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

<table>
<thead>
<tr>
<th>Batch</th>
<th>Observed AUC</th>
<th>Predicted AUC</th>
<th>Absolute % PE</th>
<th>Observed C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Predicted C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Absolute % PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x200 mg</td>
<td>8870</td>
<td>9065</td>
<td>2.20</td>
<td>362</td>
<td>341</td>
<td>5.62</td>
</tr>
<tr>
<td>A61140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x200 mg</td>
<td>8984</td>
<td>9178</td>
<td>2.16</td>
<td>314</td>
<td>307</td>
<td>2.01</td>
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<tr>
<td>A61138</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 177</td>
<td></td>
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<tr>
<td>Average % PE</td>
<td>2.18</td>
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<td>3.81</td>
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</table>

### External Predictability

<table>
<thead>
<tr>
<th>Batch</th>
<th>Observed AUC</th>
<th>Predicted AUC</th>
<th>Absolute % PE</th>
<th>Observed C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Predicted C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Absolute % PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x200 mg</td>
<td>8540</td>
<td>8722</td>
<td>2.13</td>
<td>308</td>
<td>309</td>
<td>0.36</td>
</tr>
<tr>
<td>2002B0107</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x50 mg</td>
<td>4695</td>
<td>4843</td>
<td>3.16</td>
<td>169</td>
<td>155</td>
<td>8.00</td>
</tr>
<tr>
<td>2002B0105</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Study 186</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x100 mg</td>
<td>4542</td>
<td>4677</td>
<td>2.96</td>
<td>173</td>
<td>157</td>
<td>9.53</td>
</tr>
<tr>
<td>2002B0109</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Study 186</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Internal Predictability</th>
<th>Observed AUC</th>
<th>Predicted AUC</th>
<th>Absolute % PE</th>
<th>Observed Cmax</th>
<th>Predicted Cmax</th>
<th>Absolute % PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1x200 mg A61140</td>
<td>8870</td>
<td>9080</td>
<td>2.37</td>
<td>362</td>
<td>344</td>
<td>4.95</td>
</tr>
<tr>
<td>Study 177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch 1x200 mg A61138</td>
<td>8984</td>
<td>9192</td>
<td>2.32</td>
<td>314</td>
<td>307</td>
<td>2.07</td>
</tr>
<tr>
<td>Study 177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average % PE 2.34        Average % PE 3.51

<table>
<thead>
<tr>
<th>External Predictability</th>
<th>Observed AUC</th>
<th>Predicted AUC</th>
<th>Absolute % PE</th>
<th>Observed Cmax</th>
<th>Predicted Cmax</th>
<th>Absolute % PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1x200 mg 2002B0107</td>
<td>8540</td>
<td>8772</td>
<td>2.72</td>
<td>308</td>
<td>309</td>
<td>0.36</td>
</tr>
<tr>
<td>Study 177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch 2x50 mg 2002B0105</td>
<td>4695</td>
<td>4888</td>
<td>4.10</td>
<td>169</td>
<td>170</td>
<td>0.59</td>
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<tr>
<td>Study 186</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch 1x100 mg 2002B0109</td>
<td>4542</td>
<td>4723</td>
<td>3.98</td>
<td>173</td>
<td>171</td>
<td>1.50</td>
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<tr>
<td>Study 186</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

AUC units: ng·hr/mL
Cmax 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.
3 Page(s) Withheld

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

§ 552(b)(4) Draft Labeling

§ 552(b)(5) Deliberative Process
Dissolution Method Development

The dissolution of DVS SR was evaluated in multiple media; water, 0.1N HCl, pH 4.5 buffer, pH 6.8 buffer. The sponsor selected 0.9% NaCl in water as the media of choice. The sponsor developed an IVIVC which formed the basis for dissolution specification during clinical phases of product development including stability assessment. The correlation led to the multipoint dissolution specifications, which provided measurements of the sustained release product performance. The IVIVC has been reviewed and deemed acceptable. The method and multipoint dissolution specification is as follows:

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>USP Apparatus I (baskets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>100 rpm</td>
</tr>
<tr>
<td>Media</td>
<td>900 mL 0.9% NaCl in water</td>
</tr>
<tr>
<td>Temperature</td>
<td>37°C ± 0.5°C</td>
</tr>
<tr>
<td>Specification:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Criteria (% LC Released)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td></td>
</tr>
<tr>
<td>4 hours</td>
<td></td>
</tr>
<tr>
<td>8 hours</td>
<td></td>
</tr>
<tr>
<td>12 hours</td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>NLT</td>
</tr>
</tbody>
</table>

The sponsor is proposing a dissolution specification, because a quality by design approach has been used to develop the drug product. The sponsor states that the robust formulation has demonstrated excellent control of drug release over the design space and the level of is significantly above levels where dose-dumping might be of concern. Therefore, the sponsor is proposing a 4-hour dissolution check using the same method as for the multipoint dissolution. However, for situations where product manufacturing occurs outside of an approved manufacturing design space, the multipoint dissolution criteria (above) will be used. The proposed dissolution specification is as follows:

An assessment of data was carried out, by the sponsor, to determine if a dissolution specification would suffice as a surrogate for complete profile measurement. As part of design of experiments (DOE) for determining appropriate in Desvenlafaxine Succinate 200 mg tablets, dissolution profiles were obtained from experimental runs of batches formulated with
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 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-233 100 mg Tablets](image)
Dissolution Profiles of DVS 200 mg (Registration Batches)

Dissolution Profiles of DVS-233 200 mg

Amount of Drug Released (%)

Time (Hours)

- A89767-Composite
- A89767-FCT
- A89788-Composite
- A89788-FCT
- A82580-Composite
- A82580-FCT
- A82580-Lower Limit
- A82580-Upper Limit
_____ Page(s) Withheld

\( \chi \) § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process
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)

Cumulative Percent Label Claim Dissolved

Time (Hours)

WPC = Guayama (Commercial)
MTL = Montreal (Clinical)
Absorption Profile of DVS from DVS-233 SR 50 mg Tablets

