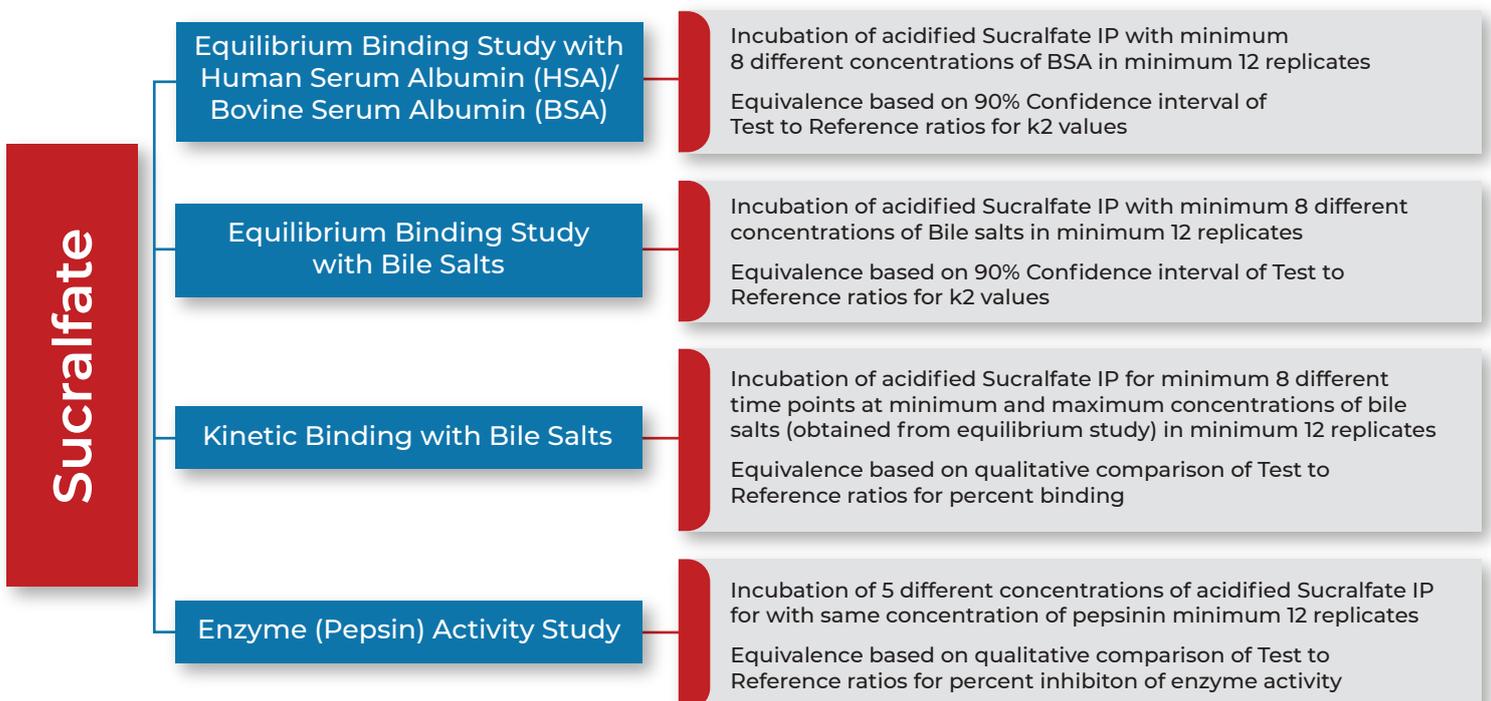


IN VITRO BIOEQUIVALENCE STUDIES FOR SUCRALFATE

About Sucralfate

- Sucralfate is a non-absorbed sucrose octasulfate complex that is prescribed for gastric and duodenal ulcers.
- Sucralfate is available in two dosage forms: tablets and suspension.
- As Sucralfate is a non-absorbed polymer, traditional in vivo bioequivalence evaluation is not feasible. Hence, the USFDA recommends evaluation of bioequivalence by in vitro binding assays performed at physiologically relevant conditions.

Assessment of In Vitro Bioequivalence: USFDA Requirements



Raptim's Experience with Sucralfate

- Raptim Research Pvt. Ltd. has optimized and validated the assay conditions and analytical methods for sample analysis.
- The methods are simple, reproducible and as per the product specific guidance for Sucralfate suspension and tablets.
- The assays are developed considering physiological conditions, properties of adsorbate and site of action.
- The pH of samples is maintained throughout the experiments with regular monitoring.
- We have submitted 2 pivotal studies and performed screening for three clients.
- We are working with sponsors in optimization of the formulation by screening the prototype using in vitro assays.

