In Vitro Binding Studies for Bioequivalence Demonstration

SBIA 2022: Advancing Generic Drug Development: Translating Science to Approval

Day 2, Session 5: In Vitro Binding Study for Locally Acting GI Drug Products

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Learning Objectives

- Understand the rationales for recommending in vitro binding studies for locally acting gastrointestinal drugs
- Become familiar with study designs of in vitro binding studies
- Calculate affinity constant (k_1) and capacity constant (k_2)

Bioequivalence

- Bioequivalence (BE) is essential for development and approval of generic drugs.
- Per 21 CFR 320.1, BE is defined as "*the absence of a significant difference in the **rate and extent** to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available **at the site of drug action** when administered at the same molar dose under similar conditions in an appropriately designed study.*"

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=320.1

BE Establishment

- The regulation 21 CFR 320.24(b) provides a list of in vivo and in vitro methods to establish BE in descending order of ~~preference~~ accuracy, sensitivity, and reproducibility:
  - Pharmacokinetic (PK) studies
  - Pharmacodynamic studies
  - ~~Clinical trials~~ Comparative clinical endpoint study
  - In vitro studies
  - Any other approach deemed adequate by FDA

In Vitro Binding Studies

- PK studies for locally acting gastrointestinal (GI) drugs
  - Drug plasma concentrations may not reflect drug concentrations at the site of action.
  - Drug plasma concentrations may be limited.
- Locally acting GI drugs bind to phosphate, potassium, or bile acids to have therapeutic efficacy.
  - Drug substances bind to phosphate, potassium, or bile acids to form insoluble complexes, which is excreted in the feces.
  - The in vitro binding studies reflect mechanism of action.

Product-Specific Guidances

- 17 product-specific guidances (PSGs) recommend in vitro binding studies.
- Classes of drug products

Bind phosphates in GI tract

- Calcium acetate
- Ferric citrate
- Ferric oxyhydroxide
- Lanthanum carbonate
- Sevelamer carbonate
- Sevelamer HCl

Bind bile acids in GI tract

- Cholestyramine
- Colesevelam HCl
- Colestipol HCl

Bind potassium in GI tract

- Sodium zirconium cyclosilicate

Bind protein and bile acids in GI tract

- Sucralfate

BE Recommendations

- The in vitro binding studies generally contain **kinetic** and **equilibrium** studies.
- Other studies may be recommended, e.g.,

Recommended Studies	Drug substance
Active pharmaceutical ingredient (API) sameness	Ferric citrate, Sucralfate, Sevelamer carbonate, Sevelamer HCl, Colesevelam HCl, Sodium zirconium cyclosilicate
Formulation characterizations	Sucralfate
Dissolution studies	Lanthanum carbonate
In vitro pepsin activity study	Sucralfate

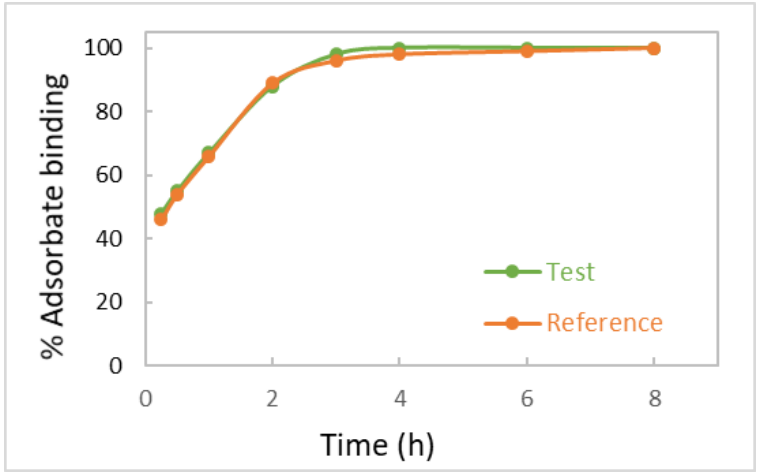
In Vitro Kinetic Binding Study

- Assess the rate of binding and the time to reach the binding equilibrium.
- Support equilibrium binding study.
- Methods:
  - Prepare two (or three) adsorbate concentrations: usually correspond to the lowest and highest concentrations (plus middle concentration) in the equilibrium study.
  - Incubate test and reference for at least eight different lengths of time. The selected time should demonstrate that maximum binding is established.

In Vitro Kinetic Binding Study

- Test/Reference bound adsorbate ratios at the various time should be compared but not subjected to the 90% confidence interval criteria.

