CLINICAL PHARMACOLOGY REVIEW

NDA	210895
Submission Date	10/30/2017
Brand Name	Welchol™
Generic Name	Colesevelam Hydrochloride
Reviewer	Mohammad (Abir) Absar, Ph.D.
Team Leader (Acting)	Jayabharathi Vaidyanathan, Ph.D.
OCP Division	Clinical Pharmacology II
OND Division	Division of Metabolism and Endocrinology Products
Applicant	Daiichi Sankyo Inc.
Formulation; Strength	Chewable bar, 3.75 gm colesevelam hydrochloride per bar
Dosage Regimen	One bar once daily
Relevant IND/NDA	IND 48034
Indication	As an adjunct to diet and exercise to (i) reduce elevated LDL-C in adults with primary hyperlipidemia as monotherapy or in combination with a HMG CoA reductase inhibitor, (ii) reduce LDL-C levels in boys and postmenarchal girls, 10 to 17 years of age, with heterozygous familial hypercholesterolemia as monotherapy or in combination with a statin after failing an adequate trial of diet therapy, (iii) improve glycemic control in adults with type 2 diabetes mellitus

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1. EXECUTIVE SUMMARY

Daiichi Sankyo has submitted an original New Drug Application (NDA) for Welchol™ (colesevelam hydrochloride (HCl)) chewable bar. The sponsor is seeking approval of the chewable bar product as an alternate dosage form to the marketed Welchol™ tablets (NDA 21176, approved on May 2000) and Welchol™ powder for suspension (NDA 22362, approved on Oct 2009), and is proposing a single Welchol™ package insert based on the currently approved labeling with appropriate sections modified to incorporate the chewable bar dosage form.

Welchol™ is a bile acid sequestrant and is indicated as an adjunct to diet and exercise to-

- Reduce elevated LDL-C in adults with primary hyperlipidemia as monotherapy or in combination with a HMG CoA reductase inhibitor
- Reduce LDL-C levels in boys and postmenarchal girls, 10 to 17 years of age, with heterozygous familial hypercholesterolemia as monotherapy or in combination with a statin after failing an adequate trial of diet therapy
- Improve glycemic control in adults with type 2 diabetes mellitus

Colesevelam HCl is a non-absorbed polymer that acts locally in the gastrointestinal tract to bind the salts of bile acids. This action causes a compensatory increase in cholesterol biosynthesis in liver and enhanced oxidation of cholesterol to bile acids and an upregulation of LDL receptors, leading to decreased serum LDL-cholesterol. The sponsor is currently marketing Welchol™ tablet formulation containing 625 mg of colesevelam HCl and the powder for suspension formulation containing 1.875 gm and 3.75 gm single-dose packet. Per the sponsor, although the tablet and powder for suspension are well-tolerated and efficacious, some patients find them difficult to swallow due to the large size of the tablets, the number of tablets (six) required to be taken, or the grittiness of the oral suspension. Hence, the sponsor is developing a chewable bar formulation to improve compliance, providing 3.75 gm of colesevelam HCl (equivalent to 6 x 625 mg tablet) in a chewable formula. The chewable bar product is being developed in three flavors: chocolate, strawberry and caramel. The final formulation is in the form of a 30 gm bar provided in child resistant (b) (4) packaging.

In the Welchol™ tablet NDA, the sponsor demonstrated comparable binding properties of the capsule and tablet formulations using in vitro assays of bile acid salt binding kinetics and binding isotherms. Similarly, for the powder for suspension formulation, the sponsor demonstrated similar equilibrium and kinetic binding properties between the tablet and powder for suspension formulations, using in vitro bile acid binding assays. This submission contains in vitro bioequivalent (BE) studies, i.e., in vitro equilibrium binding study and in vitro kinetic binding study, between each flavored colesevelam HCl chewable bar and the commercial Welchol™ tablets. For the in vitro equilibrium binding study, the Langmuir binding constants k1 (affinity constant) and k2 (capacity constant) were determined based on total bile salt binding. As recommended in the *2015 draft guidance for Colesevelam HCl Tablet*, 90% confidence interval (CI) for the capacity constant (k2) were compared between the test and reference products. In addition, the sponsor investigated the effect of chewing for the proposed chewable bar formulation to address any potential impact of chewing on bile acid binding.

1.1. Recommendations

The Office of Clinical Pharmacology/Division of Clinical Pharmacology 2 has reviewed the clinical pharmacology information provided within NDA 210895 and recommends approval.

1.2. Post Marketing Requirement

None.

1.3. Summary of Important Clinical Pharmacology Findings

The sponsor conducted two pivotal biopharmaceutic studies – in vitro equilibrium binding study and in vitro kinetic binding study. The in vitro equilibrium binding study (test method M12601 and M13400 for the chewable bars and tablets, respectively) were conducted to determine the equilibrium binding of three bile acids, glycocholic acid (GC), glycochenodeoxycholic acid (GCDC) and taurodeoxycholic acid (TDC) in each of the two dosage forms tested. The equilibrium test procedure with and without acid pretreatment was performed on twelve replicates for the Welchol™ chewable bars (of each flavor) and the Welchol™ tablets. The mean millimoles (mM) of each bile acid per gram of colesevelam HCl and the ratio of the average percent of each bile acid (GC, GCDC, and TDC) bound for all three flavors of the Welchol™ chewable bars (T) and Welchol™ tablets (R) at different concentrations with and without acid-pretreatment were calculated; the mean T/R ratio for GC, GCDC and TDC for all flavors with and without acid pre-treatment ranged from 0.95 to 1.08.

