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In vitro Bioequivalence Studies in Tablet Formulation Containing 625 mg of Colesevelam Hydrochlorides

Gulcin Tok*, Ediz Yildirim and Ali Turkyilmaz

Research and Development Center, Sanovel Pharmaceuticals, Istanbul, Turkey

Abstract

Colesevelam hydrochloride is a second generation bile acid sequestrant that is used principally for treatment of elevated LDL cholesterol. Additionally, it is indicated to improve glycemic control in adults with type 2 diabetes mellitus. Colesevelam hydrochloride is an insoluble, non-absorbed polymer that binds bile acids in the intestine, impeding their reabsorption. Because the drug is not absorbed into the systemic circulation, pharmacokinetic information is not available. The present abstract focuses on the study of *in vitro* BE in tablet formulation containing 625 mg of Colesevelam hydrochloride. Bile acid salts of Glycocholic Acid (GC), Glycochenodeoxycholic Acid (GCDC) and Taurodeoxycholic Acid (TDC) were used *in vitro* BE studies. The binding capacity HPLC method was developed and validated for these bile acid salts. The equilibrium binding study that is the pivotal BE study and *in vitro* kinetic binding study that is the support the pivotal equilibrium binding study was repeated twelve times. In the *in vitro* equilibrium binding studies, the Langmuir binding constants k_1 (affinity) and k_2 (capacity) were calculated for the three salts, individually and combined (GC+GCDC+TDC) using both nonlinear Langmuir equation and linear Langmuir equation for the test and reference products. The calculated capacity constant (k_2), the more important parameter, were obtained very similar between test and reference products in the 90% confidence interval and acceptance criteria of 80% to 120%. The test/reference ratio for capacity constant, k_2, was obtained 0.918 and 0.922 using equation 1 and 2 for total bile acid salts from the without acid pre-treatment equilibrium binding study and 0.948, 0.953 using equation 1 and 2 for total bile acid salts from the with acid pre-treatment equilibrium binding study.

Keywords: Colesevelam hydrochloride; *In vitro* bioequivalence; Bile acid sequestrant

Introduction

Colesevelam hydrochloride is a novel non-absorbed, lipid lowering polymer that binds bile acids in the intestine, impeding their reabsorption and it is used principally for treatment of elevated LDL cholesterol. The mechanism of action for the activity of colesevelam has been evaluated in several *in vitro* and *in vivo* studies. These studies have demonstrated that colesevelam binds bile acids, including glycocholic acid, the major bile acid in humans. Cholesterol is the sole precursor of bile acids. During normal digestion, bile acids are secreted into the intestine. A major portion of bile acids is then absorbed from the intestinal tract and returned to the liver via the enterohepatic circulation [1]. Colesevelam binds with bile acids in the intestine to form an insoluble complex that is eliminated in feces. This increased excretion of bile acids results in an increased oxidation of cholesterol to bile acid and a lowering of the serum cholesterol [2]. Additionally, Colesevelam hydrochloride is indicated to improve glycemic control in adults with type 2 diabetes mellitus [3,4].

Colesevelam hydrochloride is a second generation bile acid sequestrant. Second generation bile acid sequestrants have higher bile acid binding capacity then first generation bile acid sequestrants. Accordingly colesevelam hydrochloride is administered at lower doses than first-generation agents [5]. The *in vivo* studies in hamsters and rats confirmed that colesevelam is effective in enhancing the faecal excretion of bile acids. Both colesevelam hydrochloride and cholestyramine (first generation) cause a dose dependent increase in bile acid sequestration in these two rodent species. Colesevelam was at least 2-fold more potent and efficacious in increasing faecal bile acid excretion in both models than was cholestyramine. Additionally colesevelam hydrochloride is a substance of low acute toxicity. However, the safety margin in humans on repeated dosing for long periods is rather low [1].

The recommended dose of 625 mg colesevelam hydrochloride tablet is 3 tablets taken twice per day with meals or 6 tablets once per day with a meal and should be taken with a liquid [3]. When a meal is eaten, cholecystokinin is released from the endocrine cells of the intestinal mucosa. The hormone acts on vagal afferents or the nerve ganglia innervating the gallbladder and induces gallbladder contraction. At the same time it acts on the nerves innervating the sphincter of Oddi, causing it to relax. The end result is that gallbladder bile is delivered to the duodenum [6]. The affinity, capacity, and kinetics of colesevelam hydrochloride binding for GCDC, TDC, and GC were not significantly altered after suspension in water, carbonated water, Coca-Cola, Sprite, grape juice, orange juice, tomato juice, or Gatorade. The amount of bile acid sodium salt bound as a function of time was unchanged by pre-treatment with any beverage tested. The *in vitro* binding characteristics of colesevelam hydrochloride are unchanged by suspension in common beverages [7].

