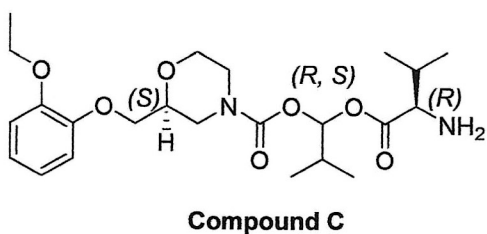
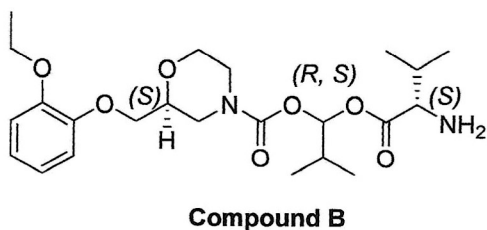
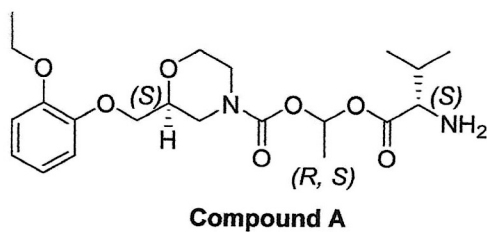


## Experimental report

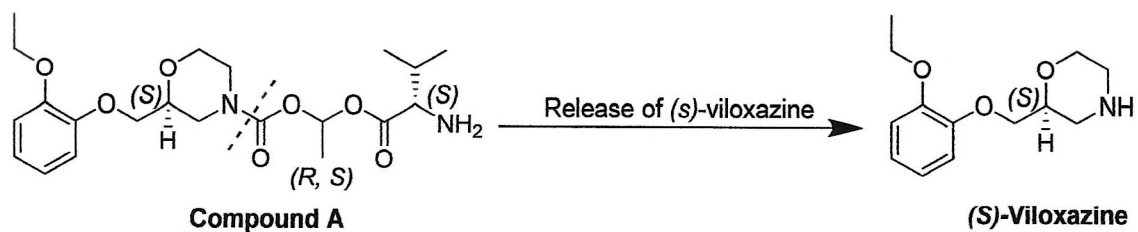
**Example 1. *In Vitro* Metabolic Conversion of Compound A, Compound B and Compound C in Human Intestinal homogenate**

An *in vitro* study was conducted to assess the metabolic stability of Compound A, Compound B and Compound C in human intestinal homogenate by monitoring both the disappearance of initial compound and formation of metabolite.

The structures of Compound A, Compound B and Compound C are shown below.



### Exemplary Reaction Scheme



### Preparation of Solutions

**Incubation Buffer (PBK):** 50 mM potassium phosphate buffer (PBK) was prepared from 1 M potassium phosphate buffer, pH 7.2.

**Test Article Solutions:** Compound A, Compound B and Compound C stock solutions at 10 mM were prepared in DMSO. The dilution of 1 mM intermediate was prepared from 10 mM stock with 90% methanol/water, then 10  $\mu$ M working solutions were prepared by diluting the 1 mM intermediates with 50 mM PBK. All working solutions were freshly prepared on the day of experiment and disposed after use.

**Positive Control Stock and Working Solution:** the positive control stock solutions, testosterone and 7-hydroxycoumarin were prepared at 10 mM in DMSO and stored at approximately -20°C. Prior to use, the stock solutions were brought to room temperature and thoroughly mixed. The working solutions were freshly prepared similarly as described for the test article working solution.

**Internal Standard Solutions and Stop Solutions:** the stock solution of internal standards, tolbutamide and labetalol, were prepared in DMSO and stored at approximately -20°C. The stop solution was prepared by spiking the stock solution (1 mg/mL) into acetonitrile to achieve a 200 ng/mL final concentration.

**Test System:** the human intestinal homogenate was diluted with 50 mM PBK buffer to make mixture solution of 0.625 mg/mL protein.

**Cofactors Solutions:** the cofactor solution was made at 10 mM of NADPH (nicotinamide-adenine dinucleotide phosphate, reduced form) in 50 mM PBK buffer.

### Assay Procedures

The incubation was carried out in 96-well plates. The intestinal homogenate mixture solution was added to 96-well plates (80  $\mu$ L/well) in duplicate. The plates were preincubated for 10 min at 37°C in a water bath and then spiked 10  $\mu$ L each of 10  $\mu$ M Compound A, Compound B and Compound C or positive control working solutions into correspondence wells, separately. Reaction was initiated by adding cofactor solution at 10  $\mu$ L/well. The plates were incubated at 37°C in a water bath with shaking. The final incubation mixture contained Compound A, Compound B and Compound C, or positive control at 1  $\mu$ M, and 0.5 mg/mL of human intestinal homogenate, and 1 mM NADPH. The final organic solvent content in the incubation was  $\leq$ 1%.

