

**Metabolic Stability of L759633 and L759,656 in Rat and  
Human Liver Microsomes**

**Southern Research Institute**  
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## 1. OBJECTIVE

The objective of the work performed was to evaluate the metabolic stability of two compounds, L759633 and L759,656 in rat and human liver microsomes at various time points over a 2 hour incubation period at 37 °C.

## 2. MATERIALS AND METHODS

L759633 and L759,656 purchased from Tocris Biosciences (Ellisville, MO) were used as received in Phase I and glucuronidation *in vitro* reactions in liver microsomes from rats and humans to determine metabolic stability. The Phase I *in vitro* reaction systems were comprised of species specific liver microsomes purchased from Xenotech LLC, (Lenexa, KS) and used at a reaction mixture concentration of 1 mg/mL. The NADPH regenerating system, purchased from BD Gentest, (Woburn, MA) used in all reactions consisted of 1.3 mM Nicotinamide Adenine Dinucleotide Phosphate (NADP<sup>+</sup>), 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 120 μM sodium citrate buffer, 3.3 mM magnesium chloride (MgCl<sub>2</sub>), 0.25 μg alamethicin, 2mM uridine 5'-diphospho-glucuronic (UDPGA) in an aqueous 50mM Tris-HCl buffer system at pH = 7.4. Each substrate was added into individual reaction tubes to give a final substrate concentration of 10 μM. The total organic solvent concentration in the reaction mixture was maintained at less than 1 % (v/v). Each reaction mixture totaling 0.5 mL was incubated at 37 °C in a shaking water bath for 2 hour. Samples at each incubation time-point (0, 5, 15 30, 60 and 120 minutes) were quenched with 1 mL of ice cold acetonitrile, vortexed well and then centrifuged at 12,500 rpm (11,000 X g) for 5 minutes and resulting supernatant was transferred to autosampler vials for analysis of the residual

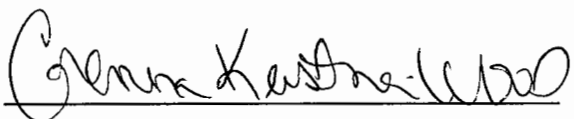
parent as compared to the time 0 point using a previously developed method (BACG-3695). In conjunction with the samples, a series of positive and negative control reactions were also conducted. The positive control reactions are based on well defined substrate/metabolite pairs to demonstrate the viability of the incubation mixture. The negative controls are used to demonstrate thermal stability of the substrate under the incubation conditions used. The positive control reactions consist of the oxidation of paclitaxel to C3' or 6 $\alpha$ -OH-paclitaxel, and glucuronidation of acetaminophen. Four separate negative control reaction were run with each substrate reaction. The first is a no substrate control in which the entire compliment of components in the *in vitro* reaction mixture is present during the incubation except the substrate. The second is a no microsome control where solvent inactivated microsomes are back added to the mixture after the reaction is quenched. The third is a no microsome control where the microsomes were not back added to the reaction mixture and the fourth is the reaction mixture without the addition of the cofactors.

## **2. RESULTS/CONCLUSIONS**

Structure for L759633 and L759,656 are shown in Figure 1. The amount of residual parent test article as compared to the time 0 points are shown in Figures 2 (human) and 3 (rat). Comparisons of the no microsome controls after a 120 minute incubation period to the time 0 samples show no loss of test article as a result of thermal degradation for either of substrates tested. Both substrates were found to be more stable in human than in rat liver microsomes. For L759633, approximately 80% was found to remain in the human *in vitro* reactions, where only approximately 40% was found in the rat *in vitro* reactions

after 120 minutes. For L759,656 approximately 73% was found to remain in the human *in vitro* reactions, where only approximately 44% was found in the rat *in vitro* reactions after 120 minutes. Analysis of the positive control reactions showed expected levels of formation of 3-OH and 6-OH paclitaxel for the lots of rat and human liver microsomes used, respectively. Additionally, expected levels of glucuronidated acetaminophen were also observed indicating acceptable viability of the reaction mixtures.