Chemical structure, molecular formula and chemical name of colesevelam hydrochloride are provided (Figure 1). It is poly (allylamine hydrochloride), cross-linked with epichlorohydrin and alkylated with (6-bromohexyl) trimethylammonium bromide and 1-bromodecane [8].

Pharmacology

Traditional absorption, distribution, metabolism, and excretion

***Corresponding author:** Gulcin Tok, Research and Development Center, Sanovel Pharmaceuticals, Istanbul, Turkey, Tel: +90 212 746 52 52/3825; Fax +90 212 746 54 30; E-mail: gulcintok@sanovel.com.tr

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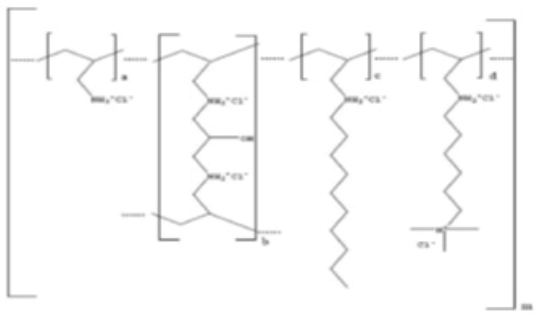


Figure 1: Chemical structure, molecular formula and chemical name of colesevelam hydrochloride.

studies were not conducted with colesevelam hydrochloride, as absorption was expected to be negligible. The results of the excretion balance studies in rat and dog (and in human) showed that the bulk of radioactivity was excreted in the faeces and no radioactivity beyond minimal quantities in any tissues, plasma or urine after a 72-h or 96-h period in dog and rat, respectively, and were consistent with the non-absorbable nature of colesevelam hydrochloride [1].

Pharmacokinetics

Colesevelam is a hydrophilic, water-insoluble polymer that is not hydrolyzed by digestive enzymes and is not absorbed. Because the drug is not absorbed into the systemic circulation, pharmacokinetic information is not available [1].

Pharmacodynamics

In the pharmacodynamic *in vitro* study, colesevelam, colestipol and cholestyramine demonstrated similar overall bile acid binding capacity when evaluated in mixed bile acid solutions. At all free ligand concentrations, colesevelam was found to bind Glycocholic Acid (GC) significantly more tightly than did cholestyramine, which in turn was more effective than colestipol. This is an important finding since GC is the major bile acid in humans. In contrast to the clear distinction for GC, the bile acid binding capacity of taurodeoxycholic acid, taurocholicdeoxycholic acid, glycodeoxycholic acid, glycocholicdeoxycholic acid and taurocholic acid were very similar for colesevelam and cholestyramine.

Colesevelam hydrochloride is a polymer that is not likely to be absorbed from the gastrointestinal tract due its particle size. The absence of specific safety pharmacology studies and secondary pharmacodynamic studies is justified by the insolubility of colesevelam and by the observation that colesevelam is not absorbed [1].

Objective

To compare *in vitro* binding characteristics of colesevelam hydrocholoride between generic product and reference product under identical experimental conditions.

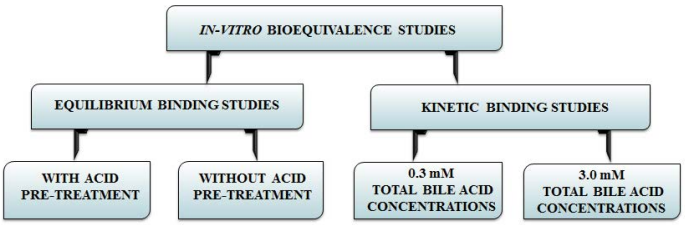
Colesevelam hydrochloride 625 mg Film Tablet which is brand name Zander, Sanovel Pharmaceuticals used for test product and Cholestagel 625 mg Film Tablet, Genzyme used for reference product in *in vitro* bioequivalence studies.

In vitro Bioequivalence Studies

The clinical efficacy of colesevelam depends on its binding capacity to intestinal bile acids.

Each bile salt-containing incubation medium should contain Glycocholic Acid (GC), Glycochenodeoxycholic Acid (GCDC) and Taurodeoxycholic Acid (TDC) [9-11].