The results of the comparison using Langmuir isotherms without acid pre-treatment were used as the pivotal parameter of bioequivalence. The results indicate that the binding capacities (k2) are comparable (±20%) for all three flavors of Welchol™ chewable bars relative to the commercial Welchol™ tablets as the

reference formulation. This was based on the sum of the mean constants of the three bile acids (GC+GCDC+TDC) for each flavor of the Welchol™ chewable bars as compared to the sum of the constants from the commercial Welchol™ tablet formulation. The individual 90% CI for the capacity constant for GC+GCDC+TDC was within 80–125% interval (Table 1).

Table 1: Statistical analysis for the total bile acid binding (GC+GCDC+TDC) without acid pre-treatment (n=12)

Parameter	Without Acid Pre-Treatment				
Capacity Constant (k_2)	Type of Chewable Bar	Dosage Form		Chewable Bars/ Tablet	90% CI for Ratio (%)
		Chewable Bars Estimate	Tablets Estimate		
	Chocolate	5.405	6.152	0.879	84.9 – 91.0
	Caramel	5.549		0.902	86.6 – 93.9
	Strawberry	6.469		1.051	101.1 – 109.3

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

The results of comparison using Langmuir isotherm with acid pre-treatment are shown in Table 2. This was also based on the sum of the mean constants of the three bile acids (GC+GCDC+TDC) for each flavor of the Welchol™ chewable bars as compared to the sum of the constants from the commercial Welchol™ tablet formulation. The individual 90% CI for the capacity constant for GC+GCDC+TDC was within 80–125% interval.

Table 2: Statistical analysis for the total bile acid binding (GC+GCDC+TDC) with acid pre-treatment (n=12)

Parameter	With Acid Pre-Treatment				
Capacity Constant (k_2)	Type of Chewable Bar	Dosage Form		Chewable Bars/ Tablet	90% CI for Ratio (%)
		Chewable Bars Estimate	Tablets Estimate		
	Chocolate	5.768	5.791	0.996	94.1 – 105.2
	Caramel	5.614		0.969	92.5 – 101.4
	Strawberry	6.099		1.053	100.3 – 110.5

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

In addition, the amount of bile acids bound and the percent bound for the two formulations were comparable. Refer to Section 2.3.1.1 for details.

The kinetic binding rate testing for the Welchol™ chewable bars and tablets were conducted using Method M12602 and M4176, respectively. Comparison of kinetic data for binding of 0.3 mM and 3.0 mM bile acid salts with Welchol™ chewable bar and tablets was conducted. In general, the binding at the initial 0.3 mM and 3.0 mM concentration was rapid for all three flavors of the chewable bars and tablets; almost no bile acid salts were left after the first few minutes of the study. The kinetic binding of 0.3 mM and 3.0 mM of each bile salt for both dosage forms were comparable. Refer to Section 2.3.1.2 for details.

To address the Agency's concern regarding the impact of chewing on the in vivo performance of colesevelam HCl, the sponsor conducted an in vitro study to evaluate the bile acid binding capacity for three

different sizes of Welchol™ chewable bar pieces using the caramel flavored bar as a representative drug product for all three flavors. The bile acid binding capacity was not impacted by size of the pieces of the chewable bar. Further, the sponsor conducted an open-label one-treatment study in healthy volunteers to assess the effect of chewing on the in vitro disintegration of Welchol™ chewable bar (Study WEL-A-U120). The mean (SD) time to complete disintegration of the bolus (chewed lump) was 24.0 (13.7) min, which is shorter than the reported half gastric emptying time (Hellmig S *et al*., J Gastroenterol Hepatol. 2006; Vasavid P *et al.* J Neurogastroenterol Motil, 2014), suggesting that following chewing the bolus would break apart and undergo complete disintegration in the stomach and consequently, colesevelam polymer will be available in the intestine to exert its therapeutic effect. Refer to Section 2.3.2 for details.

Overall, Clinical Pharmacology recommends approval of NDA 210895.

2. QUESTION-BASED REVIEW

2.1. Background

2.1.1. What is the regulatory background pertinent to this application?

The sponsor carried out the development program for Welchol™ chewable bars under IND 48034. In a written response dated 12/15/2015, the Agency deemed the sponsor's approach of demonstrating equivalence of the proposed chewable bars with commercial Welchol™ tablets using in vitro equilibrium and kinetic binding study acceptable. The sponsor was also advised to address whether there is any impact of chewing on in vivo performance of the proposed formulation.[1] During a teleconference dated 07/24/2017, the Agency reiterated its concern on the effect of chewing on the in vivo performance of the proposed chewable bar formulations. The Agency noted that the dosage form being considered is novel, so whether the in vitro assessments will adequately support safety and effectiveness of this product compared with the listed product will be closely reviewed. Although the firm stated that there is no commercially available standard equipment to perform a chewing study for a chewable bar product, the Agency stated that this could be circumvented with a clinical trial, which would directly assess the effects of the product when consumed.[2]

The NDA was submitted with comparative in vitro binding data (equilibrium and kinetic binding) of the chewable bar with Welchol™ tablet as reference. In addition, an in vitro study to address chewing was submitted. At mid-cycle, several deficiencies were noted and a Discipline Review letter was issued by DCP-2/OCP on 03/19/2018 (see Appendix A). In response to the letter, the sponsor addressed the bioanalytical method deficiency (see Section 2.4) and conducted a clinical chewing study (Study WEL-A-U120) and results were submitted to the NDA on 05/31/2018.

