0
100	1.94 ± 0.079	2.90 ± 0.12	1.5 ± 0.1	12.0 ± 2.5
250	2.25 ± 0.153	3.04 ± 0.41	1.4 ± 0.2	9.6 ± 6.0

Results are mean ± SD of three determinations each conducted on a separate day

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Table 5. IC<sub>50</sub> Values for the Inhibition of P-gp-Mediated Digoxin Efflux in Caco-2 Monolayers

Inhibitor	IC <sub>50</sub> (μM) (mean ± SD)
DVS-233	NC <sup>1</sup>
Venlafaxine	NC <sup>1</sup>
Duloxetine <sup>2</sup>	354 ± 55
Paroxetine <sup>2</sup>	262 ± 34
Sertraline <sup>2</sup>	346 ± 115
Bupropion <sup>2</sup>	768 ± 205
Amitriptyline	129 ± 6.6
Verapamil	12.2 ± 1.5

<sup>1</sup>NC - not calculated due to lack of concentration dependency and minimal inhibition (<20% control activity) at the highest concentration used (250 μM)

<sup>2</sup> extrapolated values

Results are mean ± SD of three determinations each conducted on a separate day.

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Figure 2. Inhibition of P-gp-Mediated Digoxin Efflux in Caco-2 Monolayers by DVS-233 and Venlafaxine

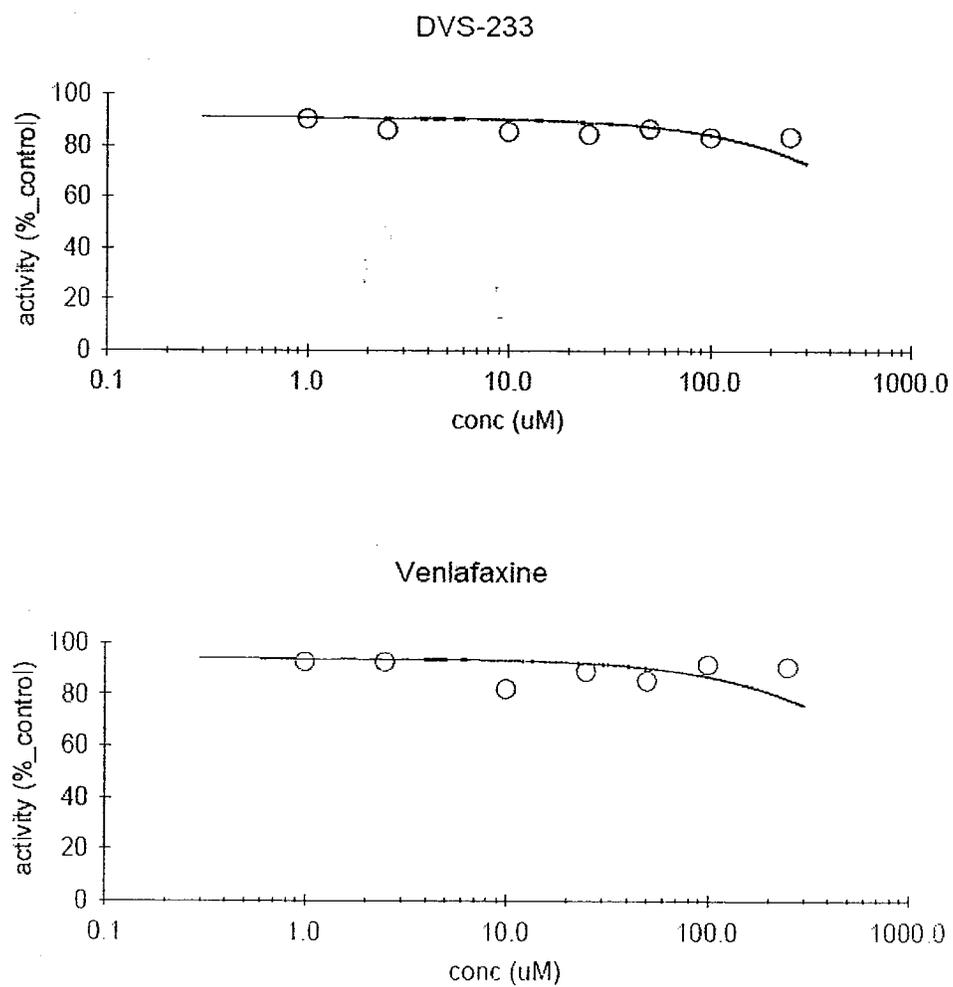


Figure 3. Inhibition of P-gp-Mediated Digoxin Efflux in Caco-2 Monolayers by S,S-Duloxetine and Paroxetine