Time (Hours)

50 mg Formulation
Batch 2002B0105
Absorption of DVS versus Dissolution of DVS Relationship for DVS SR 50 mg Tablets

- 50 mg
- 100 mg
- 75 mg
- 50 mg IVIVC
- $R^2 = 0.996$

Cumulative Percent Absorbed vs. Cumulative Percent Dissolved
Absorption of DVS versus Dissolution of DVS Relationship for DVS SR 200 mg Tablets

Cumulative Percent Dissolved

Cumulative Percent Absorbed

- Batch A61140 Formulation
- Batch A61138 Formulation
- Batch 2002B0107 Formulation
- IVIVC Model

$R^2 = 0.990$
### Dissolution of DVSSR 50 mg Tablets: Evaluation of pH

#### 6.3 Dissolution of DVS-233 SR 50 mg Tablets: Evaluation of pH

<table>
<thead>
<tr>
<th>Media</th>
<th>Time (hr)</th>
<th>AVG (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1N HCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.5 Buffer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6.8 Buffer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1N HCl/pH 6.8 Buffer</td>
<td>2</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Media</th>
<th>Batch A61140</th>
<th>Batch A61138</th>
<th>Batch A43079</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (hr)</td>
<td>AVG (%)</td>
<td>Range (%)</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1N HCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.5 Buffe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6.8 Buff</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1N HCl/pH 6.8 Buffer 2-stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>2</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>99</td>
<td></td>
</tr>
</tbody>
</table>

*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

**Dissolution of 50 mg SR Tablets – Cumulative Percent Released**

<table>
<thead>
<tr>
<th>Time (Hr)</th>
<th>Reference Profile</th>
<th>Lower Limit (-10%)</th>
<th>Upper Limit (+10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>27</td>
<td>17</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>31</td>
<td>51</td>
</tr>
<tr>
<td>8</td>
<td>62</td>
<td>51</td>
<td>72</td>
</tr>
<tr>
<td>12</td>
<td>76</td>
<td>66</td>
<td>86</td>
</tr>
<tr>
<td>24</td>
<td>96</td>
<td>86</td>
<td>106</td>
</tr>
</tbody>
</table>

**Bioavailability of 2x50 mg DVS-233 SR Tablet**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Tablet</th>
<th>Lower Limit Tablet</th>
<th>Upper Limit Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/hr/mL)</td>
<td>4798</td>
<td>4462</td>
<td>5108</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;a&lt;/sup&gt;</td>
<td>--</td>
<td>7.0</td>
<td>6.5</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>157</td>
<td>143</td>
<td>173</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;a&lt;/sup&gt;</td>
<td>--</td>
<td>9.1</td>
<td>10</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>6</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

**Dissolution of 200 mg SR Tablets – Cumulative Percent Released**

<table>
<thead>
<tr>
<th>Time (Hr)</th>
<th>Reference Profile</th>
<th>Lower Limit (-10%)</th>
<th>Upper Limit (+10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>28</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
<td>49</td>
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<td>65</td>
<td>85</td>
</tr>
<tr>
<td>24</td>
<td>98</td>
<td>88</td>
<td>108</td>
</tr>
</tbody>
</table>

**Bioavailability of 1x200 mg DVS-233 SR Tablet**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Tablet</th>
<th>Lower Limit Tablet</th>
<th>Upper Limit Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/hr/mL)</td>
<td>9558</td>
<td>8802</td>
<td>10286</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;a&lt;/sup&gt;</td>
<td>--</td>
<td>7.9</td>
<td>7.6</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>308</td>
<td>282</td>
<td>340</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;a&lt;/sup&gt;</td>
<td>--</td>
<td>8.4</td>
<td>10</td>
</tr>
<tr>
<td>% Difference&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>8</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Difference from reference
<sup>b</sup> Difference from lower limit to upper limit
Objective: To determine the plasma protein binding of Wy-45,233 in Rat, Dog and Human Plasma.

Materials and Methods: Wy-45233 was obtained form 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Post-incubation</th>
<th>Percent bound to plasma proteins&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>plasma Wy-45,233</td>
<td>N</td>
</tr>
<tr>
<td>Rat</td>
<td>1.11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2.68</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5.94</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>All concentrations</td>
<td>14</td>
</tr>
<tr>
<td>Dog</td>
<td>0.34</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.82</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.67</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>All concentrations</td>
<td>14</td>
</tr>
<tr>
<td>Human</td>
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</tr>
<tr>
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<td>0.124</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.200</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>All concentrations</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean± 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

<table>
<thead>
<tr>
<th>Pre-incubation concentration (µg/ml)</th>
<th>Post-incubation concentration of Wy-45,233 (µg/ml)</th>
<th>Percent bound to plasma proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Wy-45,233</td>
<td>Plasma</td>
<td>Buffer</td>
</tr>
<tr>
<td>3.2</td>
<td>1.19</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>1.03</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>1.17</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>1.13</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>1.04</td>
<td>0.70</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.11 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>2.62</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>2.57</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>2.85</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>2.36</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>2.99</td>
<td>1.88</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.68 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>5.33</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td>6.19</td>
<td>3.42</td>
</tr>
<tr>
<td></td>
<td>6.68</td>
<td>3.55</td>
</tr>
<tr>
<td></td>
<td>5.54</td>
<td>4.06</td>
</tr>
<tr>
<td></td>
<td>3.96</td>
<td>3.97</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.94 ± 0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<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