2.1.2. What are the clinical pharmacology studies submitted in the NDA?

The sponsor submitted the following pivotal and supportive studies in this application.

i. <u>In vitro equilibrium binding study (pivotal):</u> The equilibrium binding study for the Welchol™ chewable bar and Welchol™ tablets were performed using methods M12601 and M13400, respectively. This method determines the equilibrium of binding of three bile acids – GC, GCDC and TDC. One lot from the registration stability lots of each flavor of chewable bars was used. The tablet lot was an approved commercial lot. The experimental conditions, in general, were similar for the chewable bars and tablets, and the equilibrium binding of the bile acids GC, GCDC and TDC were conducted in simulated intestinal fluid at Ph 6.8. The equilibrium test procedure with and without acid pretreatment was performed on twelve replicates for the chewable bars (of each flavor) and tablets.

[1] IND 48034. Advice/Information Request dated 12/15/2015
[2] IND (b) (4). Meeting minute dated 08/24/2017

ii. In vitro kinetic binding study (pivotal): The kinetic bile acid binding rate testing for the Welchol™ chewable bars and Welchol™ tablets were performed using method M12602 and M4176, respectively. The kinetic binding study was conducted for each flavor of the chewable bars and tablets at the 0.3 mM and 3.0 mM bile acid concentration. For the chewable bars, 4.75 grams of each flavor was used for each sample preparation. This is equivalent to approximately 625 mg of active ingredient. Both the Welchol™ chewable bars and Welchol™ tablets were presoaked for approximately one hour before testing to remove any bias from the time that was needed for the tablet to disintegrate.

iii. In vitro chewing study (supportive): An in vitro study was conducted to evaluate the bile acid binding capacity for three different sizes of Welchol™ chewable bars using the caramel flavored bar as a representative drug product for all three flavors. The rationale for the various sizes was based on the variable nature of human chewing and the possibility that different sized pieces of the bars could be swallowed post-chewing. The bile acid binding test was performed on various sizes of the chewable bar (n=2 of each size) using three different extraction times (2, 3, and 4 h), and monitoring binding of GC and GCDC.

iv. In vivo chewing study (supportive): An open-label one-treatment study in healthy volunteers to assess the effect of chewing on the in vitro disintegration of Welchol™ chewable bar. All subjects (n=12) received a single Welchol™ chewable bar containing 3.75 gm colesevelam HCl. Each subject took 6 bite-sized pieces of the bar and chewed each piece for up to 45 seconds or until there was an urge to swallow. The subjects were then to fully expectorate the bolus into a separate sample tube. After the entire bar had been chewed and the cumulative expectorates (bolus) placed in the sample tube for all the subjects, the respective tubes were placed quickly in the USP <711> Dissolution Apparatus 3 dissolution vessels filled with 200 mL of simulated gastric fluid, the disintegration test was then conducted.

2.2. General Attributes

2.2.1.What are colesevelam hydrochloride's key physicochemical properties?

Colesevelam HCl is a high-capacity bile acid-binding molecule. It is poly(allylamine hydrochloride) cross-linked with epichlorohydrin and alkylated with 1-bromodecane and (6-bromohexyl)-trimethylammonium bromide. The chemical name of colesevelam hydrochloride is allylamine polymer with 1-chloro-2,3,-epoxypropane, [6-(allylamino)-hexyl]trimethylammonium chloride and N-allyldecylamine, hydrochloride. The chemical structure of colesevelam HCl is shown in Figure 1. No regular order of the groups is implied by the structure; cross-linking and alkylation are expected to occur randomly along the polymer chains. A large amount of the amines is protonated. The polymer is depicted in the hydrochloride form; a small amount of the halides is bromide. Colesevelam hydrochloride is hydrophilic and insoluble in water.