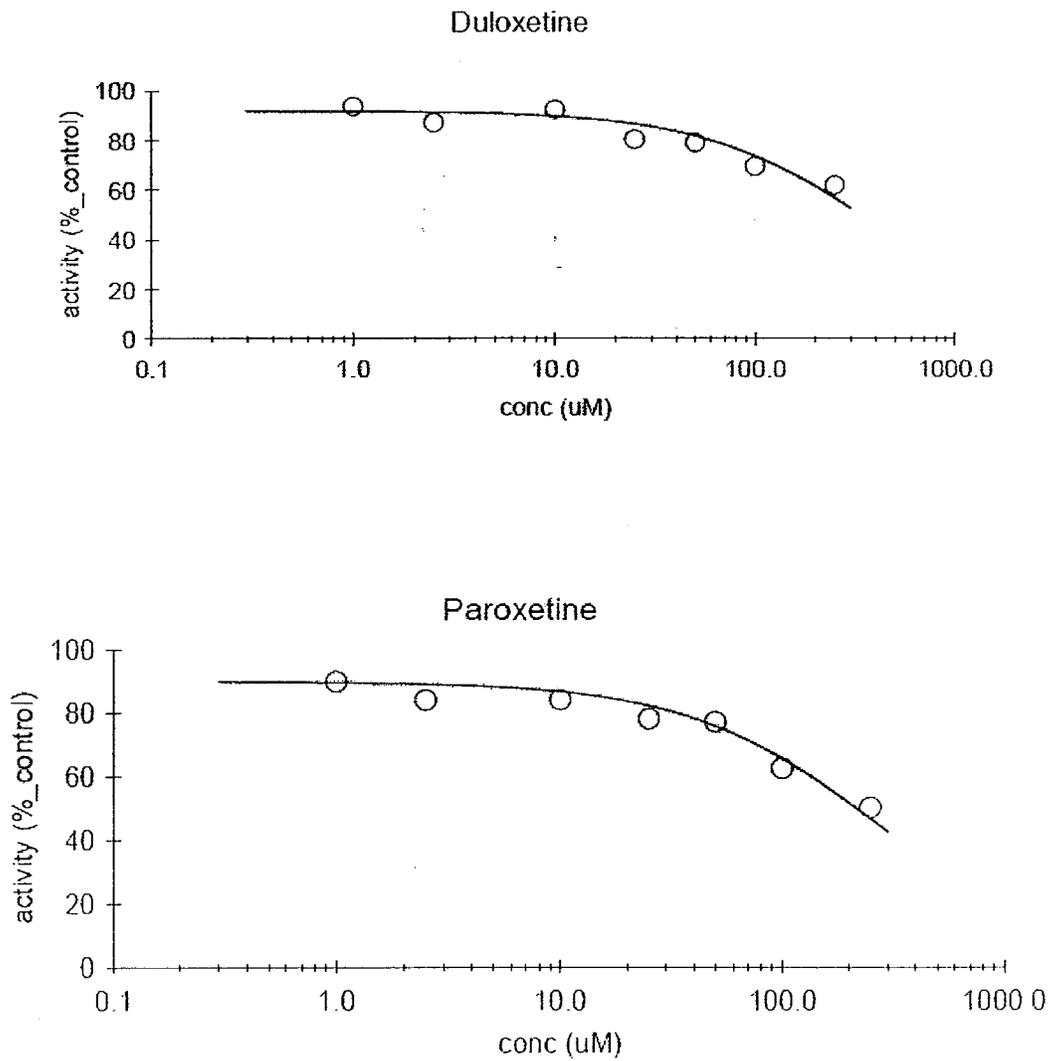


Figure 4. Inhibition of P-gp-Mediated Digoxin Efflux in Caco-2 Monolayers by Bupropion and Sertraline