<table>
<thead>
<tr>
<th>Pre-incubation concentration (μg/ml)</th>
<th>Post-incubation Wy-45,233 (μg/ml)</th>
<th>Percent bound to plasma proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Wy-45,233</td>
<td>Wy-45,233 plasma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wy-45,233 buffer</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.38</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>0.41</td>
<td>39.3</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>19.7</td>
</tr>
<tr>
<td></td>
<td>0.26</td>
<td>1.8(^a)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.34 ± 0.06(^b)</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.86</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td>0.90</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>0.67</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>0.72</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td>43.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.82 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>1.80</td>
<td>31.8</td>
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<tr>
<td></td>
<td>1.80</td>
<td>28.3</td>
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<td></td>
<td>1.84</td>
<td>24.3</td>
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<td></td>
<td>1.39</td>
<td>21.7</td>
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<tr>
<td></td>
<td>1.52</td>
<td>26.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.67 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Spurious value, not included in calculations.
\(^b\) N = 4
Table III. The Protein Binding of Wy-45,233 in Human Plasma

<table>
<thead>
<tr>
<th>Pre-incubation concentration (µg/ml)</th>
<th>Post-incubation Wy-45,233 (µg/ml)</th>
<th>Percent bound to plasma proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasma Wy-45,233 concentration</td>
<td>plasma</td>
<td>buffer</td>
</tr>
<tr>
<td>0.10</td>
<td>0.034 0.029</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>0.035 0.024</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td>0.048 0.070 b</td>
<td>-95.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.020 0.032</td>
<td>-67.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.020 0.021</td>
<td>-5.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.035 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.098 0.059</td>
<td>40.2</td>
</tr>
<tr>
<td></td>
<td>0.136 0.095</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>0.110 0.063</td>
<td>43.9</td>
</tr>
<tr>
<td></td>
<td>0.161 0.110</td>
<td>32.2</td>
</tr>
<tr>
<td></td>
<td>0.116 0.101</td>
<td>13.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.124 ± 0.025</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>0.270 0.203</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>0.196 0.124</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>0.209 0.187</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>0.175 0.093</td>
<td>47.9</td>
</tr>
<tr>
<td></td>
<td>0.146 0.109</td>
<td>26.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.200 ± 0.046</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Spurious value, not included in calculations.
<sup>b</sup><sub>N=2</sub>
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(+)-mephenytoin and dextorphan 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:
**Analytical Method:** DVS-233, its metabolites, and 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 Km 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 dextorphan. This calibration curve was linear between 2.5 and 500 nM WY-46689. The Km 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: 
\[\% \text{ inhibition} = ((a - b) / a) * 100\% ,\] 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.
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 μM 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 μM DVS-233. The K_m for WY-46689 formation was found to be approximately 290 μM DVS-233. Linear rate conditions (300 μM 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 μM) During Incubations with Membranes from E. Coli Transfected With Various cDNAs

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

\(^a\) 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 μM 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 μM) By CYP450 Isozyme Specific Inhibitors in Human Liver Microsomes

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>μM</th>
<th>Enzyme Inhibited</th>
<th>Percent Inhibition of WY-46689 formation(^a)</th>
<th>Percent Inhibition of M9 formation(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Naphthoflavone</td>
<td>1</td>
<td>CYP1A2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coumarin</td>
<td>100</td>
<td>CYP2A6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sulfaphenazole</td>
<td>10</td>
<td>CYP2C9</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>S(+) - Mephenytoin</td>
<td>100</td>
<td>CYP2C19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Quinidine</td>
<td>10</td>
<td>CYP2D6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Diethylidithiocarbamate</td>
<td>100</td>
<td>CYP2E1</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>50</td>
<td>CYP3A4</td>
<td>76</td>
<td>58</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>&quot;</td>
<td>65</td>
<td>29</td>
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<tr>
<td>&quot;</td>
<td>1</td>
<td>&quot;</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Benzylimidazolone</td>
<td>1000</td>
<td>All CYPs</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>Heat Inactivation</td>
<td>1 min at 50°C</td>
<td>FMO</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\): 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 μM concentration might be too high a concentration to be have been used in the study. Hence, inference from this study should be done with caution.
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 1-07970-272-1) 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’-diphosphoglucurononic 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 ———.
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.
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.
Summary Of In Vitro Metabolites Of DVS-233 Characterized By LC/MS

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

a: LC/MS retention times taken from or normalized to data file DVS23MH1011010.
b: R = Rat liver microsomes, D = Dog liver microsomes, H = Human liver microsomes, and HH = Human hepatocytes
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 duxoxetine (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.

IC50 values for the inhibition of the different CYP enzymes by the SSRI/SNRIs were determined using CYP-isofrom specific probe substrates at concentrations near their respective K_m values. In addition, K_i values for the inhibition of CYP2D6 activity were determined for all the drugs studied, and K_i 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 ICSo 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 duxoxetine (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 Resoruin, ethoxyresorufin, and dextorphan were purchased from 3-Mephenytoin, 4'-hydroxy-S-mephenytoin, bufuralol hydrochloride, 1'-hydroxybufuralol, 1'-hydroxymidazolam, 6α-hydroxycamptothecil, 4'-hydroxydoclofenac, 7-hydroxycoumarin and paclitaxel were purchased from Diclofenac, midazolam, testosterone, 6β-hydroxytestosterone, coumarin, α-naphthoflavone, quercetin, sulfaphenzone, tranylcypromine, 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 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.
2 Page(s) Withheld

\[ \checkmark \] § 552(b)(4) Trade Secret / Confidential

\[ \] § 552(b)(4) Draft Labeling

\[ \] § 552(b)(5) Deliberative Process
Data Calculations: For the determination of IC₅₀ 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. IC₅₀ values were calculated from plots generated from the inhibitory effect Eₘₐₓ model (model 103) utilizing WinNonlin Professional, version 4.1, with the exception of testosterone-6β-hydroxylation, where the equation was used.

For the determination of Kᵢ 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 Kᵢ values and kinetics of inhibition were determined by non-linear regression plots using simple Eₘₐₓ model (model 101) utilizing WinNonlin Professional, version 4.1, Lineweaver-Burk plots and Eadie-Hofstee plots were used with Ki values determined by plots.