Study Design



Equilibrium binding studies

An equilibrium binding study is conducted under conditions of constant time and varying concentrations of bile acid salts. This study should be conducted by incubating the test and reference products with at least eight different concentrations of total bile salts, with and without acid pre-treatment. The equilibrium binding study is considered the pivotal bioequivalence study [9,10].

Kinetic binding studies

A kinetic binding study is conducted under constant concentration of bile acid salts with varying times of observation. This study should be conducted by incubating the test and reference products for at least eight different lengths of time, with two different constant total bile salt concentrations, without acid pre-treatment. The kinetic binding study should be used to support the pivotal equilibrium binding study [9,10].

Data Treatment and Analysis

The amount of bile acid salt bound to colesevelam hydrochloride is obtained from the difference between the initial concentration of bile acid salt introduced into the system and the concentration present in the filtrate at the end of the study. The monomolecular adsorption of bile acid salt molecules from solution, at constant temperature, on to colesevelam hydrochloride is described by the following Langmuir-type nonlinear Equation 1:

$$\frac{x}{m} = \frac{k_1 . k_2 . C_{eq}}{1 + k_1 . C_{eq}} \quad (1)$$

Upon rearranging, linear Equation 2 is obtained:

$$\frac{C_{eq}}{(x/m)} = \frac{1}{k_1 . k_2} + \frac{C_{eq}}{k_2} \quad (2)$$

where:

Ceq=Concentration of the bile acid salt remaining in the solution at equilibrium;

x=The amount of bile acid salt bound to the colesevelam hydrochloride;

m=The amount of colesevelam hydrochloride used.

The constant, k_1, is defined as the adsorption coefficient or affinity constant and is related to the magnitude of the forces involved in the binding process. The Langmuir-capacity constant, k_2, indicates the apparent maximum amount of bile acid salt that can be adsorbed per unit weight of colesevelam hydrochloride.

For the *in vitro* equilibrium binding study, the Langmuir binding constants k_1 and k_2 should be determined based on total bile salt binding

(GC+GCDC+TDC). The test/reference ratio should be calculated for k_1. The 90% confidence interval (CI) should be calculated for k_2 with the acceptance criteria of 80% to 120%. For the *in vitro* kinetic binding study, the test/reference bound bile acid salt ratios at the various times should be compared but not subjected to the 90% CI criteria [10].

Bioequivalence Based on 90% CI

The Langmuir binding constant k_2 from the equilibrium binding study [9-13].

Experimental

The entire equilibrium test procedure with and without acid pre-treatment and entire kinetic test procedure with 0.3 mM and 3.0 mM aqueous solution in the presence of 0.1 M sodium chloride solution were repeated twelve times each for the reference and test tablets.

For each study, both reference and test product tablets, a tablet composite sample was prepared by grinding 10 tablets and weighing out an amount equivalent to 10 mg of active ingredient based on the average tablet weight.

Equilibrium binding studies

For stock bile acid salts solution, 40 mM bile acid salts solutions in Simulated Intestinal Fluid (SIF) without enzyme was prepared contain 3:3:1 molar proportion of Glycocholic Acid (GC), 17.14 mM, Glycochenodeoxycholic Acid (GCDC), 17.14 mM and Taurodeoxycholic Acid (TDC), 5.72 mM.

In without pre-treatment study, eight incubation flasks of the test product and eight incubation flasks of the reference product, each containing the equivalent of 10 mg colesevelam hydrochloride, were set up and soaked with SIF each incubation flask at room temperature overnight. The following day, requisite volumes of stock bile acid salts solution with the target concentrations of bile acid salts covering the ranges of 0.1-30 mM were added to each incubation flasks. Incubated all flasks at 37°C for 24 h and filtered.

In with acid pre-treatment study, eight incubation flasks of the test product and eight incubation flasks of the reference product, each containing the equivalent of 10 mg colesevelam hydrochloride, were set up and incubated in 0.1 N hydrochloric acid at 37°C for 1 h and centrifuged. The supernatant was aspirated; the pellet was resuspended with SIF until pH 6.8 was attained. The acid pre-treated colesevelam hydrochloride product was soaked in SIF at room temperature overnight. The following day, requisite volumes of stock bile acid salts solution with the target concentrations of bile acid salts covering the ranges of 0.1-30 mM were added to each incubation flask. Incubated all flasks at 37°C for 24 h and filtered.