Key Parameters for In Vitro Studies

Types of Bioassay	Key Parameters	Representative Plot for RLD (Sucralfate Suspension)
Equilibrium binding assay with Albumin	<ul style="list-style-type: none"> ● Assay performed with Bovine serum albumin ● Concentration range selected to achieve maximum binding ● Incubation time selected to achieve equilibrium binding ● Validated HPLC method for sample analysis ● Langmuir adsorption isotherm model is used for binding profiles 	<p>Langmuir profile for equilibrium binding of BSA with Sucralfate</p> <p>$y = 11.969x + 0.014$ $R^2 = 0.9994$</p>
Equilibrium binding assay with Bile salts	<ul style="list-style-type: none"> ● Assay performed with mixture of Bile acid salts (GCA : GCDA : TDCA) ● Selection of buffer and pH condition ● Concentration range selected to achieve maximum binding ● Incubation time selected to achieve equilibrium binding ● Validated LCMS method for sample analysis 	<p>Langmuir profile for equilibrium binding of bile acid with Sucralfate</p> <p>$y = 0.3509x + 1.3866$ $R^2 = 0.9875$</p>

Types of Bioassay	Key Parameters	Representative Plot for RLD (Sucralfate Suspension)																										
Kinetic binding assay with Bile salts	<ul style="list-style-type: none"> ● Assay performed with mixture of Bile acid salts (GCA : GCDA : TDCA) ● Time points selected to cover the transit time of drug under physiological condition and the time required for maximum binding ● Validated LCMS method for sample analysis 	<p style="text-align: center;">Kinetic Binding of Bile Acids with Sucralfate</p> <table border="1"> <caption>Approximate data for Kinetic Binding of Bile Acids with Sucralfate</caption> <thead> <tr> <th>Time (h)</th> <th>Bound of (x/m) of Bile acids (mmol/g)</th> </tr> </thead> <tbody> <tr><td>0</td><td>2.2</td></tr> <tr><td>1</td><td>2.5</td></tr> <tr><td>2</td><td>2.8</td></tr> <tr><td>3</td><td>3.0</td></tr> <tr><td>4</td><td>3.1</td></tr> <tr><td>5</td><td>3.1</td></tr> <tr><td>6</td><td>3.0</td></tr> <tr><td>7</td><td>2.9</td></tr> <tr><td>8</td><td>2.8</td></tr> <tr><td>12</td><td>2.4</td></tr> <tr><td>15</td><td>2.3</td></tr> <tr><td>20</td><td>2.3</td></tr> </tbody> </table>	Time (h)	Bound of (x/m) of Bile acids (mmol/g)	0	2.2	1	2.5	2	2.8	3	3.0	4	3.1	5	3.1	6	3.0	7	2.9	8	2.8	12	2.4	15	2.3	20	2.3
Time (h)	Bound of (x/m) of Bile acids (mmol/g)																											
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Enzyme (Pepsin) Activity	<ul style="list-style-type: none"> ● Assays performed with Simulated Gastric Fluid with pepsin (USP) ● Concentration range for Sucralfate selected to demonstrate maximum enzyme inhibition ● Enzyme activity evaluated using Anson's method ● Validated Spectrophotometric method using multimode reader for sample analysis 	<p style="text-align: center;">Enzyme inhibition with Sucralfate</p> <table border="1"> <caption>Approximate data for Enzyme inhibition with Sucralfate</caption> <thead> <tr> <th>Sucralfate Conc (mg/mL)</th> <th>Mean % Inhibition</th> </tr> </thead> <tbody> <tr><td>0.000</td><td>0.00</td></tr> <tr><td>1.000</td><td>10.00</td></tr> <tr><td>2.000</td><td>15.00</td></tr> <tr><td>3.000</td><td>25.00</td></tr> <tr><td>4.000</td><td>35.00</td></tr> <tr><td>5.000</td><td>45.00</td></tr> <tr><td>6.000</td><td>55.00</td></tr> <tr><td>7.000</td><td>65.00</td></tr> <tr><td>8.000</td><td>80.00</td></tr> <tr><td>9.000</td><td>90.00</td></tr> <tr><td>10.000</td><td>100.00</td></tr> </tbody> </table>	Sucralfate Conc (mg/mL)	Mean % Inhibition	0.000	0.00	1.000	10.00	2.000	15.00	3.000	25.00	4.000	35.00	5.000	45.00	6.000	55.00	7.000	65.00	8.000	80.00	9.000	90.00	10.000	100.00		
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Why work with Raptim

- Raptim Research Pvt Ltd has submitted more than 30 in vitro permeability and binding studies.
- Raptim has a team of Scientists with more than 7 years of experience with in vitro studies.
- Raptim is quick to learn, adapt and improvise on the skills and methodologies required by the regulatory agencies.

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