Results: The IC₅₀ 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
µM) and weakly inhibited CYP1A2 activity (extrapolated IC₅₀ value of 130 µM). Venlafaxine did not inhibit CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6β-hydroxylation activity at the highest concentration tested (100 µM), and inhibited CYP2D6 activity (IC₅₀ value of 69 µM). S. S Duloxetine did not inhibit CYP1A2 and CYP2C9 activity at the highest concentration tested (100 µM), weakly inhibited CYP2A6 and CYP2C8 activity (extrapolated IC₅₀ values of 270 and 180 µM, respectively), and inhibited CYP2C19, CYP2D6 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6β-hydroxylation activity (IC₅₀ values of 49, 6.0, 89 and 54 µM, respectively). Paroxetine did not inhibit CYP2C8 activity at the highest concentration tested (100 µM), weakly inhibited CYP2A6 activity (extrapolated IC₅₀ value of 210 µM), and inhibited CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6β-hydroxylation activity (IC₅₀ values of 80, 63, 70, 2.0, 32 and 59 µM, respectively). Sertraline weakly inhibited CYP2C8 and CYP2C9 activity (extrapolated IC₅₀ values of 350 and 120 µM, respectively) and inhibited CYP1A2, CYP2A6, CYP2C19, CYP2D6 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6β-hydroxylation activity (IC₅₀ values of 61, 51, 27, 5.0, 26 and 41 µM, respectively). Bupropion did not inhibit CYP2A6, CYP2C8 and CYP2C9 activity at the highest concentration tested (100 µM), weakly inhibited CYP1A2 and CYP3A-mediated midazolam-1'-hydroxylation and testosterone-6β-hydroxylation activity (extrapolated IC₅₀ values of 160, 120 and 270 µM, respectively) and inhibited CYP2C19 and CYP2D6 activity (IC₅₀ values of 43 and 28 µM, respectively). α-naphthoflavone, tranilcipromine (10 µM), quercetin, sulfaphenazole, tranilcipromine (50 µM), 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 IC₅₀ Values

<table>
<thead>
<tr>
<th>Human CYP450 Isozyme</th>
<th>Substrate</th>
<th>Concentration (µM)</th>
<th>Metabolite Monitored</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Ethoxyresorufin</td>
<td>1</td>
<td>Resorufin</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Coumarin</td>
<td>2.5</td>
<td>7-Hydroxycoumarin</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Paclitaxel</td>
<td>10</td>
<td>6α-Hydroxypaclitaxel</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Diclofenac</td>
<td>10</td>
<td>4'-Hydroxydiclofenac</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-mephenytoin</td>
<td>40</td>
<td>4'-Hydroxy-S-mephenytoin</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Bufuralol</td>
<td>5</td>
<td>1'-Hydroxybufuralol</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Midazolam</td>
<td>2.5</td>
<td>1'-Hydroxymidazolam</td>
</tr>
<tr>
<td>CYP3A b</td>
<td>Testosterone</td>
<td>50</td>
<td>6β-Hydroxytestosterone</td>
</tr>
</tbody>
</table>

a. Approximate Kₘ value
b. CYP3A activity was determined separately using testosterone as an additional probe substrate.
Estimated IC50 Values (μM) for the inhibition of CYP Enzymes by DVS-233 and Comparators in Human Liver Microsomes

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

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

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>Inhibitor</th>
<th>Conc (μM)</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>α-Naphthoflavone</td>
<td>20</td>
<td>20 ± 2.8</td>
</tr>
<tr>
<td>2A6</td>
<td>Tranylcypromine</td>
<td>10</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>2C8</td>
<td>Quercetin</td>
<td>20</td>
<td>20 ± 6.0</td>
</tr>
<tr>
<td>2C9</td>
<td>Sulfaphenazole</td>
<td>10</td>
<td>13 ± 1.4</td>
</tr>
<tr>
<td>2C19</td>
<td>Tranylcypromine</td>
<td>10</td>
<td>12 ± 1.7</td>
</tr>
<tr>
<td>2D6</td>
<td>Quinidine</td>
<td>50</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>3A</td>
<td>Ketoconazole</td>
<td>1</td>
<td>2.0 ± 1.5</td>
</tr>
</tbody>
</table>

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

Kᵢ 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 Kₘ and Vₘₐₓ values for CYP2D6-mediated bufuralol-1'-hydroxylation were 20 ± 3.6 μM and 97.4 ± 19.7 pmol min⁻¹ mg⁻¹, respectively (mean ± SD, n = 12).
### Ki Values and Mode of Inhibition for the Inhibition of CYP Enzymes by DVS-233 and Comparators in Human Microsomes

<table>
<thead>
<tr>
<th></th>
<th>CYP2D6</th>
<th>CYP2C19</th>
<th>CYP3A&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CYP3A&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVS-233</td>
<td>&gt; 300</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>93 (competitive)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>S, S Duloxetine</td>
<td>4.5 (competitive)</td>
<td>24 (competitive)</td>
<td>28 (mixed)</td>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>4.5 (competitive)</td>
<td>78 (mixed)</td>
<td>25 (non-competitive)</td>
<td>46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sertraline</td>
<td>3.8 (competitive)</td>
<td>28 (mixed)</td>
<td>43 (non-competitive)</td>
<td>23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bupropion</td>
<td>28 (competitive)</td>
<td>13 (competitive)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

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 Ki 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 Ki values are provided in the table above.

Ki 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).

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

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

<table>
<thead>
<tr>
<th>Human CYP450 Isozyme</th>
<th>Probe Substrate</th>
<th>Metabolite Monitored</th>
<th>$K_{m}$ (µM)</th>
<th>$V_{max}$ (pmol min$^{-1}$ mg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19</td>
<td>S-mephenytoin</td>
<td>4'-Hydroxy-S-mephenytoin</td>
<td>40 ± 6.0$^a$</td>
<td>50.0 ± 5.18$^a$</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Bufuralol</td>
<td>1'-Hydroxybufuralol</td>
<td>20 ± 3.6$^b$</td>
<td>97.4 ± 19.7$^b$</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Midazolam</td>
<td>1'-Hydroxymidazolam</td>
<td>4.9 ± 2.0$^c$</td>
<td>1580 ± 135$^c$</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Testosterone</td>
<td>6β-Hydroxytestosterone</td>
<td>67 ± 25$^{c,d}$</td>
<td>2770 ± 456$^c$</td>
</tr>
</tbody>
</table>

a. Values are mean ± SD of eight separate determinations each performed in duplicate
b. Values are mean ± SD of twelve separate determinations each performed in duplicate
c. Values are mean ± SD of six separate determinations each performed in duplicate
d. Represents S$_{50}$ value, gamma or $n = 1.23 ± 0.09$
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.
Title (RPT 59746): DVS-233: Inhibition of P-glycoprotein Activity in Caco-2 Monolayers: Comparator Study (Protocol 04_2291).