Kinetic binding studies

For stock bile acid salts solution, 40 mM bile acid salts solution in water was prepared contain 3:3:1 molar proportion of GC, 17.14 mM, GCDC, 17.14 mM TDC, 5.72 mM.

Twelve incubation flasks of the test product and twelve incubation flasks of the reference product, each containing the equivalent of 10 mg colesevelam hydrochloride, were set up and soaked with 0.1 M sodium chloride solution each incubation flask at room temperature overnight. The following day, requisite volumes of stock bile acid salts solution with the target concentrations, 0.3 mM or 3.0 mM, added to each incubation flasks. Incubate flasks at 37°C for twelve different time points covering 0.05-24 h (0.05, 0.1, 0.15, 0.2, 0.25, 0.50, 1, 2, 4, 8, 16, 24 h) and filtered.

Analytical method and validation

An isocratic HPLC method was developed suitable for use in bioequivalence studies and validated for GC, GCDC and TDC bile acid salts. The study parameters for both equilibrium and kinetic binding experiments were optimized and finalized for *in vitro* bioequivalence (Figure 2).

Unbound bile acids in the filtrate were determined by UV-detector (210 nm). Bile acids were separated from each other using an Inertsil ODS-3V (250 × 4.6 mm) column and the mobile phase of phosphate buffer acetonitrile mixture.

The method was accurate with peak area %RSD ≤ 2.0% and resolution ≥ 1.5 for each component. The assay solution concentration range was validated between LOQ concentration for each bile acid salt to 40 mM total bile acid salts for equilibrium study and LOQ concentration for each bile acid salt to 4 mM total bile acid salts for kinetic study. The validation consists of the following experiments:

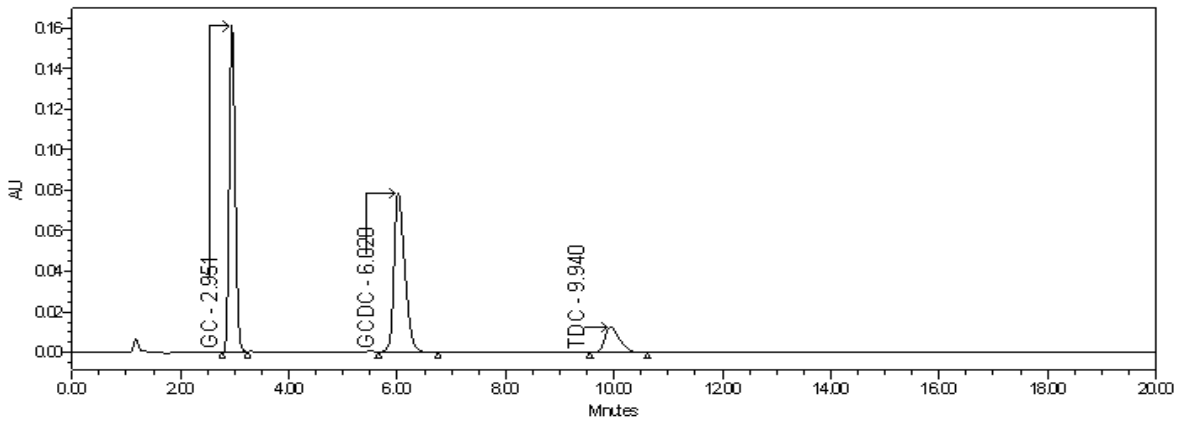


Figure 2: Chromatogram of unbound bile acids in the filtrate.

Langmuir constant	Bile acid salts	Test (T) Estimate ± SE	Reference (R) Estimate ± SE	T/R	90% CI for Ratio
Affinity constant (k_1)	GC	2.871 ± 0.025	2.965 ± 0.023	0.968	NA
	GCDC	4.695 ± 0.023	5.028 ± 0.115	0.934	NA
	TDC	16.401 ± 0.123	18.314 ± 0.709	0.896	NA
	GC+GCDC+TDC	0.867 ± 0.003	0.866 ± 0.008	1.001	NA
Capacity constant (k_2)	GC	1.059 ± 0.006	1.167 ± 0.002	0.908	89.83–91.75
	GCDC	3.622 ±0.009	3.937 ± 0.004	0.920	91.56–92.41
	TDC	1.529 ± 0.004	1.655 ± 0.001	0.924	91.95–92.77
	GC+GCDC+TDC	6.376 ± 0.021	6.946 ± 0.006	0.918	91.24–92.34

Table 1: Point estimation results of Langmuir constants calculated using Langmuir nonlinear equation (equation 1) from the without acid pre-treatment equilibrium studies of colesevelam hydrochloride 625 mg film tablet test and reference products (mean of twelve determination).