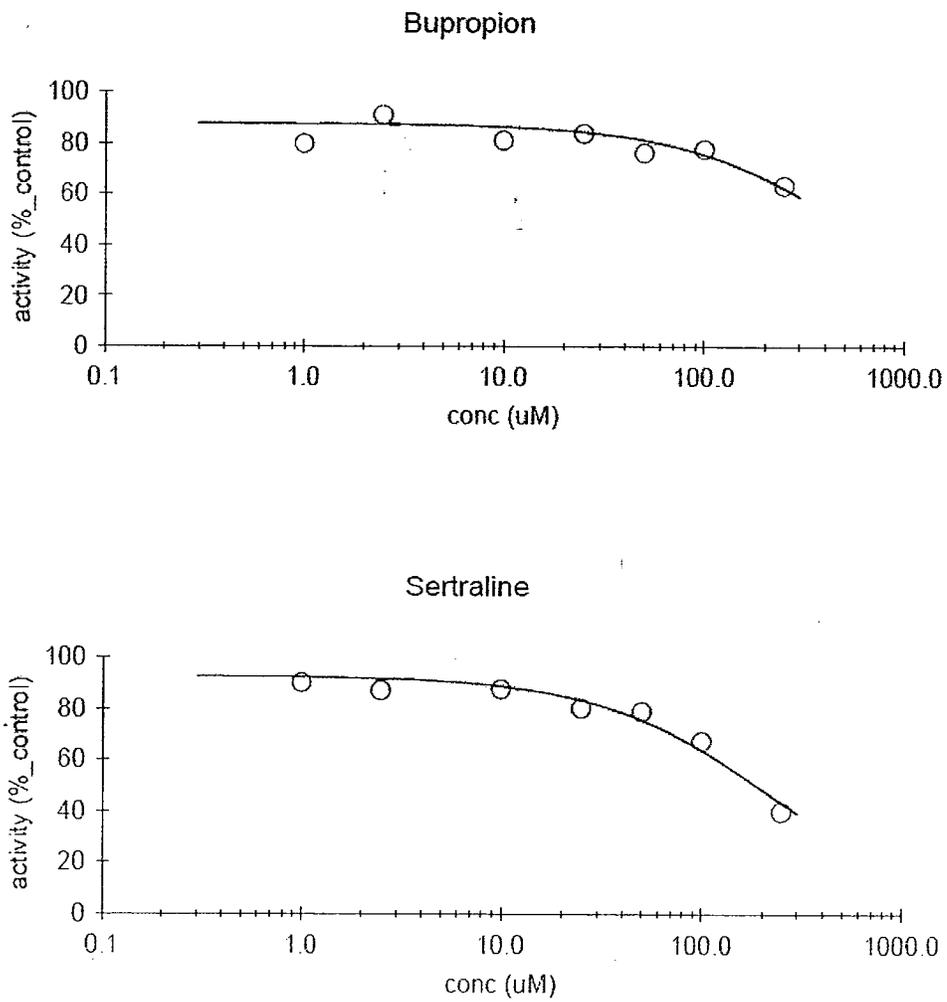
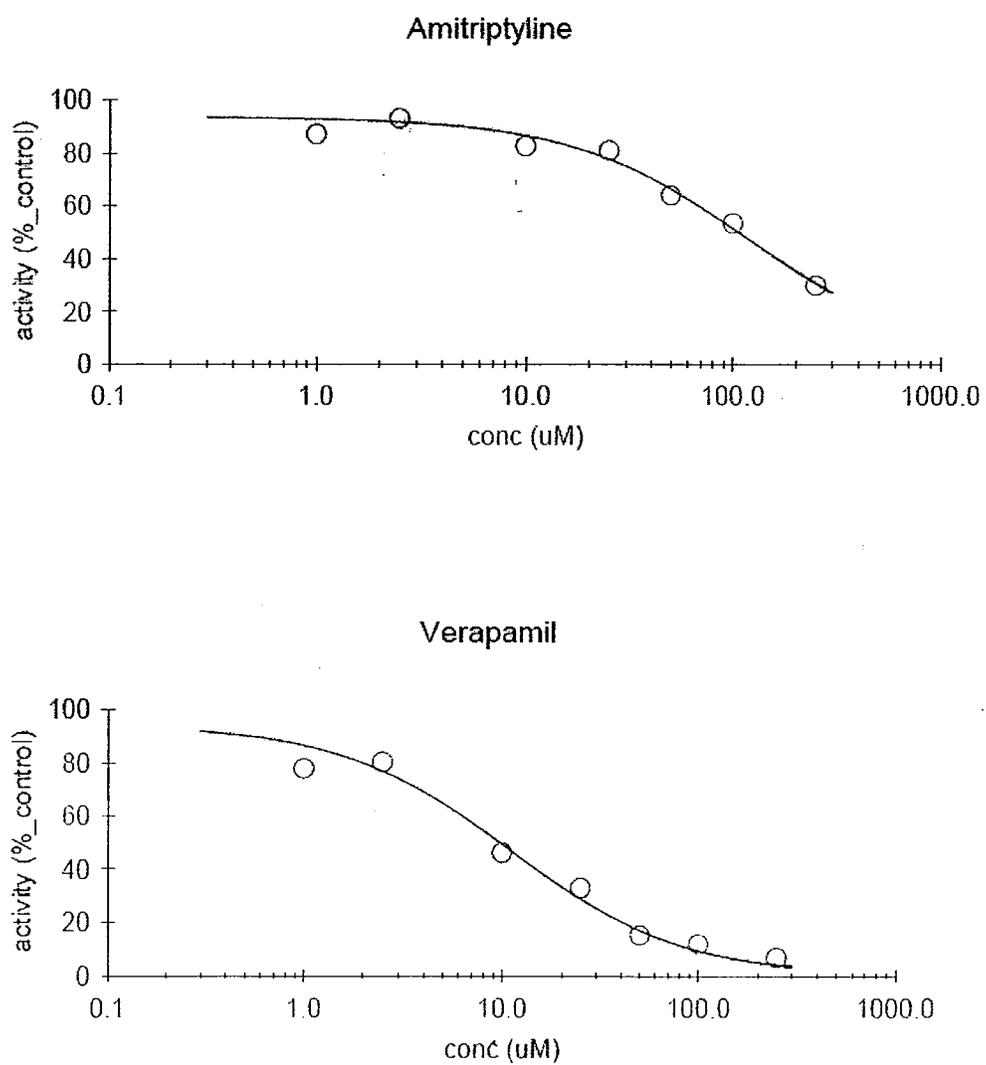


Figure 5. Inhibition of P-gp-Mediated Digoxin Efflux in Caco-2 Monolayers by Amitriptyline and Verapamil



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**Study Title:** The Disposition of <sup>14</sup>C-Labeled Venlafaxine (Wy-45,030) in Human Males Following a Single, 50 mg Oral Dose

**Background:** This study was originally submitted with the original Venlafaxine NDA. The sponsor stated that the current version contains the following corrections. The plasma to whole blood radioactivity ratio at 0.5 hr was incorrectly given as 1.1. The correct value is 1.3. This changes the 0-72 hour mean ± SD value from 1.1 ± 0.4 to 1.2 ± 0.4 and the 0 – 36 hour mean ± SD value from 1.0 ± 0.2 to 1.1 ± 0.2. Also the mean ± SD value for the Cmax of Wy-45,233 was incorrectly given as 101 ± 36 ng/ml. The sponsor reports the correct value to be 105 ± 38 ng/mL. This report describes the plasma profile of venlafaxine, Wy-45,233 and other metabolites.

**Objective:** Provide a more in depth analysis of the metabolic disposition of venlafaxine in man using a larger study population than had been in previous human study (GTR 12983).

**Study Design:** Ten healthy male subjects completed the study. Thirty minutes after a standard medium-fat breakfast, one capsule was taken by each subject with 200 mL of water. Venous blood samples was collected at specified times for up to 120 hours after dosing. Pre-dose urines and complete urine collections were obtained at specified intervals. The final specific activity of <sup>14</sup>C-Labeled Venlafaxine was approximately 1 µCi/mg venlafaxine (free base).

