1. **Objective:**

   1.1. To determine the oral bioavailability of the Sponsor’s test article(s), after intravenous (IV) or oral (PO) administration in male Sprague-Dawley rats (Non-crossover).

2. **Required from Sponsor**

   2.1. Test article in powder form
   2.2. Molecular mass (exact mass) of each test article and its salt form/purity
   2.3. MSDS or handling and storage information, e.g., light sensitive, store at -20°C, etc
   2.4. If applicable, instructions for dose vehicle preparation

3. **Deliverables**

   3.1. *Express Plus* report containing results outlined below:
       A. In-life observations on each rat
       B. A table containing test article concentrations in plasma samples at each time point
       C. Plasma concentration vs. time graphs
       D. A table containing test article concentrations in dosing solutions (N=3)
       E. A table containing appropriate pharmacokinetic parameters for each dose route
           a. AUC, half-life, $C_{\text{max}}$, $t_{\text{max}}$ and Mean residence time (MRT) for each dose route
           b. The clearance and volume of distribution of test article after intravenous administration as applicable
           c. Bioavailability after oral dosing
       F. Appendices:
           a. Animal data sheets
           b. Analytical methodology

   **NOTE:** Raw bioanalytical data (e.g. chromatograms and analytical method summary) will not be included in the basic report. These can be obtained for an additional fee.

4. **Substrate**

   4.1. Test article in an appropriate dose vehicle suitable for dosing by IV and PO route individually

5. **Assay System**

   5.1. Conscious, male Sprague-Dawley rats weighing between 200 and 400 grams
   5.2. Animals will be fasted overnight prior to dosing and until 4 hours post-dose
   5.3. The animals may be fitted with jugular vein cannulae as appropriate
   5.4. Water is offered ad libitum to the rats
6. Assay Conditions

6.1. Analytical method development
A. MS optimization for suitable sensitivity
B. LC evaluation for suitable specificity
C. Standard curve with 6-8 points

6.2. In-life study design (Non-crossover):
A. Each test article will be assessed through two dose routes, intravenous (IV) and oral (PO)
B. N=3 per dose group per test article
C. Total of 6 rats per test article
D. Blood sampling site: Jugular vein (or other suitable vessel)

Table 1: Study design for each test article

<table>
<thead>
<tr>
<th>Dose Group</th>
<th>Dosing Route</th>
<th>N=</th>
<th>Dose (mg/kg)</th>
<th>Blood Sampling Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IV</td>
<td>3</td>
<td>TBD</td>
<td>Pre-dose, 5, 15 min, 1, 2, 4, and 8 hrs post dose</td>
</tr>
<tr>
<td>2</td>
<td>PO</td>
<td>3</td>
<td>TBD</td>
<td>Pre-dose, 15 min, 1, 2, 4, and 8 hrs post dose</td>
</tr>
</tbody>
</table>

E. Samples will be collected into tubes with containing sodium heparin
F. Prepare plasma from each sample and freeze
G. LC-MS/MS analysis of test article in plasma samples

6.3. Sample analysis
A. Determine the concentrations of test article in dosing solutions and incurred samples using a generic LC-MS/MS method with a minimum 6-8 point calibration curve
B. The analytical rigor does not include a pre-study validation, and QCs will not be used for sample analysis
C. Dosing solutions will be normalized in matched matrix (rat plasma) and analyzed (n=3) in the same analytical batch as the incurred samples
D. Non-compartmental analysis is used to determine PK parameters for each test article
E. Batch Acceptance Criteria
   a. At least 60% of the calibration standards must be within ±20% of the nominal values to accept the analytical run, except at the Lower Limit of Quantification (LLOQ), where ±25% is acceptable