Objective: To determine IC₅₀ 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 ——.
**Analytical Method:** To an aliquot of medium from the apical (100 μL) or basolateral (250-300 μL) compartment was added (5 mL) and mixtures were counted for 10 min or until % σ 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 \( P_{app} = \frac{dQ/dt \times 10}{A \times C_0} \), where \( dQ/dt \) is the rate of drug appearance in the receiver compartment (umole/sec), \( C_0 \) is the initial drug concentration in the donor compartment (μM), and \( A \) is the surface area of the monolayer (cm²). These values (mean ± SD of n=3) were calculated in both A→B (apical to basolateral) and B→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 = \( (1 - (i_{A→B} - l_{A→B} / ab_{A→B} - aa_{A→B})) \times 100\% \), 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 the following tables and figures. DVS-233 and venlafaxine showed minimal inhibition (less than 20% control activity) of P-gp-mediated
digoxin efflux at the highest concentration used (250 μM), and IC\textsubscript{50} values could not be defined due to lack of concentration dependency. The IC\textsubscript{50} values for the inhibition of P-gp-mediated digoxin efflux by amitriptyline was 129 μM, and by S,S-duloxetine, paroxetine, sertraline, bupropion were extrapolated to be greater than 250 μM (highest concentration tested). The corresponding % inhibition at a putative inhibitor concentration of 250 μM were approximately 70, 40, 47, 45 and 36%, respectively. Verapamil, a known P-gp inhibitor, had an IC\textsubscript{50} value of 12.2 ± 1.5 μM under the same conditions used and showed 90% inhibition at an inhibitor concentration of 250 μM.

**Summary**: With the exception of verapamil which is a relatively potent inhibitor of P-gp activity (IC\textsubscript{50} value of 12.2 μM), all the other drugs evaluated in this study showed low inhibition of P-gp activity (extrapolated IC\textsubscript{50} values of >100 μM or not definable). Using a fluorimetric assay with calcein-acetoxyxymethylene as a P-gp substrate and two different cell systems (L-MDR1 cells and primary porcine brain endothelial cells), verapamil (IC\textsubscript{50} values of approximately 2-19 μM), paroxetine and sertraline (IC\textsubscript{50} values not definable to approximately 30 μM) showed higher inhibition of P-gp activity than O-desmethylvenlafaxine and venlafaxine (IC\textsubscript{50} values not definable). The sponsor stated that the relatively high IC\textsubscript{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.
Table 1. Inhibition of Pgp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of DVS-233 and Venlafaxine

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

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

Results are mean ± SD of three determinations each conducted on a separate day.
Table 2. Inhibition of P-gp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of S,S-Duloxetine and Paroxetine

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

<table>
<thead>
<tr>
<th>Paroxetine</th>
<th>( P_{\text{app}} ) (A to B), x 10-6 cm/sec (Mean ± SD)</th>
<th>( P_{\text{app}} ) (B to A), x 10-6 cm/sec (Mean ± SD)</th>
<th>( \frac{P_{\text{app}}(B \text{ to } A)}{P_{\text{app}}(A \text{ to } B)} ) Ratio</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.320 ± 0.017</td>
<td>8.65 ± 0.08</td>
<td>27.1 ± 1.4</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>0.291 ± 0.008</td>
<td>7.59 ± 0.41</td>
<td>26.1 ± 2.1</td>
<td>87.6 ± 4.6</td>
</tr>
<tr>
<td>2.5</td>
<td>0.295 ± 0.007</td>
<td>7.30 ± 0.13</td>
<td>24.7 ± 1.0</td>
<td>84.9 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>0.321 ± 0.023</td>
<td>7.13 ± 0.32</td>
<td>22.3 ± 2.6</td>
<td>81.7 ± 3.8</td>
</tr>
<tr>
<td>25</td>
<td>0.390 ± 0.012</td>
<td>6.80 ± 0.31</td>
<td>17.0 ± 0.8</td>
<td>76.6 ± 3.2</td>
</tr>
<tr>
<td>50</td>
<td>0.477 ± 0.018</td>
<td>6.39 ± 0.47</td>
<td>13.4 ± 1.4</td>
<td>71.0 ± 5.9</td>
</tr>
<tr>
<td>100</td>
<td>0.745 ± 0.027</td>
<td>5.48 ± 0.36</td>
<td>7.4 ± 0.7</td>
<td>56.9 ± 4.8</td>
</tr>
<tr>
<td>250</td>
<td>2.63 ± 0.332</td>
<td>7.08 ± 0.06</td>
<td>2.7 ± 0.3</td>
<td>53.3 ± 3.8</td>
</tr>
</tbody>
</table>

Results are mean ± SD of three determinations each conducted on a separate day.
Table 3. Inhibition of P-gp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of Sertraline and Bupropion