**Data Analysis:** Percent of the administered dose of <sup>14</sup>C-Labeled Venlafaxine appearing as each metabolite during each urine collection interval was calculated as follows:

$$X = \frac{(A/B) \times C}{D}$$

Where X = percent of dose appearing as the metabolite

A = dpm in radioactive peak of the metabolite

B = total dpm recovered from chromatograph

C = dpm in the total urine of the collection period

D = dpm administered as <sup>14</sup>C-Labeled Venlafaxine

The plasma concentration of venlafaxine metabolites other than Wy-45,233 was calculated from the difference between the total radioactivity and the sum of the concentrations of venlafaxine and Wy-45,233 in the plasma. For venlafaxine and Wy-45,233 the lower limit of quantitation was 10 ng/mL; detectable peaks below this limit were assigned a value of 5 ng/mL.

**Results:** Primary among the species differences in the metabolic fate of venlafaxine is the predominance of the unconjugated form of O-desmethyl-venlafaxine (Wy-45-233) in man. The time course for total radioactivity in the whole blood and plasma, as well as the time course for plasma to whole blood ratio are presented in the figures in the Attachments. The summary data are provided in the following Table and Figure

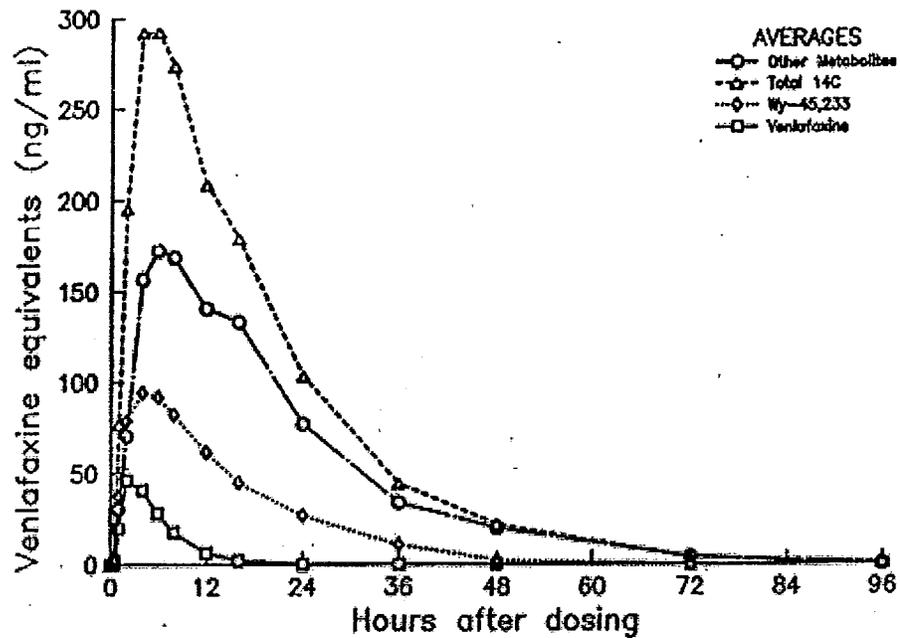
Table VII. Plasma Concentrations of Venlafaxine, Wy-45,233, Other Metabolites and Total Radioactivity in Ten Male Subjects Following a Single Oral 50 mg Dose of [<sup>14</sup>C]Venlafaxine, as the Hydrochloride Salt.

Hours after dosing	Plasma concentration (mean ± SD ng equivalents/ml)			
	Venlafaxine	Wy-45,233	Other metabolites	Total radioactivity
0.5	BQL <sup>a</sup>	3 ± 6	3 ± 8	5 ± 8
1	20 ± 16	37 ± 36	30 ± 18	77 ± 41
2	46 ± 16	79 ± 32	70 ± 36	196 ± 51
4	41 ± 20	94 ± 42	157 ± 56	292 ± 61
6	28 ± 18	92 ± 39	172 ± 61	292 ± 65
8	18 ± 13	82 ± 31	169 ± 59	274 ± 61
12	6 ± 7	62 ± 20	141 ± 41	209 ± 46
16	2 ± 4	45 ± 17	134 ± 34	179 ± 40
24	BQL	27 ± 12	77 ± 21	104 ± 24
36	BQL	11 ± 7	34 ± 14	45 ± 16
48	BQL	2 ± 4	20 ± 5	22 ± 8
72	BQL	BQL	4 ± 4	4 ± 4
96	BQL	BQL	BQL	BQL
120	BQL	BQL	BQL	BQL

<sup>a</sup>BQL = below quantifiable limits

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## Plasma Concentrations in Human Subjects Receiving 50 mg of <sup>14</sup>C Venlafaxine



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The maximum plasma venlafaxine concentrations were reached between 2 and 4 hour after dosing. This is about 16% of the peak radioactivity concentration. The time course of urinary, fecal and total recovery is shown in the attachment. About 98% was recovered in the urine and 1.9% of the dose was recovered in feces. Of the total recovery radioactivity recovered, 75% was recovered by 24 hours after dosing and 94% had been accounted for by 48 hours after dosing. Most of the administered venlafaxine was metabolized by O-demethylation to Wy-45233;  $29.4 \pm$

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5.0% of the dose appeared in the urine as Wy-45233 and  $26.4 \pm 9.0\%$  as Wy-45, 233 glucuronide. Wy-46689, the N, O-didesmethyl metabolite of venlafaxine was also found in significant amounts: 9.8% of the dose appeared in the urine as unconjugated Wy-46,869 and  $6.2 \pm 1.3\%$  as the glucuronide. Unidentified metabolite peaks in urine comprised  $0.9 \pm 0.4\%$  of the dose.