Bile acid salts	Test			Reference			T/R	
	k_1	k_2	r^2	k_1	k_2	r^2	k_1	k_2
GC	8.451	10.300	0.9877	0.953	1.040	0.9942	0.820	0.917
GCDC	8.009	8.144	0.9983	3.452	3.741	0.9984	0.984	0.923
TDC	22.609	26.915	0.9981	1.515	1.634	0.9986	0.840	0.928
GC+GCDC+TDC	1.395	1.418	0.9955	6.031	6.543	0.9963	0.984	0.922

Table 2: Mean values of affinity (k_1), capacity (k_2) and linear regression (r^2) for GC, GCDC, TDC and total bile acid salts calculated using Langmuir linear equation (equation 2) from the equilibrium studies without acid pre-treatment of colesevelam hydrochloride 625 mg film tablet test and reference products (mean of twelve determination).

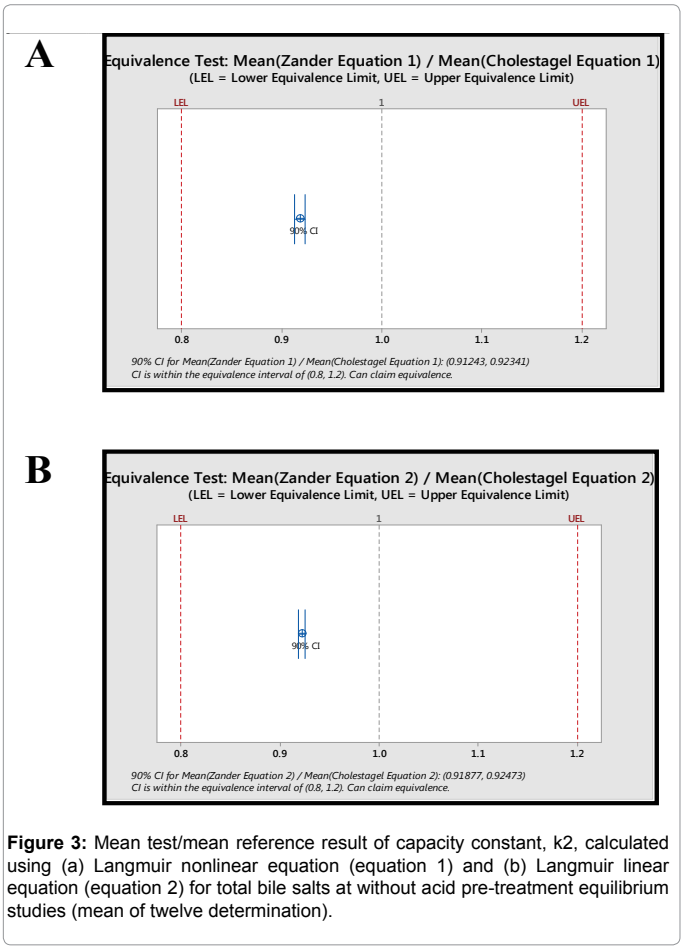


Figure 3: Mean test/mean reference result of capacity constant, k2, calculated using (a) Langmuir nonlinear equation (equation 1) and (b) Langmuir linear equation (equation 2) for total bile salts at without acid pre-treatment equilibrium studies (mean of twelve determination).

specificity, linearity of individual bile acid salts, LOQ confirmation, accuracy/recovery of samples, precision/repeatability, intermediate precision, standard and sample solution stability, and filter evaluation.

Results

Equilibrium binding studies

Without acid pre-treatment: As indicated in the Tables 1 and 2; Figure 3, both the equation 1 and the equation 2 results in 90% CI for individual bile acid and total bile acid were within the 80-120% interval used in the approval of ANDA [11].

The Langmuir constants k_1 (GC+GCDC+TDC) and k_2 (GC+GCDC+TDC) for test and reference was comparable ± 20%.

With acid pre-treatment: As indicated in the Tables 3 and 4; Figure 4, both the Equation 1 and the Equation 2 results the 90% CI for individual bile acid and total bile acid was within the 80-120%.

The results indicate that the percent binding and the binding capacity of both test and reference products at several initial concentrations (Table 5) (ranging from 0.1-30 mM) are very similar and there was no significant difference in the binding performances of test and reference products in both with and without pre-treatment equilibrium studies (Figure 5).