<table>
<thead>
<tr>
<th>Sertraline (uM)</th>
<th>$P_{app}$ (A to B), x 10^{-6} \text{ cm/sec} (Mean ± SD)</th>
<th>$P_{app}$ (B to A), x 10^{-6} \text{ cm/sec} (Mean ± SD)</th>
<th>$P_{app}$ (B to A)/$P_{app}$ (A to B) Ratio</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.357 ± 0.020</td>
<td>8.64 ± 0.31</td>
<td>24.3 ± 2.2</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>0.334 ± 0.097</td>
<td>7.62 ± 0.13</td>
<td>22.9 ± 1.0</td>
<td>88.1 ± 2.2</td>
</tr>
<tr>
<td>2.5</td>
<td>0.381 ± 0.076</td>
<td>7.48 ± 0.35</td>
<td>20.2 ± 4.3</td>
<td>85.7 ± 3.5</td>
</tr>
<tr>
<td>10</td>
<td>0.372 ± 0.061</td>
<td>7.53 ± 0.13</td>
<td>20.6 ± 3.4</td>
<td>86.4 ± 1.9</td>
</tr>
<tr>
<td>25</td>
<td>0.381 ± 0.043</td>
<td>6.77 ± 0.18</td>
<td>17.9 ± 1.9</td>
<td>77.2 ± 2.8</td>
</tr>
<tr>
<td>50</td>
<td>0.448 ± 0.042</td>
<td>6.83 ± 0.37</td>
<td>15.3 ± 1.1</td>
<td>77.1 ± 3.3</td>
</tr>
<tr>
<td>100</td>
<td>0.582 ± 0.071</td>
<td>5.80 ± 0.18</td>
<td>10.0 ± 0.9</td>
<td>63.1 ± 3.7</td>
</tr>
<tr>
<td>250</td>
<td>2.32 ± 1.59</td>
<td>6.93 ± 0.31</td>
<td>4.0 ± 2.3</td>
<td>55.4 ± 14</td>
</tr>
</tbody>
</table>

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

Results are mean ± SD of three determinations each conducted on a separate day.
Table 4. Inhibition of P-gp-mediated Digoxin Efflux in Caco-2 Monolayers in the Absence or Presence of Amitriptyline and Verapamil

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

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

Results are mean ± SD of three determinations each conducted on a separate day.
Table 5. IC₅₀ Values for the Inhibition of P-gp-Mediated Digoxin Efflux in Caco-2 Monolayers

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVS-233</td>
<td>NC¹</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>NC¹</td>
</tr>
<tr>
<td>Duloxetine²</td>
<td>354 ± 55</td>
</tr>
<tr>
<td>Paroxetine²</td>
<td>262 ± 34</td>
</tr>
<tr>
<td>Sertraline²</td>
<td>346 ± 115</td>
</tr>
<tr>
<td>Bupropion²</td>
<td>768 ± 205</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>129 ± 6.6</td>
</tr>
<tr>
<td>Verapamil</td>
<td>12.2 ± 1.5</td>
</tr>
</tbody>
</table>

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

²extrapolated values

Results are mean ± SD of three determinations each conducted on a separate day.
Figure 2. Inhibition of P-gp-Mediated Digoxin Efflux in Caco-2 Monolayers by DVS-233 and Venlafaxine

DVS-233

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

**Duloxetine**

activity (% of control) vs. conc (μM)

**Paroxetine**

activity (% of control) vs. conc (μM)
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.
Study Title: The Disposition of $^{14}$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 $^{14}$C-Labeled Venlafaxine was approximately 1 μCi/mg venlafaxine (free base).

Data Analysis: Percent of the administered dose of $^{14}$C-Labeled Venlafaxine appearing as each metabolite during each urine collection period was calculated as follows:

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

Where $X = \text{percent of dose appearing as the metabolite}$
$A = \text{dpm in radioactive peak of the metabolite}$
$B = \text{total dpm recovered from chromatograph}$
$C = \text{dpm in the total urine of the collection period}$
$D = \text{dpm administered as $^{14}$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, Wv-45,233, Other Metabolites and Total Radioactivity in Ten Male Subjects Following a Single Oral 50 mg Dose of [14C]Venlafaxine, as the Hydrochloride Salt.

<table>
<thead>
<tr>
<th>Hours after dosing</th>
<th>Plasma concentration (mean ± SD ng equivalents/ml)</th>
<th>Other metabolites</th>
<th>Total radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>BQL(^a)</td>
<td>3 ± 6</td>
<td>3 ± 8</td>
</tr>
<tr>
<td>1</td>
<td>20 ± 16</td>
<td>37 ± 36</td>
<td>30 ± 18</td>
</tr>
<tr>
<td>2</td>
<td>46 ± 16</td>
<td>79 ± 32</td>
<td>70 ± 36</td>
</tr>
<tr>
<td>4</td>
<td>41 ± 20</td>
<td>94 ± 42</td>
<td>157 ± 56</td>
</tr>
<tr>
<td>6</td>
<td>28 ± 18</td>
<td>92 ± 39</td>
<td>172 ± 61</td>
</tr>
<tr>
<td>8</td>
<td>18 ± 13</td>
<td>82 ± 31</td>
<td>169 ± 59</td>
</tr>
<tr>
<td>12</td>
<td>6 ± 7</td>
<td>62 ± 20</td>
<td>141 ± 41</td>
</tr>
<tr>
<td>16</td>
<td>2 ± 4</td>
<td>45 ± 17</td>
<td>134 ± 34</td>
</tr>
<tr>
<td>24</td>
<td>BQL</td>
<td>27 ± 12</td>
<td>77 ± 21</td>
</tr>
<tr>
<td>36</td>
<td>BQL</td>
<td>11 ± 7</td>
<td>34 ± 14</td>
</tr>
<tr>
<td>48</td>
<td>BQL</td>
<td>2 ± 4</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>72</td>
<td>BQL</td>
<td>BQL</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>96</td>
<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
</tr>
<tr>
<td>120</td>
<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
</tr>
</tbody>
</table>

\(^a\)BQL = below quantifiable limits

APPEARS THIS WAY ON ORIGINAL
Plasma Concentrations in Human Subjects Receiving 50 mg of 14C Venlafaxine

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 ±
5.0% of the dose appeared in the urine as Wy-45233 and 26.4 ± 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 ± 1.3% as the glucuronide. Unidentified metabolite peaks in urine comprised 0.9 ± 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
### Table II. Ratio of the Radioactive Concentration of Plasma and Whole Blood in 10 Male Subjects Following a Single Oral 50 mg Dose of 
of $^{14}$CVenlafaxine, as the Hydrochloride Salt.