Summary: Over 92% of the dose was recovered in urine, showing that venlafaxine was well absorbed from the GI tract. The metabolite Wy-45,233 was 5-fold greater than venlafaxine. This difference was attributed to higher plasma levels and to slower elimination of Wy-45,233 relative to venlafaxine. Exposure to venlafaxine metabolites other than Wy-45,233 was much greater than the exposure to either Wy-45,233 and venlafaxine. The primary route of excretion for dose-related material is via the kidneys into the urine. By 48 hours after dosing, 87% of the dose had appeared in the urine. Seventy-two percent of the dose was present in the urine as products of O-demethylation. Of this, 40% (29.4% of the dose) escaped further metabolism and appeared in the urine as Wy-45,233. Another 36% (26.4% of the dose) was conjugated to form Wy-45,233 glucuronide. The final 22% (16% of the dose) appeared in the urine without further metabolism and 39% (6.2% of the dose) appeared as Wy-46,689 glucuronide.

*Reviewer comments: The disposition of venlafaxine was adequately characterized. The pharmacologically active metabolite, Wy-45,233 (Desvenlafaxine) was the most abundant single metabolite. The primary route of excretion is via the kidneys. The disposition of the active metabolite (Desvenlafaxine) can be inferred from this study.*

## Attachments

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Table II. Ratio of the Radioactive Concentration of Plasma and Whole Blood in 10 Male Subjects Following a Single Oral 50 mg Dose of [<sup>14</sup>C]Venlafaxine, as the Hydrochloride Salt.

Hours after dosing	Whole blood [ <sup>14</sup> C] (dpm/ml) <sup>a</sup>	Plasma [ <sup>14</sup> C] (dpm/ml) <sup>a</sup>	Plasma radioactivity
			Whole blood radioactivity
0	0	0	
0.5	11 ± 34	14 ± 17	1.3
1	224 ± 121	177 ± 96	0.8
2	526 ± 126	453 ± 118	0.9
4	719 ± 124	677 ± 141	0.9
6	684 ± 117	678 ± 151	1.0
8	635 ± 191	654 ± 136	1.0
12	402 ± 105	483 ± 107	1.2
16	380 ± 119	416 ± 94	1.1
24	258 ± 44	240 ± 55	0.9
36	76 ± 86	103 ± 37	1.4
48	23 ± 49	51 ± 18	2.2
72	BQL <sup>b</sup>	12 ± 5	-
96	BQL	BQL	-
120	BQL	BQL	-
Mean (0-72 hr)			1.2 ± 0.4
Mean (0-36 hr)			1.1 ± 0.2

<sup>a</sup>Mean ± SD

<sup>b</sup>BQL = below quantifiable limits (<10 dpm)

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Table VII. Plasma Concentrations of Venlafaxine, Wy-45,233, Other Metabolites and Total Radioactivity in Ten Male Subjects Following a Single Oral 50 mg Dose of [<sup>14</sup>C]Venlafaxine, as the Hydrochloride Salt.

Hours after dosing	Plasma concentration (mean ± SD ng equivalents/ml)			
	Venlafaxine	Wy-45,233	Other metabolites	Total radioactivity
0.5	BQL <sup>a</sup>	3 ± 6	3 ± 8	5 ± 8
1	20 ± 16	37 ± 36	30 ± 18	77 ± 41
2	46 ± 16	79 ± 32	70 ± 36	196 ± 51
4	41 ± 20	94 ± 42	157 ± 56	292 ± 61
6	28 ± 18	92 ± 39	172 ± 61	292 ± 65
8	18 ± 13	82 ± 31	169 ± 59	274 ± 61
12	6 ± 7	62 ± 20	141 ± 41	209 ± 46
16	2 ± 4	45 ± 17	134 ± 34	179 ± 40
24	BQL	27 ± 12	77 ± 21	104 ± 24
36	BQL	11 ± 7	34 ± 14	45 ± 16
48	BQL	2 ± 4	20 ± 5	22 ± 8
72	BQL	BQL	4 ± 4	4 ± 4
96	BQL	BQL	BQL	BQL
120	BQL	BQL	BQL	BQL

<sup>a</sup>BQL = below quantifiable limits

Table x.  $C_{max}$  and AUC of Venlafaxine, Wy-45,233, Other Metabolites and Total Radioactivity in the Plasma of Ten Male Subjects Following a Single Oral 50 mg Dose of [ $^{14}C$ ]Venlafaxine, as the Hydrochloride Salt.

Subject	$C_{max}$ (ng Venlafaxine equiv./ml)				AUC <sub>0-24 hr</sub> (ng Venlafaxine equiv. hr/ml)			
	Venlafaxine	Wy-45,233	Other Metabolites	Total radioactivity	Venlafaxine	Wy-45,233	Other Metabolites	Total radioactivity
1	59	119	119	257	454	1763	2143	4299
2	49	176	282	431	277	2162	4236	6597
3	67	121	92	239	519	1706	1588	3812
4	15	122	269	352	49	1350	3399	4791
5	40	126	168	296	286	1749	2974	5002
6	14	102	231	330	46	1474	3961	5463
7	55	87	208	327	263	1212	3352	4827
8	87	38	165	272	661	625	3095	4380
9	49	70	141	220	359	832	2377	3566
10	49	85	178	270	219	1020	2713	3943
Mean	48	109	185	299	313	1389	2984	4668
±SD	±22	±38	±62	±63	±195	±476	±476	±813

Table XIV. Mean Values for Percent of Radioactivity in 10 Male Subjects Following a Single Oral Dose of [<sup>14</sup>C]Venlafaxine, as the Hydrochloride Salt<sup>a</sup>.