Incubation time (h)	T/R ratios
0.25	1.04
0.5	1.02
1	1.02
2	0.99
3	1.02
4	1.02
6	1.01
8	1.00

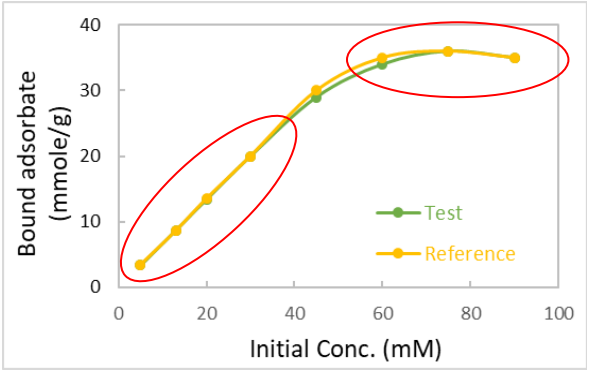


In Vitro Equilibrium Binding Study

- Evaluate affinity and capacity binding constant
- Considered as the **pivotal BE study**
- Conducted under conditions of constant time and varying adsorbate concentration

Methods for In Vitro Equilibrium Binding Study

- Incubate test and reference with at least eight different concentrations of adsorbate.
  - The concentration should be selected to ensure the binding curve is well defined and captures the maximum binding.



- Measure unbound adsorbate concentration.
- Data are analyzed using the Langmuir equation to determine binding affinity constant (k_1) and capacity constant (k_2).
- BE is based on 90% confidence interval of k_2 with the acceptance criterion of 80% to 120% (untransformed data).

Media pH for In Vitro Equilibrium Binding Studies

- The media pH should be physiologically relevant and sensitive to detect any binding capacity differences between the test and reference products.

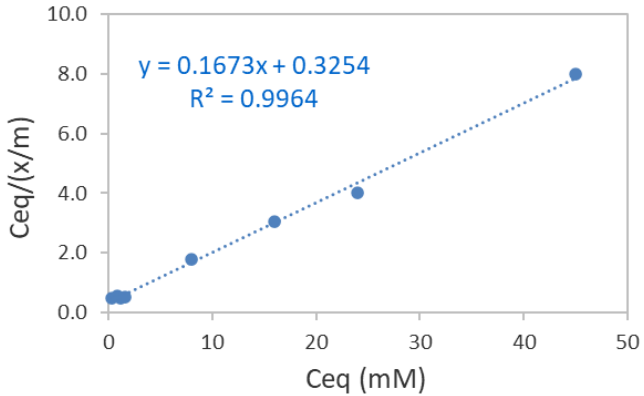
Mechanism	Drug substance	pH recommendation in PSG
Bind phosphate in GI tract	Ferric citrate; Ferric oxyhydroxide	pH 1.2, 3.0, 7.5
	Lanthanum carbonate	pH 1.2, 3.0, 5.0
	Sevelamer carbonate; Sevelamer HCl	pH 4.0, 7.0
Bind bile acids in GI tract	Cholestyramine	pH 6.8
	Colesevelam HCl	pH 6.8
	Colestipol HCl	pH 6.8
Bind potassium in GI tract	Sodium zirconium cyclosilicate	pH 1.2, 4.5 and 6.8
Binds protein and bile acids	Sucralfate	Not specified

Data Analysis: Langmuir Equation

- Langmuir Equation: describes the equilibrium between adsorbate and adsorbent system.
- Langmuir Equation: $\frac{x}{m} = \frac{k_1 k_2 C_{eq}}{1 + k_1 C_{eq}} \longrightarrow \frac{C_{eq}}{x/m} = \frac{1}{k_1 k_2} + \frac{1}{k_2} C_{eq}$
  - X: the amount of adsorbate bound to the drug substance
  - m: the amount of drug substance used
  - C_{eq}: adsorbate concentration remaining in the solution at equilibrium
- Plot linear regression on $\frac{C_{eq}}{x/m}$ vs. C_{eq}
  - k_1 = slope/intercept
  - k_2 = 1/slope

Affinity Constant and Capacity Constant Calculation

Initial adsorbate conc (mM)	Ceq (unbound adsorbate conc.; mM)	Bound adsorbate (mM)	x (bound adsorbate; mmole)	m (used drug substance; g)	Ceq/(x/m)
2	0.3	1.7	0.51	0.8	0.47
5	0.85	4.15	1.245	0.8	0.55
8	1.2	6.8	2.04	0.8	0.47
10	1.6	8.4	2.52	0.8	0.51
20	8	12	3.6	0.8	1.78
30	16	14	4.2	0.8	3.05
40	24	16	4.8	0.8	4.00
60	45	15	4.5	0.8	8.00



k_1 = slope/intercept = 0.51

k_2 = 1/slope = 6.08

Challenge Question #1

Which of the following is NOT the adsorbate for in vitro binding studies:

A. Phosphate

B. Potassium

C. Calcium

D. Bile acids

Challenge Question #2

The BE assessment is based on 90% confidence interval of:

A. Affinity constant (k_1)

B. Capacity constant (k_2)

C. Test/Reference bound adsorbate ratio

D. All of the above

Summary

- In vitro binding studies reflect the drug mechanism of action and can be used to demonstrate BE for certain locally acting GI drug products.
- The in vitro binding studies generally contain kinetic and equilibrium studies.
- Study design factors (e.g., incubation time, adsorbate concentration) should be considered for the method development of in vitro binding study.
- Generic drug applicants may seek correspondence with the Agency to clarify BE recommendation in PSGs or propose alternative BE approaches.

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