At specified time points, i.e., 5, 10, 20, 30 and 60 minutes, NCF60 (no cofactor at 60 min, without adding NADPH replaced with 50 mM PBK buffer) reaction was stopped by adding three volumes of stop solution. The time zero ( $T_0$ ) samples were prepared by adding three volumes of stop solution to intestinal homogenate samples, followed by addition of test article or control, and cofactor solution 10  $\mu$ L/well.

All sample plates were mixed well by shaking for approximately 10 minutes and centrifuged at 3220 x g for 20 minutes. Subsequently, 100  $\mu$ L of supernatant was removed from each well, diluted with 100  $\mu$ L pure water and analyzed by LC/MS/MS.

## Results

The results of metabolic conversion of Compound A, Compound B and Compound C in human intestinal homogenate, including percent remaining,  $t_{1/2}$  (min), and intrinsic clearance values ( $CL_{int(HIH)}$ ) are summarized in **Table 1a**, formation of metabolite viloxazine is summarized in **Table 1b**.

**Table 1a.** Metabolic Stability of Compound A, Compound B and Compound C in Human Intestinal Homogenate at 60-Minute Incubation.

| Compound ID | Time (min) | Analyte Peak area/ IS<br>Peak area | %Remaining | t <sub>1/2</sub><br>(min) | CL <sub>int(HIH)</sub><br>(µL/min/mg) |
|-------------|------------|------------------------------------|------------|---------------------------|---------------------------------------|
|             |            | Mean (n=2)                         |            |                           |                                       |
| Compound A  | 0          | 0.269                              | 100        | <2.5                      | N/A                                   |
|             | 5          | 0.000                              | 0          |                           |                                       |
|             | 10         | 0.000                              | 0          |                           |                                       |
|             | 20         | 0.000                              | 0          |                           |                                       |
|             | 30         | 0.000                              | 0          |                           |                                       |
|             | 60         | 0.000                              | 0          |                           |                                       |
|             | NCF60      | 0.000                              | 0          | N/A                       |                                       |
| Compound B  | 0          | 0.275                              | 100        | 10.3                      | 135                                   |
|             | 5          | 0.020                              | 7.3        |                           |                                       |
|             | 10         | 0.020                              | 7.1        |                           |                                       |
|             | 20         | 0.017                              | 6.1        |                           |                                       |
|             | 30         | 0.007                              | 2.7        |                           |                                       |
|             | 60         | 0.002                              | 0.57       |                           |                                       |
|             | NCF60      | 0.002                              | 0.8        | N/A                       |                                       |
| Compound C  | 0          | 0.286                              | 100        | 7.20                      | 192                                   |
|             | 5          | 0.013                              | 4.4        |                           |                                       |
|             | 10         | 0.009                              | 3.0        |                           |                                       |
|             | 20         | 0.004                              | 1.3        |                           |                                       |
|             | 30         | 0.002                              | 0.67       |                           |                                       |
|             | 60         | 0.000                              | 0.08       |                           |                                       |
|             | NCF60      | 0.000                              | 0.12       | N/A                       |                                       |

**Table 1b.** Formation of Viloxazine in Human Intestinal Homogenate at 60-Minute Incubation.

| Species | Time (min) | Viloxazine Formed (Analyte Peak area/IS Peak area) |                 |                 |
|---------|------------|----------------------------------------------------|-----------------|-----------------|
|         |            | From Compound A                                    | From Compound B | From Compound C |
| Human   | 0          | 0.50                                               | 0.17            | 0.18            |
|         | 5          | 1.17                                               | 1.00            | 1.11            |
|         | 10         | 1.22                                               | 1.00            | 1.10            |
|         | 20         | 1.21                                               | 0.99            | 1.19            |
|         | 30         | 1.21                                               | 1.04            | 1.01            |
|         | 60         | 1.19                                               | 0.97            | 1.10            |
|         | NCF60      | 1.22                                               | 1.06            | 1.17            |

The results of positive controls in human intestinal homogenate, including percent remaining,  $t_{1/2}$  (min), and intrinsic clearance values ( $C_{\text{Lint(HIH)}}$ ) are summarized in Table 1c.

**Table 1c. Metabolic Stability of Positive Controls in Human Intestinal Homogenate**

| Compound          | Species | %Remaining at 60 min | $t_{1/2}$ (min) | $C_{\text{Lint(HIH)}}$ ( $\mu\text{L/min/mg}$ ) |
|-------------------|---------|----------------------|-----------------|-------------------------------------------------|
| Testosterone      | Human   | 63.7                 | 91.2            | 15.1                                            |
| 7-hydroxycoumarin |         | 94.2                 | >187            | <7.4                                            |

Compound A, Compound B and Compound C were rapidly metabolized in human intestinal homogenate, formation of metabolite viloxazine at 5 minutes was significantly higher than that of time zero but did not increase further with incubation time.

### **Example 2. Evaluation of Pharmacokinetics Following Single Oral Administration to Male and Female Beagle Dogs**

A study was carried out to evaluate the pharmacokinetic (PK) properties of Compound A, Compound B and Compound C, and S(-)- and R(+)-viloxazine following single oral (PO) administration to male and female beagle dogs.