Route	Percent of Dose									Total (0-120)
	0-4 <sup>b</sup>	4-8	8-12	12-24	24-48	48-72	72-96	96-120		
Urine	Mean	8.63	18.8	14.3	26.9	18.4	3.93	0.82	0.31	92.1
	SD	5.45	7.59	3.66	6.17	4.22	2.00	0.52	0.26	8.1
	n	9	9	9	9	9	9	9	9	9
Feces	Mean		0.6 <sup>c</sup>			0.9	0.4	0.2	0.2	1.93
	SD		0.3			0.7	0.2	0.2	0.4	0.68
	n		8			8	9	7	7	9
Urine plus Feces	Mean		69.2 <sup>c</sup>			19.2	4.33	1.0	0.5	94.1
	SD		7.35			3.8	2.0	0.6	0.5	8.4
	n		9			9	9	9	9	9

<sup>a</sup>Data for subjects 1-8, 10 only.

<sup>b</sup>Hours after dosing.

<sup>c</sup>0-24 hr time period.

Table XV. Urinary Metabolites Excreted by 9 Male Subjects up to 48 hr Following a Single Oral 50 mg Dose of [<sup>14</sup>C]Venlafaxine, as the Hydrochloride Salt<sup>a</sup>.

Subject	Venlafaxine	Percent of Administered Dose								
		Wy-45,233	Gluc.	Wy-46,689	Gluc.	A <sup>b</sup>	B <sup>c</sup>	Wy-46,965	Wy-45,494	Other <sup>d</sup>
1										
2										
3										
4										
5										
6										
7										
8										
10										
Mean	4.7	29.4	26.4	9.8	6.2	2.7	2.2	1.0	1.0	0.9
±SD	3.1	5.0	9.0	2.7	1.3	1.5	0.8	0.6	1.6	0.4

<sup>a</sup>Subject 9 was excluded due to a missing urine sample.

<sup>b</sup>Metabolite A was an unidentified conjugate.

<sup>c</sup>Metabolite B was an unidentified metabolite, not a glucuronide or sulfate conjugate.

<sup>d</sup>Other - the sum of minor unidentified metabolite peaks.

Plasma Concentrations in Human Subjects Receiving  
50 mg of <sup>14</sup>C Venlafaxine

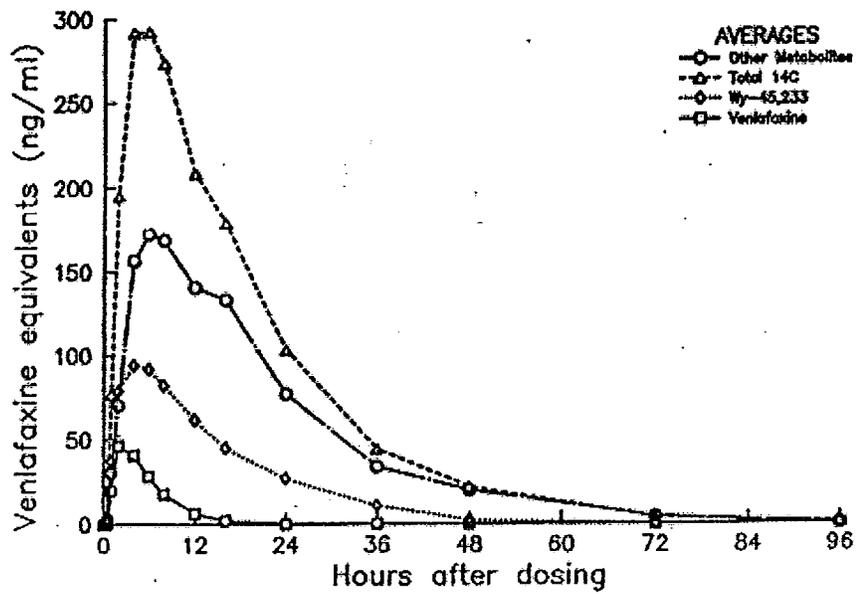


Table XIV. Mean Values for Percent of Radioactivity in 10 Male Subjects Following a Single Oral Dose of [<sup>14</sup>C]Venlafaxine, as the Hydrochloride Salt<sup>a</sup>.

Route	Percent of Dose									
	0-4 <sup>b</sup>	4-8	8-12	12-24	24-48	48-72	72-96	96-120	Total (0-120)	
Urine	Mean	8.63	18.8	14.3	26.9	18.4	3.93	0.82	0.31	92.1
	SD	5.45	7.59	3.66	6.17	4.22	2.00	0.52	0.26	8.1
	n	9	9	9	9	9	9	9	9	9
Feces	Mean		0.6 <sup>c</sup>			0.9	0.4	0.2	0.2	1.93
	SD		0.3			0.7	0.2	0.2	0.4	0.68
	n		8			8	9	7	7	9
Urine plus Feces	Mean		69.2 <sup>c</sup>			19.2	4.33	1.0	0.5	94.1
	SD		7.35			3.8	2.0	0.6	0.5	8.4
	n		9			9	9	9	9	9

<sup>a</sup>Data for subjects 1-8, 10 only.

<sup>b</sup>Hours after dosing.

<sup>c</sup>0-24 hr time period.