Kinetic binding studies

Comparison of kinetic data of binding of 0.3 mM and 3.0 mM total bile acid salts concentration.

As indicated in the Figure 6 each product achieved equilibrium between 15-30 min for 0.3 mM and 3.0 mM kinetic studies (Tables 6 and 7).

Discussion

Because the colesevelam hydrochloride is not absorbed into the systemic circulation, *in vivo* bioequivalence is not available. Thence, equivalence to the reference product was compared *in vitro* condition.

Mean test/mean reference (T/R) of twelve determination result of capacity constant, k_2, was obtained 0.918 (91.24-92.34% CI) using equation 1 and 0.922 (91.88-92.47% CI) equation 2 for total bile salts at without acid pre-treatment equilibrium studies. Equation 1 and equation 2 results were very close to each other and both results

Langmuir constant	Bile acid salts	Test (T) Estimate ± SE	Reference (R) Estimate ± SE	T/R	90% CI for Ratio
Affinity constant (k_1)	GC	3.202 ± 0.120	2.596 ± 0.079	1.233	NA
	GCDC	6.700 ± 0.149	5.966 ± 0.071	1.173	NA
	TDC	21.892 ± 0.440	19.140 ± 0.297	1.144	NA
	GC+GCDC+TDC	1.118 ± 0.021	1.001 ± 0.013	1.117	NA
Capacity constant (k_2)	GC	1.242 ± 0.004	1.349 ± 0.005	0.921	91.29–92.83
	GCDC	3.513 ±0.007	3.695 ± 0.005	0.951	94.69–95.49
	TDC	1.506 ± 0.003	1.561 ± 0.002	0.965	96.04–96.95
	GC+GCDC+TDC	6.419 ± 0.016	6.770 ± 0.010	0.948	94.35–95.30

Table 3: Point estimation results of Langmuir constants calculated using Langmuir nonlinear equation (equation 1) from the equilibrium studies with acid pre-treatment of colesevelam hydrochloride 625 mg film tablet test and reference products (mean of twelve determination).

Bile acid salts	Test			Reference			T/R	
	k_1	k_2	r^2	k_1	k_2	r^2	k_1	k_2
GC	6.962	1.159	0.9926	6.281	1.245	0.9933	1.108	0.931
GCDC	16.995	3.315	0.9985	10.667	3.485	0.9987	1.593	0.951
TDC	32.368	1.493	0.9981	29.288	1.535	0.9985	1.105	0.973
GC+GCDC+TDC	2.010	6.058	0.9969	1.801	6.358	0.9967	1.116	0.953

Table 4: Mean values of affinity (k_1), capacity (k_2) and linear regression (r^2) for GC, GCDC, TDC and total bile acid salts calculated using Langmuir linear equation (equation 2) from the equilibrium studies with acid pre-treatment of Colesevelam hydrochloride 625 mg film tablet test and reference products (mean of twelve determination).

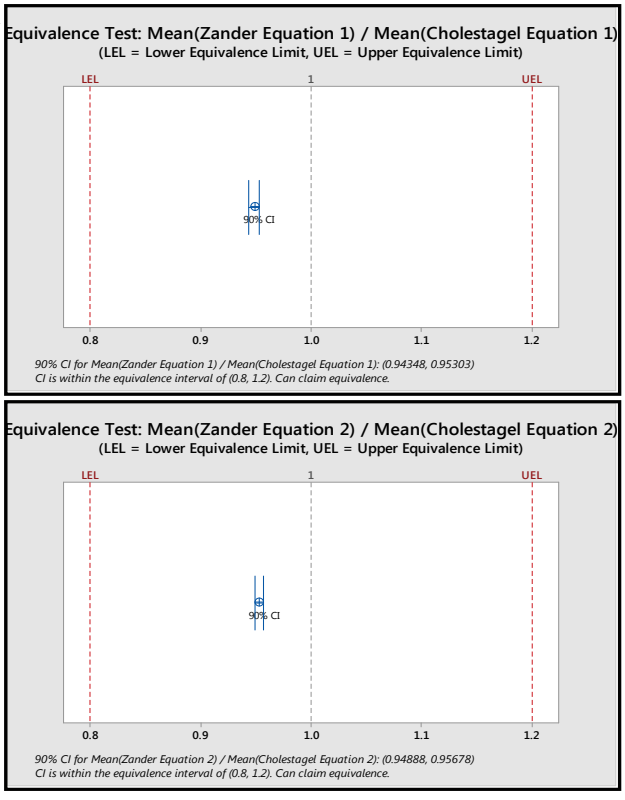


Figure 4: Mean test/mean reference result of capacity constant, k_2, calculated using (a) Langmuir nonlinear equation (equation 1) and (b) Langmuir linear equation (equation 2) for total bile salts at with acid pre-treatment equilibrium studies (mean of twelve determination).