**Study Design:** Compound A, Compound B and Compound C each was given to two male and two female beagle dogs at 80 mg/kg by oral gavage. The animals were fasted overnight prior to dosing. Food was returned 4 hours post-dose. Animals had access to water freely the entire time throughout the study. Blood samples were collected from each animal at 0.5, 1, 2, 4, 6, 8 and 24 hours post-dose, and then centrifuged to extract plasma for the determination of the concentrations of Compound A, Compound B and Compound C and the two viloxazine isomers, S(-)- and R(+)-viloxazine. The concentrations of the analytes in the plasma were quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The bioanalytical assay provided a lower limit of quantification (LLOQ) of 1.5 ng/mL and an upper limit of quantification (ULOQ) of 1500 ng/mL for the two viloxazine isomers, and a LLOQ of 3.0 ng/mL and an ULOQ of 3000 ng/mL for the three Compounds. The data of plasma concentration vs. time were analyzed using Phoenix WinNonlin 8.3 to determine the PK properties of the analytes. The non-compartmental analysis model and the linear log trapezoidal method were applied to the PK calculation.

**Test Article Formulation:** Dose solutions were prepared freshly on the day of study prior to dosing. The formulations of Compounds A, B, and C were prepared the same way using HPLC water as vehicle. The target concentration of Compound A, Compound B and Compound C was 16 mg/mL; when administered at the nominal dose volume of 5 mL/kg, it yielded a target dosage of 80 mg/kg of Compound A, Compound B and Compound C.

### **Results**

**Clinical Observation:** Compound A, Compound B and Compound C at the administered dosages was well tolerated by all animals. No adverse effects were observed throughout the study.

With respect to Compound A and Compound B group, following oral administration at 80 mg/kg, their concentration in the plasma was mostly below the LLOQ in both the male and female beagle dogs. Therefore, the PK properties of Compound A and Compound B were not determined. The

corresponding PK parameters are summarized in **Tables 2a-2c**. The plasma concentration-time profiles of Compound C and S(-)- and R(+)-viloxazine are shown in **FIGS.1-6**.

**Table 2a.** Mean PK parameters of S(-)-viloxazine and R(+)-viloxazine following single PO administration of Compound A to male (D1001, D1002) and female (D1501, D1502) beagle dogs at 80 mg/kg.

| Time (h)                              | S(-)-viloxazine |        | R(+)-viloxazine |        |
|---------------------------------------|-----------------|--------|-----------------|--------|
|                                       | Male            | Female | Male            | Female |
| <b>Rs<sub>q</sub>_adj</b>             | 0.998           | 0.970  | 0.982           | 0.989  |
| <b>C<sub>max</sub> (ng/mL)</b>        | 10032           | 11922  | 983             | 1064   |
| <b>T<sub>max</sub> (h)</b>            | 1.25            | 1.00   | 1.25            | 1.00   |
| <b>T<sub>1/2</sub> (h)</b>            | 3.56            | 2.24   | 4.08            | 2.58   |
| <b>T<sub>last</sub> (h)</b>           | 24.0            | 16.0   | 24.0            | 16.0   |
| <b>AUC<sub>0-last</sub> (ng·h/mL)</b> | 57582           | 59434  | 4185            | 3976   |
| <b>AUC<sub>0-inf</sub> (ng·h/mL)</b>  | 58192           | 61297  | 4254            | 4076   |
| <b>MRT<sub>0-last</sub> (h)</b>       | 5.34            | 3.89   | 4.85            | 3.50   |
| <b>MRT<sub>0-inf</sub> (h)</b>        | 5.59            | 4.17   | 5.25            | 3.77   |

**Table 2b.** Mean PK parameters of S(-)-viloxazine and R(+)-viloxazine following single PO administration of Compound B to male (D2001, D2002) and female (D2501, D2502) beagle dogs at 80 mg/kg.

| Time (h)                              | S(-)-viloxazine |        | R(+)-viloxazine |        |
|---------------------------------------|-----------------|--------|-----------------|--------|
|                                       | Male            | Female | Male            | Female |
| <b>Rs<sub>q</sub>_adj</b>             | 0.986           | 0.999  | 0.978           | 0.993  |
| <b>C<sub>max</sub> (ng/mL)</b>        | 5112            | 5039   | 524             | 542    |
| <b>T<sub>max</sub> (h)</b>            | 1.50            | 1.50   | 1.50            | 1.00   |
| <b>T<sub>1/2</sub> (h)</b>            | 2.98            | 2.70   | 3.26            | 3.54   |
| <b>T<sub>last</sub> (h)</b>           | 16.0            | 24.0   | 16.0            | 16.0   |
| <b>AUC<sub>0-last</sub> (ng·h/mL)</b> | 27111           | 32021  | 2061            | 2581   |
| <b>AUC<sub>0-inf</sub> (ng·h/mL)</b>  | 27898           | 32188  | 2123            | 2736   |
| <b>MRT<sub>0-last</sub> (h)</b>       | 4.00            | 4.82   | 3.71            | 3.90   |
| <b>MRT<sub>0-inf</sub> (h)</b>        | 4.55            | 4.91   | 4.27            | 4.94   |