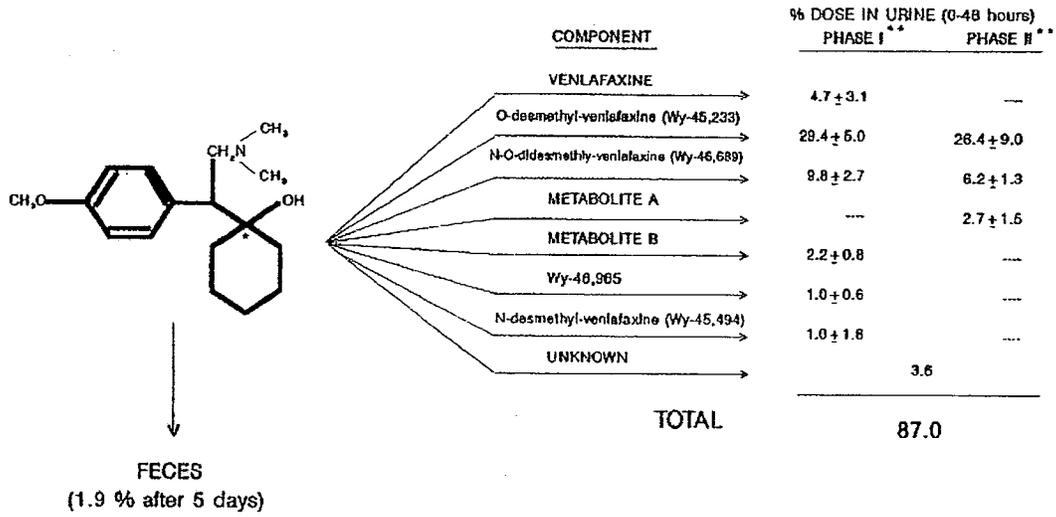
Table XI: Percent of Administered Radioactivity Excreted into Urine of Male Subjects Following a Single Oral 50 mg Dose of [<sup>14</sup>C]Venlafaxine, as the Hydrochloride Salt.

Subject	Percent of Dose								Total (0-120)
	0-4 <sup>a</sup>	4-8	8-12	12-24	24-48	48-72	72-96	96-120	
1									99.9
2									81.8
3									85.0
4									85.7
5									100.1
6									99.5
7									100.1
8									82.8
9									61.8
10								.6	94.5

<sup>a</sup>Hours after dosing.

<sup>b</sup>NS = no sample obtained.

Metabolic Pathways of Venlafaxine in Healthy Subjects



\* Position of label

\*\* Phase I denotes oxidative metabolism  
 Phase II denotes conjugation metabolism

Pharmacokinetics of Wy-45,233 in Male Subjects Receiving a Single Oral 50 mg Dose of [<sup>14</sup>C] Venlafaxine, as the Hydrochloride Salt

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 ON ORIGINAL

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Subject	$t_{\max}$ (hr)	$C_{\max}$ (ng/ml)
1	6.0	114
2	4.0	169
3	4.0	116
4	4.0	117
5	6.0	121
6	6.0	98
7	4.0	84
8	6.0	37
9	2.0	67
10	2.0	82
Mean±SD	4.4 ±1.6	100.5 ±35.7

---

Concentrations of Wy-45,233 in Plasma of 10 Male Subjects Following a Single Oral 50 mg Dose of [<sup>14</sup>C] Venlafaxine, as the Hydrochloride Salt as the Hydrochloride Salt

Hours after dosing	WY-45,233 (ng/ml)									
	001 <sup>a</sup>	002	003	004	005	006	007	008	009	010
0 (pre-dose)	ND <sup>b</sup>	12	ND							
0.5										
1										
2										
4										
6										
8										
12										
16										
24										
36										
48										
72										
96										
120										

<sup>a</sup>Subject Number

<sup>b</sup>ND = not detected

— ng/ml was lower limit of quantitation

\*[] = Spurious value; not used in calculations

4.4 OCP Filing Form

Office of Clinical Pharmacology and Biopharmaceutics			
<u>New Drug Application Filing and Review Form</u>			
I. General Information About the Submission			
	Information		Information
NDA Number	21-992	Brand Name	TBD
OCPB Division (I, II, III)	I	Generic Name	Desvenlafaxine ER Tablets
Medical Division	DPP	Drug Class	<p>■</p> <p>nt i- D e p r e s s i v e</p>
OCPB Reviewer	<p>■</p> <p>ofi Ku mi</p>	Indication(s)	<p>■</p> <p>aj or D e p r e s s i v e D i s o r d e r</p>
OCPB Team Leader	Raman Baweja	Dosage Form	Tablet
		Dosing Regimen	100 mg/day
Date of Submission	12/22/05	Route of Administration	Oral
Estimated Due Date of OCPB Review	7/28/06	Sponsor	Wyeth

PDUFA Due Date	10/6/06	Priority Classification	standard	
Division Due Date	1/25/06			
<p>○ Information Clin. Pharm. and Biopharm.</p>				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
<b>STUDY TYPE</b>				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
<b>I. Clinical Pharmacology</b>				
Mass balance:	X	1		
Isozyme characterization:	X	1		
Blood/plasma ratio:				
Plasma protein binding:	X	1		
<b>Pharmacokinetics (e.g., Phase I) -</b>				
Healthy Volunteers-				
single dose:	X	9		
multiple dose:		1		
Patients-				
single dose:				
multiple dose:				
<b>Dose proportionality -</b>				
fasting / non-fasting single dose	X	1		
fasting / non-fasting multiple dose				
<b>Drug-drug interaction studies -</b>				
In-vivo effects on primary drug	X	1		
In-vivo effects of primary drug	X	3		
In-vitro				
<b>Subpopulation studies -</b>				
ethnicity:	X	1		
gender	X	1		
pediatrics				
geriatrics	X	1		
renal impairment	X	1		
hepatic impairment	X	1		
<b>PD:</b>				