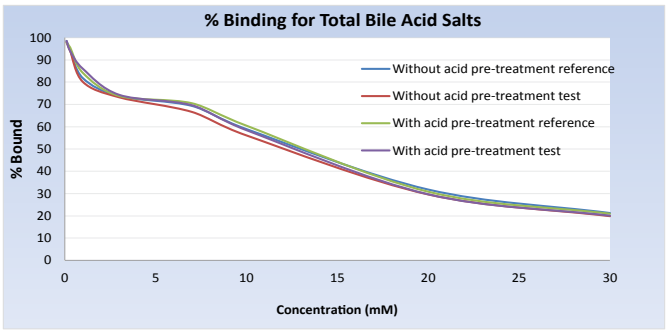


Figure 5: Results of the percent binding versus concentration for total bile acid salts at with and without acid pre-treatment equilibrium binding studies (mean of twelve determinations).

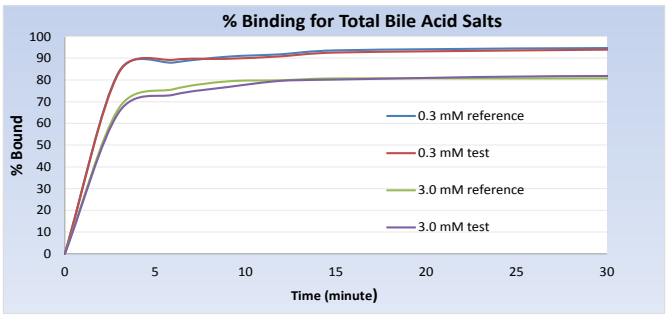


Figure 6: Results of the percent binding vs. time for total bile acid salts at 0.3 mM and 3.0 mM kinetic studies (mean of twelve determinations).

calculated with equations 1 and equation 2 were obtained very similar between test and reference products in the 90% confidence interval and acceptance criteria of 80-120%.

These parameter indicated in the approval of ANDA to pivotal bioequivalence study [11]. The results obtained from without acid pre-treatment equilibrium binding studies meet the acceptance criteria [9-11].

Additionally T/R ratio of twelve determination result of capacity constant, k_2, was obtained 0.948 (94.35-95.30% CI) using equation 1 and 0.953 (94.88-95.68%) equation 2 for total bile salts at with acid

Concentration (mM, Total bile acids)	Without acid pre-treatment			With acid pre-treatment		
	Test Av. x/m	Reference Av. x/m	T/R	Test Av. x/m	Reference Av. x/m	T/R
0.1	0.0969	0.0974	0.99	0.0974	0.0965	1.01
0.3	0.2791	0.2800	1.00	0.2787	0.2833	0.98
1	0.7920	0.8086	0.98	0.8497	0.8314	1.02
3	2.1713	2.1874	0.99	2.2028	2.1877	1.01
7	4.6125	4.8006	0.96	4.8168	4.8790	0.99
10	5.5473	5.8279	0.95	5.7754	5.9750	0.97
20	5.8537	6.2884	0.93	5.8643	6.0935	0.96
30	5.8882	6.3056	0.93	5.9513	6.2066	0.96

Table 5: Results of binding capacity of the equilibrium studies. Average micromoles total bile acid salts bound per 10 mg colesevelam hydrochloride (x/m) (mean of twelve determinations).

Test				Reference			T/R
Time (Hour)	Av. x/m	%CV	Av. %Bound	Av. x/m	%CV	Av. %Bound	
0.05	0.2488	4.1831	83.4540	0.2499	3.9379	83.8365	1.00
0.10	0.2661	2.2006	89.2541	0.2623	1.5726	88.0054	1.01
0.15	0.2675	2.0025	89.7242	0.2704	1.1614	90.7026	0.99
0.20	0.2709	0.7342	90.8805	0.2738	1.2664	91.8330	0.99
0.25	0.2761	2.0225	92.6123	0.2791	1.2786	93.6126	0.99
0.50	0.2800	0.8312	93.9397	0.2822	1.4577	94.6763	0.99
1	0.2861	0.6182	95.9863	0.2834	0.5315	95.0535	1.01
2	0.2863	0.8390	96.0314	0.2859	1.0938	95.9071	1.00
4	0.2865	1.0939	96.0926	0.2873	0.9346	96.3837	1.00
8	0.2861	0.6812	95.9741	0.2870	1.2848	96.2779	1.00
16	0.2881	0.7881	96.6353	0.2859	0.8168	95.9028	1.01
24	0.2879	0.6789	96.5614	0.2843	0.5938	95.3714	1.01