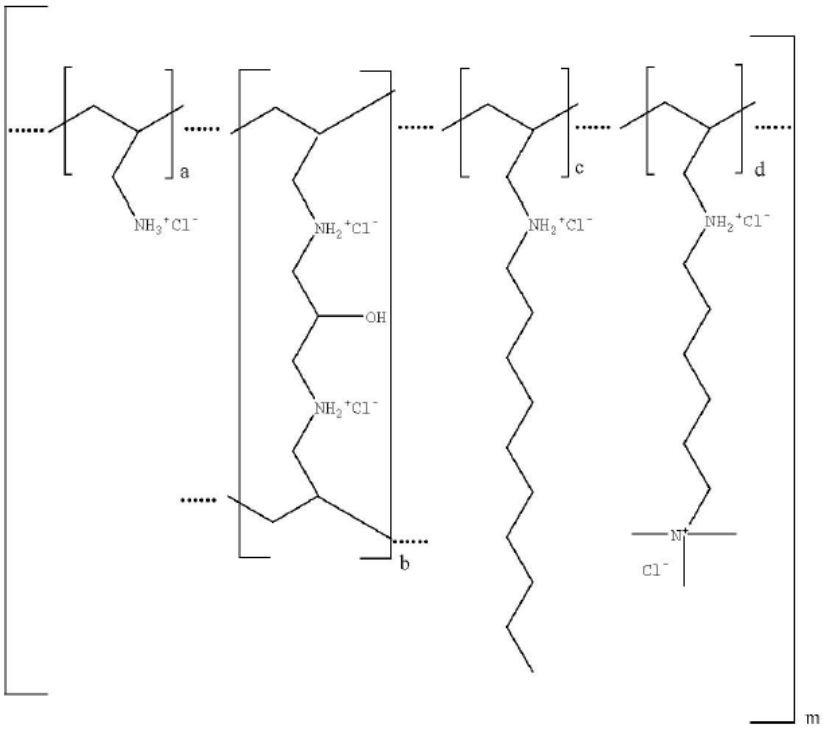


Figure 1: Chemical structure of colesevelam HCl where in (a) represents allyl amine monomer units that have not been alkylated by either of the 1-bromodecane or (6-bromohexyl)-trimethylammonium bromide alkylating agents or cross-linked by epichlorohydrin; (b) represents allyl amine units that have undergone cross-linking with epichlorohydrin; (c) represents allyl amine units that have been alkylated with a decyl group; (d) represents allyl amine units that have been alkylated with a (6-trimethylammonium) hexyl group, and m represents a number ≥ 100 to indicate an extended polymer network. A small amount of the amines is dialkylated, and are not depicted in the formula above.

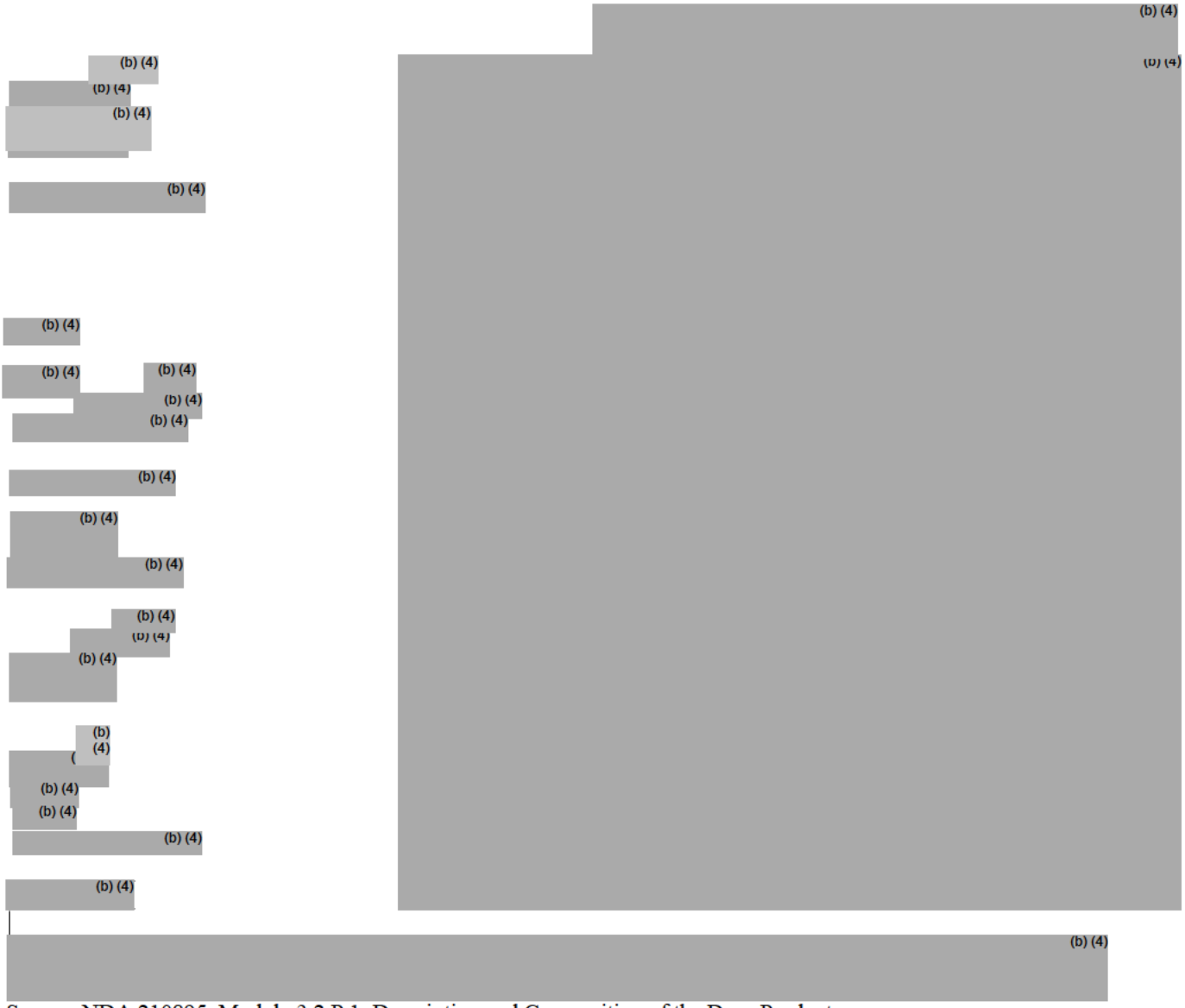
2.2.2. What is the formulation for the drug product?

Welchol™ chewable bars are available in three flavors – chocolate, strawberry and caramel – containing 3.75 gm of colesevelam HCl (anhydrous) in a 30 gm bar (Figure 2). Each bar is individually packaged in child-resistant white foil laminate (b) (4) wrappers.