Phase 2:	X	1		
Phase 3:		1		
<b>PK/PD:</b>				
Phase 1 and/or 2, proof of concept				
Phase 3 clinical trial.		1		
<b>Population Analyses -</b>				
Data rich:	X	1		
Data sparse:	X	3		
<b>II. Biopharmaceutics</b>				
Absolute bioavailability:	X	1		
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:	X	1		
<b>Bioequivalence studies -</b>				
traditional design, single / multi dose:				
replicate design, single / multi dose:				
<b>Food-drug interaction studies:</b>	X	2		
<b>Dissolution:</b>				
(IVIVC):	X	1		
Bio-wavier request based on BCS				
BCS class				
<b>III. Other CPB Studies</b>				
Genotype/phenotype studies:				
Effect of DVS on QTc	X	1		
Pediatric development plan				
Literature References				
<b>Total Number of Studies</b>		<b>21</b>		
<i>Filability and QBR comments</i>				
	"X" if yes		<b>omments</b>	
Application filable ?			Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?	
Comments sent to firm ?	X		Comments have been sent to firm (or attachment included). FDA letter date if applicable.	
QBR questions (key issues to be considered)			<ol style="list-style-type: none"> <li>1. What is the relationship between exposure to DVS and efficacy/safety?</li> <li>2. Are there intrinsic factors that affect the pharmacokinetics of DVS?</li> <li>3. Are there any extrinsic factors that affect the PK of DVS?</li> <li>4. What is the Bioavailability and metabolic characteristics of DVS?</li> <li>5. Is the IVIVC reported by the sponsor substantiated by the data?</li> </ol>	
Other comments or information not included above			This application is all electronic. Link to EDR	
Primary reviewer Signature and Date			Kofi A. Kumi	

Secondary reviewer Signature and Date	
---------------------------------------	--

CC: NDA 21-992, HFD-850 (Electronic Entry or Lee), HFD-130, HFD-860 (Mehta, Baweja, KumiK), CDR (B. Murphy)

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Jogarao Gobburu  
10/26/2006 11:36:03 AM  
BIOPHARMACEUTICS

Raman Baweja  
10/26/2006 03:41:17 PM  
BIOPHARMACEUTICS

*Office of Clinical Pharmacology and Biopharmaceutics*  
*New Drug Application Filing and Review Form*

**General Information About the Submission**

	Information		Information
NDA Number	21-992	Brand Name	TBD
OCPB Division (I, II, III)	I	Generic Name	Desvenlafaxine ER Tablets
Medical Division	DPP	Drug Class	Anti-Depressive
OCPB Reviewer	Kofi Kumi	Indication(s)	Major Depressive Disorder
OCPB Team Leader	Raman Baweja	Dosage Form	Tablet
		Dosing Regimen	100 mg/day
Date of Submission	12/22/05	Route of Administration	Oral
Estimated Due Date of OCPB Review	7/28/06	Sponsor	Wyeth
PDUFA Due Date	10/6/06	Priority Classification	Standard
Division Due Date	8/25/06		

**Clin. Pharm. and Biopharm. Information**

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
<b>STUDY TYPE</b>				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
<b>I. Clinical Pharmacology</b>				
Mass balance:	X	1		
Isozyme characterization:	X	1		
Blood/plasma ratio:				
Plasma protein binding:	X	1		
Pharmacokinetics (e.g., Phase I) -				
<i>Healthy Volunteers-</i>				
single dose:	X	9		
multiple dose:	X	1		
<i>Patients-</i>				
single dose:				
multiple dose:				
<b>Dose proportionality -</b>				
fasting / non-fasting single dose:	X	1		
fasting / non-fasting multiple dose:				
<b>Drug-drug interaction studies -</b>				
In-vivo effects on primary drug:	X	1		
In-vivo effects of primary drug:	X	3		
In-vitro:				
<b>Subpopulation studies -</b>				
ethnicity:	X	1		
gender:	X	1		
pediatrics:				
geriatrics:	X	1		
renal impairment:	X	1		
hepatic impairment:	X	1		
<b>PD:</b>				

Phase 2:	X	1		
Phase 3:	X	1		
<b>PK/PD:</b>				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:	X	1		
<b>Population Analyses -</b>				
Data rich:	X	1		
Data sparse:	X	3		
<b>II. Biopharmaceutics</b>				
<b>Absolute bioavailability:</b>	X	1		
<b>Relative bioavailability -</b>				
solution as reference:				
alternate formulation as reference:	X	1		
<b>Bioequivalence studies -</b>				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
<b>Food-drug interaction studies:</b>	X	2		
<b>Dissolution:</b>				
<b>(IVIVC):</b>	X	1		
<b>Bio-wavier request based on BCS</b>				
<b>BCS class</b>				
<b>III. Other CPB Studies</b>				
<b>Genotype/phenotype studies:</b>				
<b>Effect of DVS on QTc</b>	X	1		
<b>Pediatric development plan</b>				
<b>Literature References</b>				
<b>Total Number of Studies</b>		21		
<b>Filability and QBR comments</b>				
	<b>"X" if yes</b>	<b>Comments</b>		
<b>Application filable ?</b>	X	Reasons if the application <u>is not</u> filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?		
<b>Comments sent to firm ?</b>	X	Comments have been sent to firm (or attachment included). FDA letter date if applicable.		
<b>QBR questions (key issues to be considered)</b>		<ol style="list-style-type: none"> <li>1. What is the relationship between exposure to DVS and efficacy/safety?</li> <li>2. Are there intrinsic factors that affect the pharmacokinetics of DVS?</li> <li>3. Are there any extrinsic factors that affect the PK of DVS?</li> <li>4. What is the Bioavailability and metabolic characteristics of DVS?</li> <li>5. Is the IVIVC reported by the sponsor substantiated by the data?</li> </ol>		
<b>Other comments or information not included above</b>		This application is all electronic. Link to EDR		
<b>Primary reviewer Signature and Date</b>	Kofi A. Kumi			
<b>Secondary reviewer Signature and Date</b>				

CC: NDA 21-992, HFD-850 (Electronic Entry or Lee), HFD-130, HFD-860 (Mehta, Baweja, KumiK), CDR (B. Murphy)

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/s/

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Kofi Kumi  
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Raman Baweja  
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BIOPHARMACEUTICS  
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