Table 6: Results of the kinetics study of the binding of the total bile acid salts to Colesevelam hydrochloride in 0.3 mM aqueous bile salt solution in the presence of added 0.1 M NaCl. Average micromoles total bile acid salts bound per 10 mg colesevelam hydrochloride (x/m) and average % bound (mean of twelve determinations).

Test				Reference			T/R
Time (Hour)	Av. x/m	%CV	Av. %Bound	Av. x/m	%CV	Av. %Bound	
0.05	1.9467	0.0217	65.1983	2.0017	0.0165	67.0610	0.97
0.10	2.1832	0.0054	73.1706	2.2602	0.0172	75.7207	0.97
0.15	2.2945	0.0096	76.7145	2.3680	0.0176	79.3315	0.97
0.20	2.3779	0.0029	79.6140	2.3836	0.0027	79.8556	1.00
0.25	2.3906	0.0082	80.1770	2.4090	0.0096	80.7057	0.99
0.50	2.4410	0.0035	81.8262	2.4096	0.0070	80.7239	1.01
1	2.4248	0.0125	81.0222	2.4302	0.0070	81.4162	1.00
2	2.4513	0.0025	82.1018	2.4408	0.0047	81.7721	1.00
4	2.4285	0.0069	81.2411	2.4211	0.0017	81.1124	1.00
8	2.4440	0.0073	81.8772	2.4338	0.0018	81.5368	1.00
16	2.4498	0.0048	82.0151	2.4260	0.0037	81.2737	1.01
24	2.4366	0.0048	81.6263	2.4340	0.0025	81.5424	1.00

Table 7: Results of the kinetics study of the binding of the total bile acid salts to Colesevelam hydrochloride in 3.0 mM aqueous bile salt solution in the presence of added 0.1 M NaCl. Average micromoles total bile acid salts bound per 10 mg colesevelam hydrochloride (x/m) and average % bound (mean of twelve determinations).

pre-treatment equilibrium binding studies. These results are also in the acceptance criteria.

Based on these data generic Colesevelam 625 mg Film Tablet (Zander) is comparable to Cholestagel 625 mg Film Tablet in the equilibrium study of bile acid salt binding in SIF with and without acid pre-treatment.

In the kinetic binding study, the binding at the initial both 0.3 mM and 3.0 mM concentration was extremely rapid for both test and reference product. The results for both concentrations were extremely reproducible.

Additionally, the kinetic binding of 0.3 mM and 3.0 mM bile salts for both test and reference product was nearly identical. T/R ratio was obtained between 0.99–1.01 for 0.3 mM concentration and 0.97–1.01 for 3.0 mM concentration. Therefore, based on these data test and reference products are comparable in terms of its *in vitro* binding characteristics.

Concentration of bile acid in the intestine varies according to fasting state [6]. For this reason, in the equilibrium studies, the binding characteristics of the test product to the reference product at 8 different concentrations in the concentration range of 0.1-30 mM were compared and it was found that the test product was comparable to

the reference product at all concentrations. In addition, results of the kinetic studies shown that maximum binding to bile acids achieved in 15-30 min. When taken tablets with a meal, it takes much longer than 30 min to pass through the intestine [14]. Therefore, the tablets perform maximum binding pass through the intestine. The results of binding capacity shown in Table 5 indicate that test and reference products at 8 concentrations ranging from 0.1 to 30 mM are comparable. T/R ratio of twelve determination result of binding capacity was obtained ranging from 0.93-1.00 for without acid pre-treatment equilibrium study and 0.96-1.02 with acid pre-treatment equilibrium study.

An important parameter that affects the variation between repetitions is the waiting time for soaked with SIF each incubation flask at room temperature overnight. It should be repeated in the same way for less variation between repetitions.

Conclusion

All results obtained from equilibrium binding studies proved that generic Colesevelam 625 mg Film Tablet (Zander) bioequivalent to reference product Cholestagel 625 mg Film Tablet. In addition to the equilibrium binding results, the results of kinetic binding studies supported to bioequivalence.

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