**Table 2c.** Mean PK parameters of Compound C, S(-)-viloxazine and R(+)-viloxazine following single PO administration to male (D3001, D3002) and female (D3501, D3502) beagle dogs at 80 mg/kg.

| Time (h)                              | Compound C |        | S(-)-viloxazine |        | R(+)-viloxazine |        |
|---------------------------------------|------------|--------|-----------------|--------|-----------------|--------|
|                                       | Male       | Female | Male            | Female | Male            | Female |
| <b>Rs<sub>q</sub>_adj</b>             | 0.991      | 0.924  | 0.979           | 0.987  | 0.977           | 0.984  |
| <b>C<sub>max</sub> (ng/mL)</b>        | 775        | 308    | 6059            | 4247   | 495             | 371    |
| <b>T<sub>max</sub> (h)</b>            | 0.500      | 0.500  | 0.750           | 1.00   | 0.750           | 0.500  |
| <b>T<sub>1/2</sub> (h)</b>            | 2.94       | 4.73   | 2.65            | 2.06   | 3.03            | 2.46   |
| <b>T<sub>last</sub> (h)</b>           | 16.0       | 16.0   | 16.0            | 16.0   | 8.00            | 16.0   |
| <b>AUC<sub>0-last</sub> (ng·h/mL)</b> | 1186       | 572    | 28629           | 21120  | 1824            | 1441   |
| <b>AUC<sub>0-inf</sub> (ng·h/mL)</b>  | 1203       | 591    | 31005           | 21354  | 2154            | 1466   |
| <b>MRT<sub>0-last</sub> (h)</b>       | 2.58       | 3.16   | 3.59            | 3.47   | 2.88            | 3.42   |
| <b>MRT<sub>0-inf</sub> (h)</b>        | 2.80       | 4.78   | 4.43            | 3.67   | 4.44            | 3.67   |

FIG. 1 shows plasma concentration-time profiles of S(-)-viloxazine following single PO administration of Compound A to male (top) and female (middle) beagle dogs at 80 mg/kg, as well as the corresponding mean (bottom) plasma concentration-time profiles.

FIG. 2 shows plasma concentration-time profiles of R(+)-viloxazine following single PO administration of Compound A to male (top) and female (middle) beagle dogs at 80 mg/kg, as well as the corresponding mean (bottom) plasma concentration-time profiles.

FIG. 3 shows plasma concentration-time profiles of S(-)-viloxazine following single PO administration of Compound B to male (top) and female (middle) beagle dogs at 80 mg/kg, as well as the corresponding mean (bottom) plasma concentration-time profiles.

FIG. 4 shows plasma concentration-time profiles of R(+)-viloxazine following single PO administration of Compound B to male (top) and female (middle) beagle dogs at 80 mg/kg, as well as the corresponding mean (bottom) plasma concentration-time profiles.

FIG. 5 shows plasma concentration-time profiles of Compound C following single PO administration to male (top) and female (middle) beagle dogs at 80 mg/kg, as well as the corresponding mean (bottom) plasma concentration-time profiles.

FIG. 6 shows plasma concentration-time profiles of S(-)-viloxazine following single PO administration of Compound C to male (top) and female (middle) beagle dogs at 80 mg/kg, as well as the corresponding mean (bottom) plasma concentration-time profiles.

FIG. 7 shows plasma concentration-time profiles of R(+)-viloxazine following single PO administration of Compound C to male (top) and female (middle) beagle dogs at 80 mg/kg, as well as the corresponding mean (bottom) plasma concentration-time profiles.

The data showed that the  $C_{max}$  and  $AUC_{0-last}$  of the S(-)-viloxazine were consistently higher (about 10x) than those of the R(+)-viloxazine following PO administration of Compounds A, B, and C, indicating a better yield of the S(-)-isomer than the R(+)-isomer for all three compounds. The PK properties of either isomer were comparable among the three compounds. The only difference between the three compounds was that the plasma concentrations of Compound C were well above LLOQ, while the plasma concentrations of Compounds A and B were mostly below the LLOQ. The study did not find significant differences in any measured PK parameters between the male and female dogs.