### 3.0 Approvals



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11/02/07

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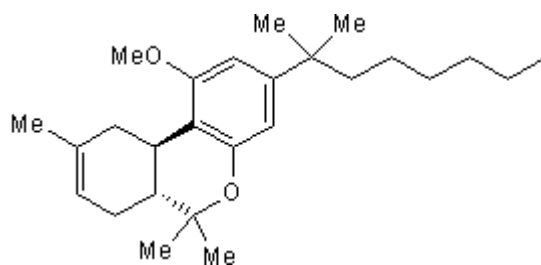
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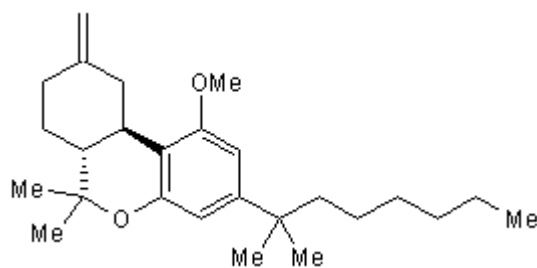
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Figure 1

Structures of L759633 and L759,656



L759633



L759,656

Figure 2

Metabolic Stability of L759633 and L759,656 in Human Liver Microsomes

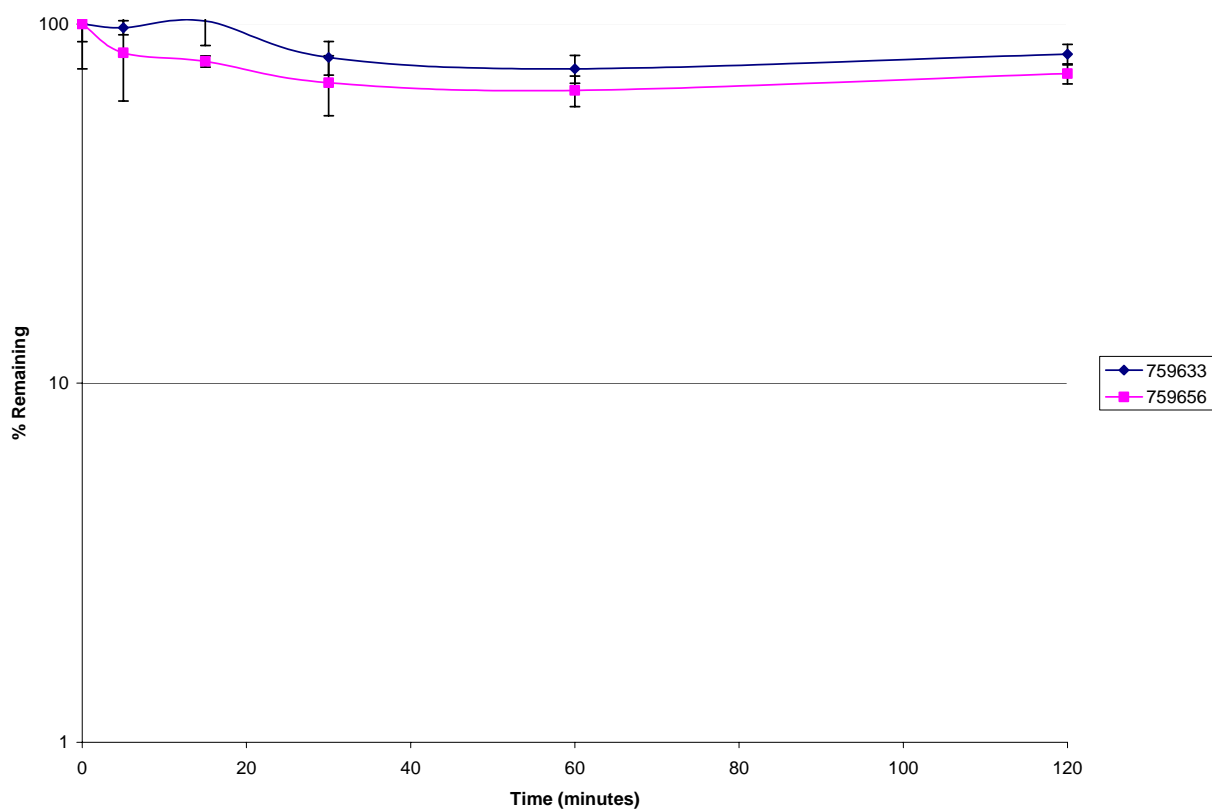


Figure 3

Metabolic Stability of L759633 and L759,656 in Rat Liver Microsomes