Item	Chocolate Bar	Strawberry Bar	Caramel Bar
Description (Appearance)	Brown, Oblong, rectangular	Pink Oblong, rectangular	Tan Oblong, rectangular
Dosage Form	Chewable bar	Chewable bar	Chewable bar
Picture			
Weight	30 g	30 g	30 g

Figure 2: Description of the Welchol™ bars. Source: NDA 210895, Module 3.2.P.1.

Table 3: Welchol™ chewable bars composition



Source: NDA 210895, Module 3.2.P.1. Description and Composition of the Drug Product

2.2.3. What are the proposed mechanism of action and therapeutic indications?

Primary Hyperlipidemia: Colesevelam hydrochloride is a non-absorbed, lipid-lowering polymer that binds bile acids in the intestine, impeding their reabsorption. As the bile acid pool becomes depleted, the hepatic enzyme, cholesterol 7-α-hydroxylase, is upregulated, which increases the conversion of cholesterol to bile acids. This causes an increased demand for cholesterol in the liver cells, resulting in the dual effect of increasing transcription and activity of the cholesterol biosynthetic enzyme, HMG-CoA reductase, and increasing the number of hepatic LDL receptors. These compensatory effects result in increased clearance of LDL-C from the blood, resulting in decreased serum LDL-C levels. Serum TG levels may increase or remain unchanged.

Type 2 Diabetes Mellitus: The mechanism by which Welchol™ improves glycemic control is unknown.

2.2.4. What are the proposed dosages and routes of administration?

The proposed dose of Welchol™ chewable bar is one bar containing 3.75 gm of colesevelam HCl once daily. Chewable bar should be taken with a meal. The approved dose of Welchol™ tablets is 6 tablets once daily or 3 tablets twice daily. Welchol™ tablets should be taken with a meal and liquid. The approved recommended dose of Welchol™ powder for suspension is one 3.75 gm packet once daily or one 1.875 gm packet twice daily.

2.3.General Clinical Pharmacology

2.3.1. What are the key findings of the pivotal in vitro BE studies?

2.3.1.1.Equilibrium binding

The equilibrium binding study for the Welchol™ chewable bar and Welchol™ tablets were performed using methods M12601 and M13400, respectively. Sponsor followed recommendations outlined in the product specific draft guidance for Colesevelam HCl (revised on Jan 2016). One lot of each flavor for the registration stability lots of Welchol™ chewable bars and one approved commercial lot of Welchol™ tablets were used for the study. The equilibrium binding of the bile acids were conducted in simulated intestinal fluid at pH 6.8. The equilibrium test procedure with and without acid pretreatment was performed on n=12 replicates for the Welchol™ chewable bars (of each flavor) and the Welchol™ tablets.

The initial concentrations of bile acids GC, GCDC and TDC were the same for both the Welchol™ chewable bars and Welchol™ tablets. For the chewable bars, each flavor was separately tested for each sample set of eight concentrations ranging from 0.1 to 30 mM. For the Welchol™ tablets, a tablet composite sample was prepared by grinding 20 tablets and weighing out an amount equivalent to 80 mg of active ingredient based on the average tablet weight. The set of 8 concentrations ranging from 0.1 to 30 mM was prepared.

The equilibrium reaction with and without acid pre-treatment were prepared in centrifuge tubes placed in a heated orbital shaker set at 37 °C at 280 rpm for at least 24 hours. Aliquots of the test solutions are collected, filtered after 24 hours and analyzed by HPLC. The eight levels of bile acid concentrations tested were 0.1, 0.3, 1, 3, 7, 10, 20 and 30 mM solutions. The amount of bile acid salt bound to the active ingredient (colesevelam) was calculated from the difference between the initial concentrations of the bile acid salts added and the concentrations present in the filtrate at the end of the binding experiment. This information was then used to evaluate the binding parameters by using the Langmuir equation shown below.

$$\frac{x}{m} = \frac{k1k2Ceq}{1 + k1Ceq}$$

Where Ceq is the concentration of the bile acid salt remaining in solution (unbound), x is the amount of bile acid salt bound to the Welchol™ and m is the amount of Welchol™ used. The x/m ratio is the binding capacity of the drug product. The constant k1 is defined as the adsorption coefficient or affinity constant and is related to the relative strength of the binding. The constant k2 is the capacity constant and indicates the apparent maximum binding capacity of the tested material.

Equilibrium binding without acid pretreatment

Based on the guidance, the parameters to be reported for both the test and reference products include, percent binding of bile acid salt, micromoles of bile acid salts bound, affinity constant k1, capacity constant k2 and the coefficient of determination r2 (when linear regression is used to determine k1 and k2). Tables 4 to 7 contain the average results from 12 replicate determinations for Welchol™ chewable bars and tablets.

Table 4: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Chewable bar (Chocolate) formulation in pH 6.8 phosphate buffer without acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)
0.1	0.04	0.00	0.04	0.04	0.00	0.04	0.01	0.00	0.01
0.3	0.12	0.00	0.11	0.13	0.00	0.13	0.04	0.00	0.04
1.0	0.39	0.13	0.26	0.42	0.04	0.39	0.14	0.00	0.14
3.0	1.18	0.61	0.58	1.27	0.17	1.10	0.42	0.03	0.39
7.0	2.76	1.68	1.09	2.96	0.50	2.46	0.98	0.09	0.89
10.0	3.95	2.76	1.19	4.23	1.16	3.06	1.40	0.23	1.16
20.0	7.90	7.07	0.82	8.46	5.35	3.11	2.80	1.44	1.35
30.0	11.84	10.88	0.96	12.69	9.56	3.13	4.19	2.81	1.38