**FIG. 1**

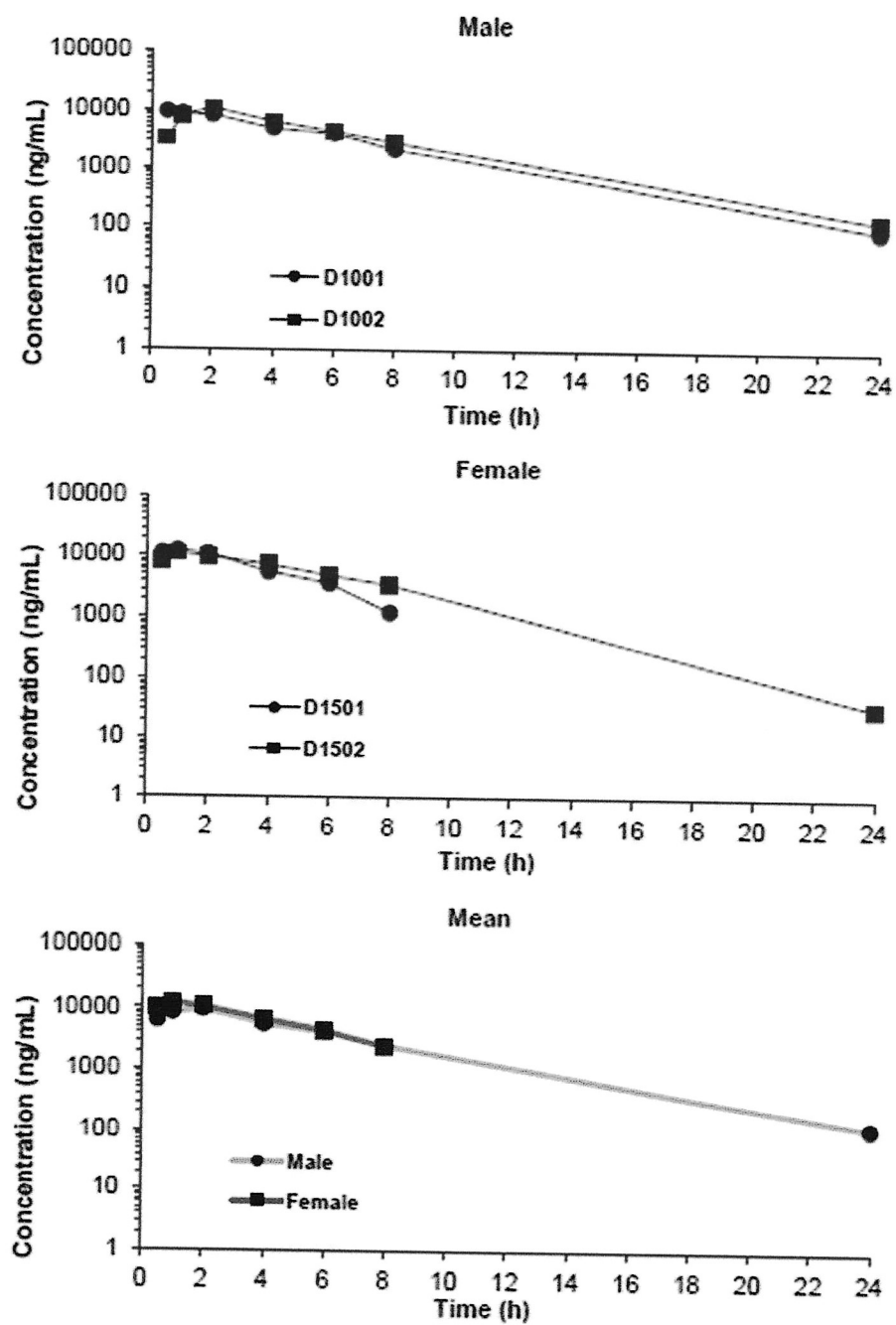


FIG. 2

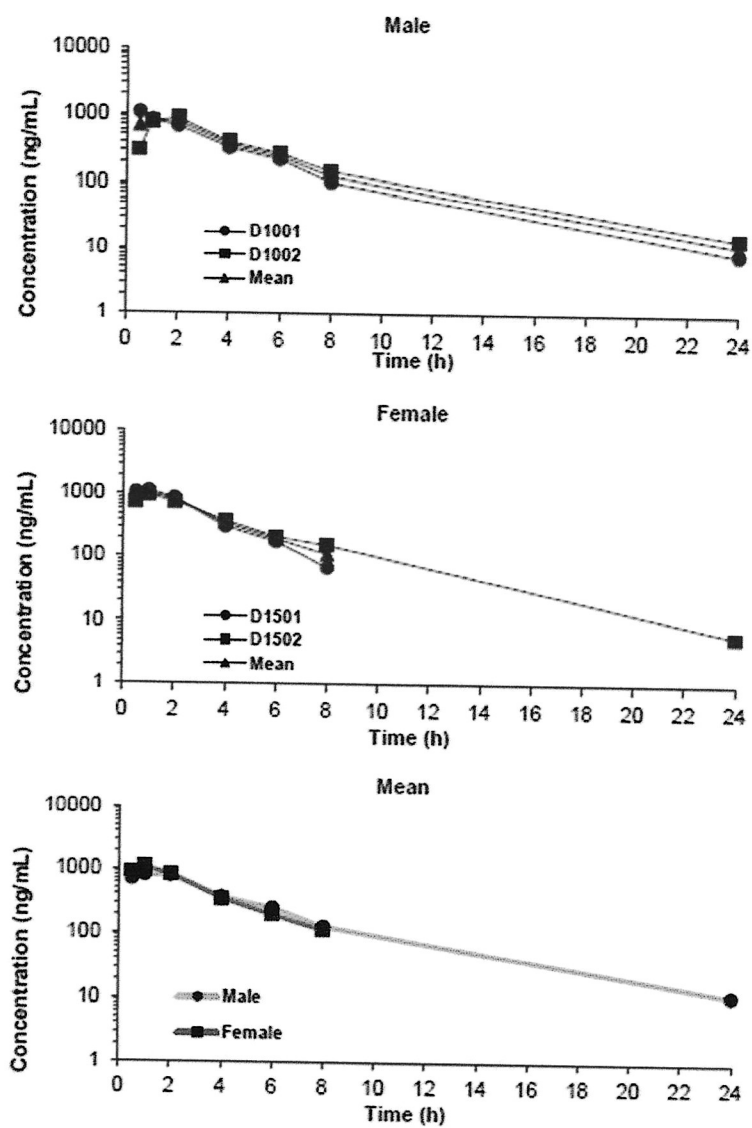