<table>
<thead>
<tr>
<th>Hours after dosing</th>
<th>Whole blood $^{14}$C (dpm/ml)$^a$</th>
<th>Plasma $^{14}$C (dpm/ml)$^b$</th>
<th>Plasma radioactivity</th>
<th>Whole blood radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>11 ± 34</td>
<td>14 ± 17</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>224 ± 121</td>
<td>177 ± 96</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>526 ± 126</td>
<td>453 ± 118</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>719 ± 124</td>
<td>677 ± 141</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>684 ± 117</td>
<td>578 ± 151</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>635 ± 191</td>
<td>654 ± 136</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>402 ± 105</td>
<td>483 ± 107</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>380 ± 119</td>
<td>416 ± 94</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>258 ± 44</td>
<td>240 ± 55</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>76 ± 86</td>
<td>103 ± 37</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>23 ± 49</td>
<td>51 ± 18</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>BQL$^b$</td>
<td>12 ± 5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>BQL</td>
<td>BQL</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>BQL</td>
<td>BQL</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Mean (0-72 hr) 1.2 ± 0.4
Mean (0-36 hr) 1.1 ± 0.2

$^a$Mean ± SD

$^b$BQL = below quantifiable limits (<10 dpm)
Table VII. Plasma Concentrations of Venlafaxine, Wv-45,233, Other Metabolites and Total Radioactivity in Ten Male Subjects Following a Single Oral 50 mg Dose of [14C]Venlafaxine, as the Hydrochloride Salt.

<table>
<thead>
<tr>
<th>Hours after dosing</th>
<th>Plasma concentration (mean ± SD mg equivalents/ml)</th>
<th>Other metabolites</th>
<th>Total radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>BQL</td>
<td>3 ± 6</td>
<td>3 ± 8</td>
</tr>
<tr>
<td>1</td>
<td>20 ± 16</td>
<td>37 ± 36</td>
<td>30 ± 18</td>
</tr>
<tr>
<td>2</td>
<td>46 ± 16</td>
<td>79 ± 32</td>
<td>70 ± 36</td>
</tr>
<tr>
<td>4</td>
<td>41 ± 20</td>
<td>94 ± 42</td>
<td>157 ± 56</td>
</tr>
<tr>
<td>6</td>
<td>28 ± 18</td>
<td>92 ± 39</td>
<td>172 ± 61</td>
</tr>
<tr>
<td>8</td>
<td>18 ± 13</td>
<td>82 ± 31</td>
<td>169 ± 59</td>
</tr>
<tr>
<td>12</td>
<td>6 ± 7</td>
<td>62 ± 20</td>
<td>141 ± 41</td>
</tr>
<tr>
<td>16</td>
<td>2 ± 4</td>
<td>45 ± 17</td>
<td>134 ± 34</td>
</tr>
<tr>
<td>24</td>
<td>BQL</td>
<td>27 ± 12</td>
<td>77 ± 21</td>
</tr>
<tr>
<td>36</td>
<td>BQL</td>
<td>11 ± 7</td>
<td>34 ± 14</td>
</tr>
<tr>
<td>48</td>
<td>BQL</td>
<td>2 ± 4</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>72</td>
<td>BQL</td>
<td>BQL</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>96</td>
<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
</tr>
<tr>
<td>120</td>
<td>BQL</td>
<td>BQL</td>
<td>BQL</td>
</tr>
</tbody>
</table>

*BQL = below quantifiable limits*
Table X.  \(C_{\text{max}}\) 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 \([14\text{C}]\)Venlafaxine, as the Hydrochloride Salt.

<table>
<thead>
<tr>
<th>Subject</th>
<th>(C_{\text{max}}) (ng Venlafaxine equiv./ml)</th>
<th>AUC0-24 hr (ng Venlafaxine equiv. hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Other</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>59</td>
<td>119</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>176</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>121</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>122</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>126</td>
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<tr>
<td>6</td>
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<td>102</td>
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<tr>
<td>7</td>
<td>55</td>
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<tr>
<td>8</td>
<td>87</td>
<td>30</td>
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<tr>
<td>9</td>
<td>49</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>85</td>
</tr>
<tr>
<td>Mean</td>
<td>48</td>
<td>109</td>
</tr>
<tr>
<td>±SD</td>
<td>±27</td>
<td>±38</td>
</tr>
</tbody>
</table>

579
Table XIV. Mean Values for Percent of Radioactivity in 10 Male Subjects Following a Single Oral Dose of $[^{14}C]$Venlafaxine, as the Hydrochloride Salt$^a$.

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

$^a$Data for subjects 1-8, 10 only.

$^b$Hours after dosing.

$^c$0-24 hr time period.
Table XV. Urinary Metabolites Excreted by 9 Male Subjects up to 48 hr Following a Single Oral 50 mg Dose of [$^{14}C]$Venlafaxine, as the Hydrochloride Salt.

<table>
<thead>
<tr>
<th>Percent of Administered Dose</th>
<th>Wy-45,233</th>
<th>Wy-46,689</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject Venlafaxine</td>
<td>Wy-45,233</td>
<td>Wy-46,689</td>
</tr>
<tr>
<td>Gluc.</td>
<td>Wy-46,689</td>
<td>Gluc.</td>
</tr>
<tr>
<td>Metabolite A</td>
<td>Wy-46,965</td>
<td></td>
</tr>
<tr>
<td>Metabolite B</td>
<td>Wy-45,494</td>
<td></td>
</tr>
</tbody>
</table>

<p>| | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>6</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean 4.7 29.4 26.4 9.0 6.2 2.7 2.2 1.0 1.0 0.9

$^a$Subject 9 was excluded due to a missing urine sample.

$^b$Metabolite A was an unidentified conjugate.

$^c$Metabolite B was an unidentified metabolite, not a glucuronide or sulfate conjugate.