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 5: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Chewable bar (Strawberry) formulation in pH 6.8 phosphate buffer without acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mM/g)	Initial (mM)	Unbound (mM)	Bound (mM/g)	Initial (mM)	Unbound (mM)	Bound (mM/g)
0.1	0.04	0.00	0.04	0.04	0.00	0.04	0.01	0.00	0.01
0.3	0.12	0.01	0.11	0.13	0.00	0.13	0.04	0.00	0.04
1.0	0.41	0.15	0.26	0.42	0.04	0.38	0.14	0.00	0.14
3.0	1.23	0.65	0.58	1.27	0.17	1.10	0.42	0.03	0.39
7.0	2.86	1.63	1.23	2.97	0.44	2.53	0.98	0.07	0.91
10.0	4.09	2.70	1.39	4.24	0.95	3.29	1.40	0.17	1.23
20.0	8.18	7.16	1.03	8.49	5.04	3.45	2.80	1.28	1.53
30.0	12.28	11.00	1.27	12.73	9.15	3.57	4.21	2.54	1.66

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 6: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Chewable bar (Caramel) formulation in pH 6.8 phosphate buffer without acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)
0.1	0.04	0.00	0.04	0.04	0.00	0.04	0.01	0.00	0.01
0.3	0.12	0.01	0.11	0.12	0.00	0.12	0.04	0.00	0.04
1.0	0.41	0.16	0.25	0.41	0.04	0.37	0.14	0.00	0.14
3.0	1.23	0.65	0.58	1.24	0.17	1.07	0.42	0.03	0.39
7.0	2.87	1.71	1.16	2.89	0.47	2.43	0.98	0.08	0.90
10.0	4.10	2.79	1.31	4.13	1.07	3.07	1.40	0.22	1.18
20.0	8.20	7.33	0.87	8.27	5.21	3.05	2.79	1.42	1.37
30.0	12.29	11.20	1.09	12.40	9.27	3.13	4.19	2.78	1.41

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 7: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Tablet formulation in pH 6.8 phosphate buffer without acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)
0.1	0.02	0.00	0.04	0.02	0.00	0.04	0.01	0.00	0.01
0.3	0.05	0.02	0.11	0.05	0.00	0.12	0.04	0.00	0.04
1.0	0.16	0.16	0.25	0.17	0.03	0.39	0.14	0.01	0.13
3.0	0.49	0.61	0.62	0.50	0.12	1.14	0.42	0.03	0.39
7.0	1.15	1.68	1.20	1.18	0.40	2.53	0.98	0.09	0.89
10.0	1.64	2.84	1.26	1.68	1.02	3.18	1.40	0.24	1.16
20.0	3.28	6.98	1.23	3.36	5.02	3.38	2.80	1.49	1.31
30.0	4.92	10.79	1.52	5.04	9.14	3.46	4.20	2.95	1.25

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Tables 8 to 11 contain the average results from 12 replicate determinations for the percent bound for Welchol™ chewable bars and tablets, respectively.

Table 8: Percent binding results for Welchol™ chewable bar (Chocolate) in the equilibrium binding study without acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	100.0	1.9	100.0	2.0	100.0	0.7
0.3	96.3	5.6	100.0	6.0	100.0	2.2
1.0	66.9	12.8	91.6	18.2	100.0	7.3
3.0	48.6	28.1	86.4	51.7	93.0	20.3
7.0	39.3	53.0	83.2	116.2	91.0	46.4
10.0	30.2	57.9	72.5	144.5	83.3	60.6
20.0	10.4	40.2	36.8	146.5	48.5	70.7
30.0	8.1	46.7	24.7	147.6	33.1	72.2

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 9: Percent binding results for Welchol™ chewable bar (Strawberry) in the equilibrium binding study without acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	100.0	2.0	100.0	2.0	100.0	0.7
0.3	89.0	5.3	99.4	5.9	100.0	2.2
1.0	62.9	12.5	90.2	18.1	100.0	7.3
3.0	47.4	28.3	86.7	51.9	93.1	20.4
7.0	43.2	60.2	85.2	119.1	92.5	47.2
10.0	34.1	67.9	77.6	154.9	87.8	64.0
20.0	12.6	50.0	40.7	162.6	54.5	79.6
30.0	9.9	61.8	28.2	168.5	39.5	86.4

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 10: Percent binding results for Welchol™ chewable bar (Caramel) in the equilibrium binding study without acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	100.0	2.0	100.0	2.0	100.0	0.7
0.3	88.2	5.3	100.0	5.9	100.0	2.2
1.0	60.7	12.1	89.9	17.5	100.0	7.3
3.0	47.1	28.2	86.6	50.6	93.3	20.4
7.0	40.2	56.4	83.8	114.6	91.4	46.7
10.0	32.6	63.9	74.1	144.6	84.5	61.6
20.0	10.6	42.3	36.9	144.1	49.2	71.7
30.0	8.9	53.3	25.2	147.6	33.7	73.5

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 11: Percent binding results for Welchol™ Tablet in the equilibrium binding study without acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	93.8	1.9	100.0	2.0	100.0	0.7
0.3	86.5	5.2	97.5	5.8	100.0	2.2
1.0	61.5	12.4	91.8	18.2	95.3	7.0
3.0	49.8	30.4	90.1	53.6	93.9	20.6
7.0	40.7	58.3	86.1	119.5	91.0	46.5
10.0	29.5	61.5	75.3	149.8	82.6	60.4
20.0	13.5	59.8	39.4	159.2	46.7	68.1
30.0	10.8	74.1	26.4	163.0	29.9	65.4

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

The amount of bile acid bound was then used to evaluate the binding parameters by using the Langmuir equation mentioned above. Table 12 summarizes the affinity (k1) and capacity (k2) constants without acid pretreatment.