**FIG. 3**

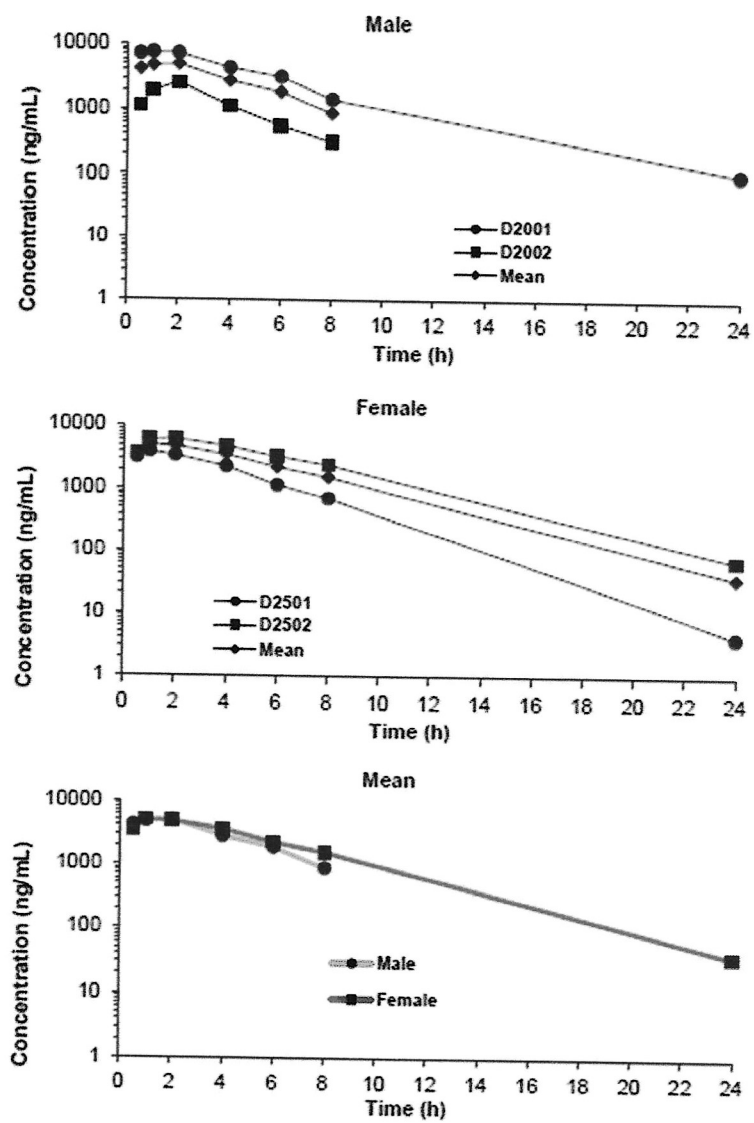


**FIG. 4**

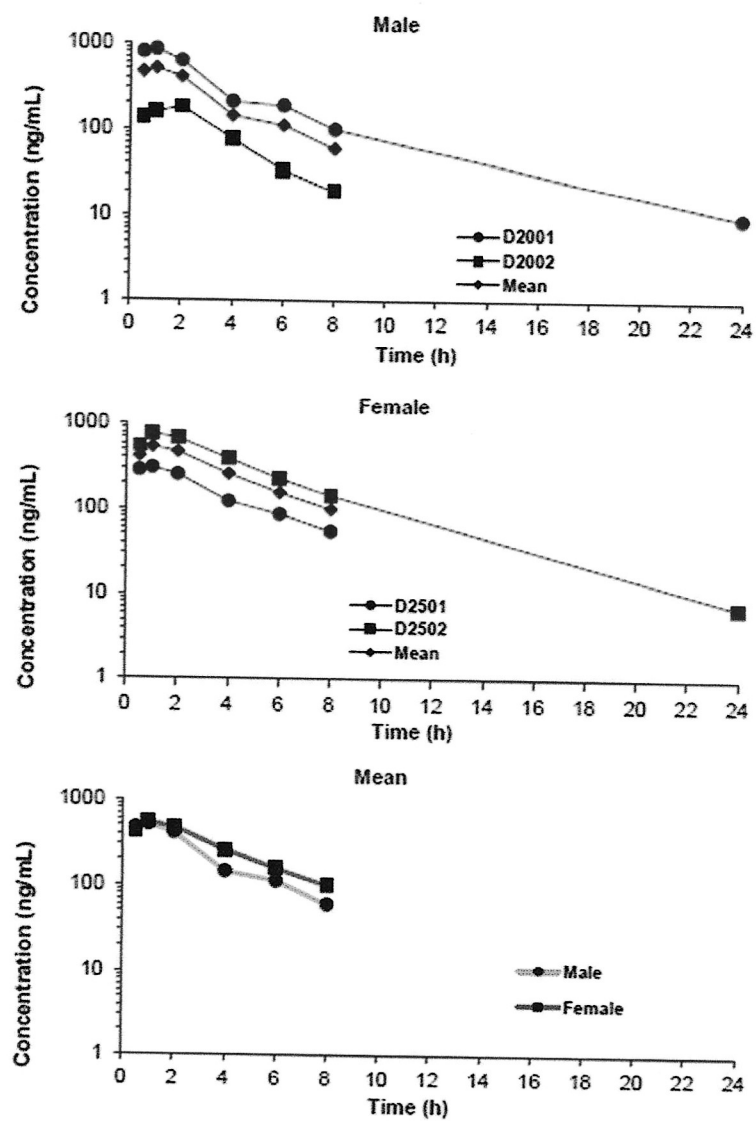