$^d$Other - the sum of minor unidentified metabolite peaks.
Plasma Concentrations in Human Subjects Receiving 50 mg of 14C Venlafaxine
Table XIV. Mean Values for Percent of Radioactivity in 10 Male Subjects Following a Single Oral Dose of $^{14}C$Venlafaxine, as the Hydrochloride Salt$^a$.

<table>
<thead>
<tr>
<th>Route</th>
<th>0-4$^b$</th>
<th>4-8</th>
<th>8-12</th>
<th>12-24</th>
<th>24-48</th>
<th>48-72</th>
<th>72-96</th>
<th>96-120</th>
<th>Total (0-120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>8.63</td>
<td>18.1</td>
<td>14.3</td>
<td>26.9</td>
<td>18.4</td>
<td>3.93</td>
<td>0.82</td>
<td>0.31</td>
<td>92.1</td>
</tr>
<tr>
<td>Urine SD</td>
<td>5.45</td>
<td>7.59</td>
<td>3.66</td>
<td>6.17</td>
<td>4.22</td>
<td>2.00</td>
<td>0.52</td>
<td>0.26</td>
<td>8.1</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Mean</td>
<td>0.6$^c$</td>
<td>0.9</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>Feces SD</td>
<td>0.3</td>
<td>0.7</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Urine Mean</td>
<td>69.2$^c$</td>
<td>19.2</td>
<td>4.33</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>94.1</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>plus SD</td>
<td>7.35</td>
<td>3.8</td>
<td>2.0</td>
<td>0.6</td>
<td>0.5</td>
<td>0.5</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Data for subjects 1-8, 10 only.

$^b$Hours after dosing.

$^c$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 [14C]Venlafaxine, as the Hydrochloride Salt.

<table>
<thead>
<tr>
<th>Subject</th>
<th>0-4h</th>
<th>4-8</th>
<th>8-12</th>
<th>12-24</th>
<th>24-48</th>
<th>48-72</th>
<th>72-96</th>
<th>96-120</th>
<th>Total (0-120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.9</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>81.6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>85.0</td>
</tr>
<tr>
<td>4</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.7</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>100.1</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.5</td>
</tr>
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\(a\) Hours after dosing.
\(b\) NS = no sample obtained.

Metabolic Pathways of Venlafaxine in Healthy Subjects
Pharmacokinetics of Wy-45,233 in Male Subjects Receiving a Single Oral 50 mg Dose of $[^{14}C]$ Venlafaxine, as the Hydrochloride Salt
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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 [14C] Venlafaxine, as the Hydrochloride Salt as the Hydrochloride Salt

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*Subject Number

bND = not detected

ng/ml was lower limit of quantitation

[] = Spurious value; not used in calculations
### Office of Clinical Pharmacology and Biopharmaceutics

**New Drug Application Filing and Review Form**

1. **General Information About the Submission**

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<th>Number of studies reviewed</th>
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#### 1. Clinical Pharmacology

- **Mass balance:**
  - X
  - 1

- **Isoenzyme characterization:**
  - X
  - 1

- **Blood/plasma ratio:**
  - X
  - 1

- **Pharmacokinetics (e.g., Phase I):**
  - Healthy Volunteers:
    - Single dose: X
      - 9
    - Multiple dose: X
      - 1

  - Patients:
    - Single dose:
    - Multiple dose:

- **Dose proportionality:**
  - Fasting / non-fasting single dose: X
    - 1
  - Fasting / non-fasting multiple dose: X

- **Drug-drug interaction studies:**
  - In-vivo effects on primary drug: X
    - 1
  - In-vivo effects of primary drug: X
    - 3
  - In-vitro:

- **Subpopulation studies:**
  - Ethnicity: X
    - 1
  - Gender: X
    - 1
  - Pediatrics:
  - Geriatrics: X
    - 1
  - Renal impairment: X
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  - Hepatic impairment: X
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589
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**Population Analyses -**

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**II. Biopharmaceutics**

**Absolute bioavailability:**

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**Relative bioavailability -**

| alternate formulation as reference: | X | 1 |

**Bioequivalence studies -**

| traditional design, single / multi dose: |   |   |
| replicate design, single / multi dose:   |   |   |

**Food-drug interaction studies:**

| X | 2 |

**Dissolution:**

| (IVIVC): | X | 1 |
| Bio-waiver request based on BCS |   |   |
| BCS class |   |   |

**III. Other CPB Studies**

| Genotype/phenotype studies: |   |   |
| Effect of DVS on QTc | X | 1 |
| Pediatric development plan |   |   |
| Literature References |   |   |

**Total Number of Studies:**

| 21 |

---

**Filability and QBR comments**

| "X" if yes |   |

**Comments**

- 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?

**Application filable?**

|   |   |

**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)**

1. What is the relationship between exposure to DVS and efficacy/safety?
2. Are there intrinsic factors that affect the pharmacokinetics of DVS?
3. Are there any extrinsic factors that affect the PK of DVS?
4. What is the Bioavailability and metabolic characteristics of DVS?
5. Is the IVIVC reported by the sponsor substantiated by the data?

**Other comments or information not included above**

| This application is all electronic. Link to EDR |

**Primary reviewer Signature and Date**

| Kofi A. Kumi |   |

---

590
Secondary reviewer Signature and Date

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

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

/s/

Kofi Kumi
10/26/2006 11:34:28 AM
BIOPHARMACEUTICS

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

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**Fitability and QBR comments**

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**QBR questions (key issues to be considered)**

1. What is the relationship between exposure to DVS and efficacy/safety?
2. Are there intrinsic factors that affect the pharmacokinetics of DVS?
3. Are there any extrinsic factors that affect the PK of DVS?
4. What is the Bioavailability and metabolic characteristics of DVS?
5. Is the IVIVC reported by the sponsor substantiated by the data?

**Other comments or information not included above**

This application is all electronic. Link to EDR

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**Primary reviewer Signature and Date**

Kofi A. Kumi

**Secondary reviewer Signature and Date**

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