Table 12: Comparison of affinity (k1) and capacity (k2) constants without acid pre-treatment

Dosage form	k_1				
	GC	**GCDC**	**TDC**		
Chocolate	14.47	5.44	19.91		
Strawberry	3.14	3.70	13.24		
Caramel	5.17	4.74	19.61		
Tablets	2.22	5.75	26.82		
Dosage form	k_2				
	GC	GCDC	TDC	GC+GCDC+TDC	Chewable Bar/Tablets (T/R)
Chocolate	0.91	3.14	1.36	5.41	0.88
Strawberry	1.21	3.65	1.64	6.50	1.06
Caramel	1.00	3.15	1.39	5.54	0.90
Tablets	1.39	3.47	1.28	6.14	

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

As recommended in the guidance, 90% CI for the T/R ration was calculated for the capacity constant (k2) for each flavor of the chewable bar (Table 13).

Table 13: Statistical analysis of total bile acid binding (GC+GCDC+TDC) without acid pretreatment (n=12)

Parameter	Without Acid Pre-Treatment				
Capacity Constant (k_2)	Type of Chewable Bar	Dosage Form		Chewable Bars/ Tablet	90% CI for Ratio (%)
		Chewable Bars Estimate	Tablets Estimate		
	Chocolate	5.405	6.152	0.879	84.9 – 91.0
	Caramel	5.549		0.902	86.6 – 93.9
	Strawberry	6.469		1.051	101.1 – 109.3

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Equilibrium binding with acid pretreatment

The equilibrium binding with acid pretreatment was conducted using the same method except that 5 mL of 0.1 N HCl was added to each test tube and soaked at 37 °C for one hour. The subsequent steps were similar to that used for study without acid pretreatment. Tables 14 to 17 contain the average results from 12 replicate determinations for Welchol™ chewable bars and tablets.

Table 14: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Chewable bar (Chocolate) formulation in pH 6.8 phosphate buffer with acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)
0.1	0.04	0.00	0.04	0.04	0.00	0.04	0.01	0.00	0.01
0.3	0.12	0.01	0.11	0.12	0.00	0.12	0.04	0.00	0.04
1.0	0.41	0.15	0.26	0.41	0.04	0.37	0.14	0.00	0.14
3.0	1.24	0.63	0.61	1.24	0.16	1.08	0.42	0.02	0.40
7.0	2.89	1.73	1.16	2.89	0.47	2.41	0.98	0.09	0.89
10.0	4.12	2.84	1.29	4.12	1.14	2.98	1.40	0.25	1.15
20.0	8.25	7.33	0.92	8.25	5.25	3.01	2.80	1.53	1.28
30.0	12.37	11.03	1.35	12.37	9.14	3.24	4.20	2.91	1.29

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 15: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Chewable bar (Strawberry) formulation in pH 6.8 phosphate buffer with acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mM/g)	Initial (mM)	Unbound (mM)	Bound (mM/g)	Initial (mM)	Unbound (mM)	Bound (mM/g)
0.1	0.04	0.00	0.04	0.04	0.00	0.04	0.01	0.00	0.01
0.3	0.12	0.02	0.11	0.12	0.00	0.12	0.04	0.00	0.04
1.0	0.41	0.16	0.25	0.41	0.04	0.37	0.14	0.00	0.14
3.0	1.23	0.64	0.59	1.24	0.16	1.09	0.42	0.03	0.39
7.0	2.87	1.71	1.16	2.90	0.47	2.44	0.98	0.08	0.90
10.0	4.10	2.79	1.31	4.15	1.12	3.04	1.40	0.23	1.17
20.0	8.20	7.04	1.17	8.29	5.14	3.16	2.80	1.45	1.36
30.0	12.30	10.86	1.44	12.44	9.14	3.31	4.20	2.79	1.41

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 16: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Chewable bar (Caramel) formulation in pH 6.8 phosphate buffer with acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)
0.1	0.04	0.00	0.04	0.04	0.00	0.04	0.01	0.00	0.01
0.3	0.12	0.02	0.11	0.13	0.00	0.13	0.04	0.00	0.04
1.0	0.41	0.16	0.25	0.42	0.04	0.38	0.14	0.00	0.14
3.0	1.23	0.64	0.59	1.26	0.16	1.10	0.42	0.03	0.40
7.0	2.87	1.72	1.15	2.93	0.48	2.45	0.98	0.09	0.90
10.0	4.10	2.82	1.28	4.19	1.17	3.03	1.40	0.24	1.16
20.0	8.20	7.22	0.98	8.37	5.37	3.01	2.80	1.49	1.32
30.0	12.29	11.02	1.27	12.56	9.49	3.07	4.20	2.89	1.32

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 17: Summary of bile acid salt equilibrium binding data (n=12) for Welchol™ Tablet formulation in pH 6.8 phosphate buffer with acid pretreatment