FIG. 5

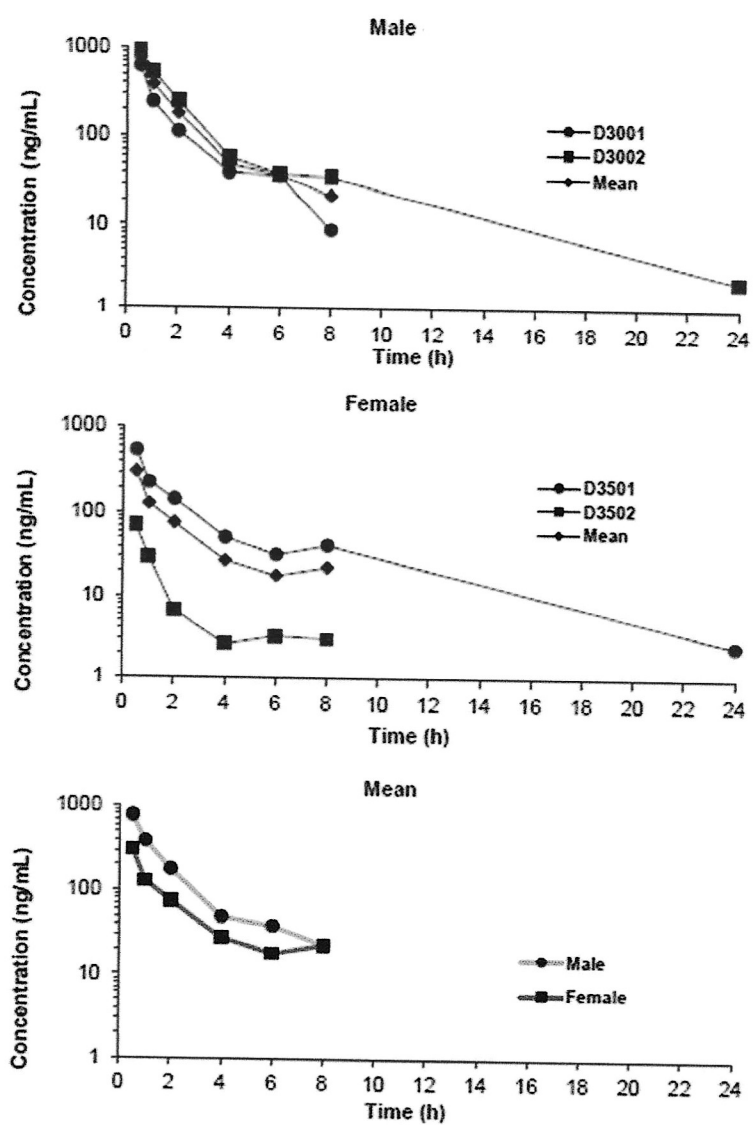


**FIG. 6**

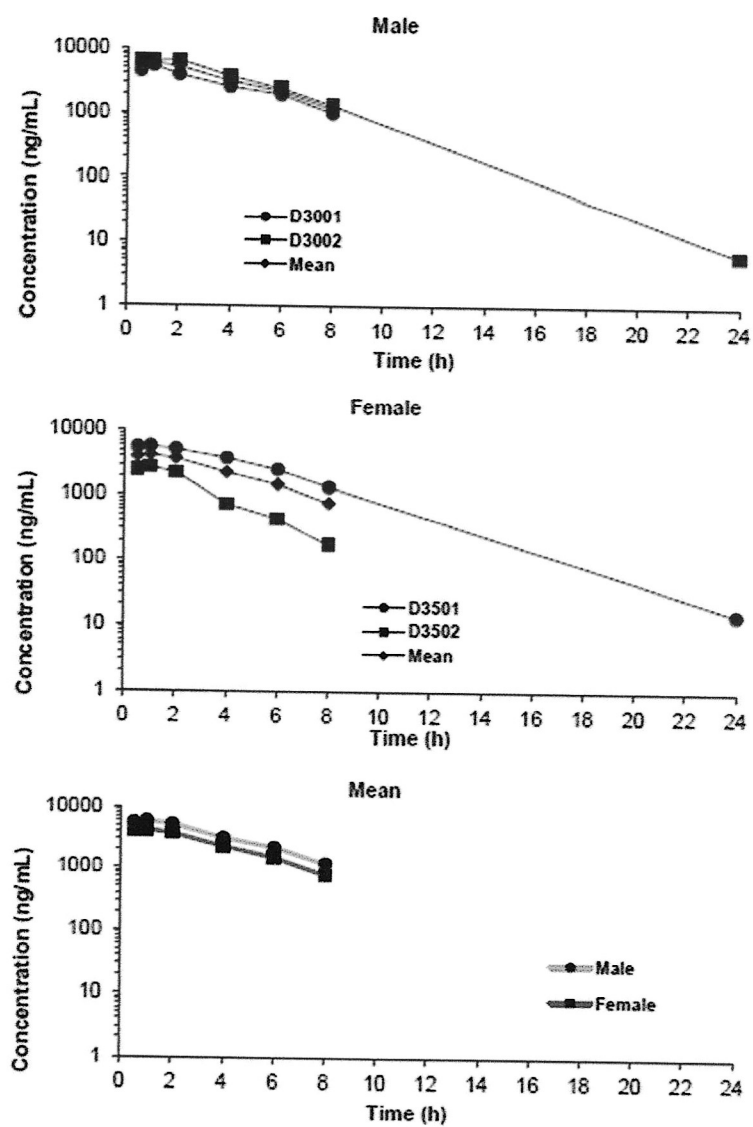
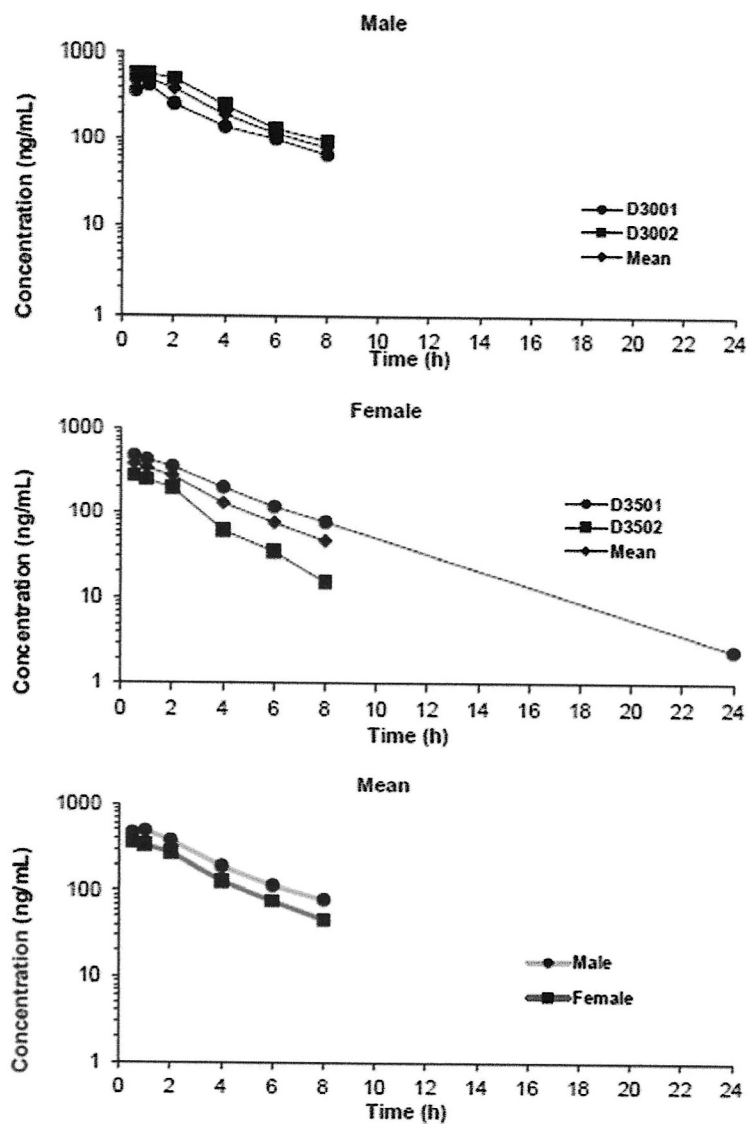


**FIG. 7**



Signed:

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