Total Initial Conc. (mM)	GC			GCDC			TDC		
	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)	Initial (mM)	Unbound (mM)	Bound (mmol/g)
0.1	0.04	0.00	0.04	0.04	0.00	0.04	0.01	0.00	0.01
0.3	0.12	0.02	0.11	0.12	0.00	0.12	0.04	0.00	0.04
1.0	0.41	0.16	0.25	0.41	0.03	0.38	0.14	0.01	0.13
3.0	1.24	0.62	0.62	1.23	0.12	1.11	0.42	0.02	0.40
7.0	2.89	1.71	1.18	2.87	0.38	2.49	0.98	0.08	0.90
10.0	4.13	2.93	1.20	4.10	1.01	3.10	1.40	0.23	1.17
20.0	8.26	7.16	1.10	8.21	5.03	3.18	2.80	1.46	1.33
30.0	12.38	11.06	1.32	12.31	9.18	3.14	4.19	2.90	1.30

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Tables 18 to 21 contain the average results from 12 replicate determinations for the percent bound for Welchol™ chewable bars and tablets, respectively.

Table 18: Percent binding results for Welchol™ chewable bar (Chocolate) in the equilibrium binding study with acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	100.0	2.0	100.0	1.9	100.0	0.7
0.3	89.5	5.4	100.0	5.8	100.0	2.2
1.0	63.2	12.8	90.4	17.6	100.0	7.3
3.0	49.1	29.9	87.3	51.1	94.1	20.7
7.0	39.7	56.4	83.6	113.9	91.2	46.7
10.0	30.8	62.8	72.3	140.7	82.5	60.3
20.0	10.6	44.8	36.3	141.8	45.6	66.6
30.0	10.4	65.8	26.1	152.7	30.8	67.4

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 19: Percent binding results for Welchol™ chewable bar (Strawberry) in the equilibrium binding study with acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	100.0	2.0	100.0	2.0	93.3	0.7
0.3	87.4	5.2	99.5	5.8	97.5	2.1
1.0	60.7	12.1	89.5	17.6	98.1	7.2
3.0	47.6	28.6	86.9	51.2	93.4	20.6
7.0	40.6	56.7	83.9	114.9	91.4	46.8
10.0	31.9	64.0	73.1	143.3	83.3	61.1
20.0	14.2	56.9	38.1	149.3	48.9	71.0
30.0	11.7	70.4	26.5	156.1	33.5	73.8

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 20: Percent binding results for Welchol™ chewable bar (Caramel) in the equilibrium binding study with acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	100.0	2.0	100.0	2.0	100.0	0.7
0.3	87.0	5.2	99.8	5.9	100.0	2.2
1.0	60.2	12.0	89.6	17.7	100.0	7.3
3.0	47.9	28.8	87.2	51.8	93.8	20.6
7.0	39.9	55.9	83.5	115.4	91.3	46.7
10.0	31.2	62.4	72.2	142.7	82.9	60.7
20.0	11.9	47.6	35.9	141.8	46.9	68.7
30.0	10.3	62.1	24.8	145.0	31.3	68.7

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 21: Percent binding results for Welchol™ Tablet in the equilibrium binding study with acid pretreatment

Total Initial Conc. (mM)	GC		GCDC		TDC	
	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol	% Bile Acid Salt Bound vs. Initial Amount	% Bile Acid Salt Bound vs. Weight of Welchol
0.1	93.4	1.9	100.0	1.9	100.0	0.7
0.3	86.4	5.2	97.7	5.7	98.3	2.2
1.0	60.4	12.2	91.5	17.7	95.3	6.9
3.0	49.9	30.2	90.2	52.4	94.2	20.6
7.0	40.8	57.4	86.6	117.4	91.7	46.8
10.0	29.0	58.3	75.4	146.0	83.4	60.8
20.0	13.3	53.4	38.7	150.0	47.5	69.6
30.0	10.7	64.5	25.5	148.1	30.8	67.6

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

The amount of bile acid bound was then used to evaluate the binding parameters by using the Langmuir equation mentioned above. Table 22 summarizes the affinity (k1) and capacity (k2) constants with acid pretreatment.

Table 22: Comparison of affinity (k1) and capacity (k2) constants with acid pre-treatment

Dosage form	k_1				
	GC	GCDC	TDC		
Chocolate	1.90	3.92	24.71		
Strawberry	1.71	3.76	27.59		
Caramel	2.49	4.79	28.88		
Tablets	2.57	7.46	25.44		
Dosage form	k_2				
	GC	GCDC	TDC	GC+GCDC+TDC	Chewable Bars/Tablets (T/R)
Chocolate	1.21	3.27	1.30	5.78	1.00
Strawberry	1.41	3.37	1.30	6.08	1.05
Caramel	1.18	3.13	1.30	5.61	0.97
Tablets	1.27	3.20	1.32	5.79	

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

The 90% CI for the T/R ratio was calculated for the capacity constant (k2) for each flavor of the chewable bar, as presented in Table 23.

Table 23: Statistical analysis of total bile acid binding (GC+GCDC+TDC) without acid pretreatment (n=12)

Parameter	With Acid Pre-Treatment				
Capacity Constant (k_2)	Type of Chewable Bar	Dosage Form		Chewable Bars/ Tablet	90% CI for Ratio (%)
		Chewable Bars Estimate	Tablets Estimate		
	Chocolate	5.768	5.791	0.996	94.1 – 105.2
	Caramel	5.614		0.969	92.5 – 101.4
	Strawberry	6.099		1.053	100.3 – 110.5

Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Based on these data, each flavor of the Welchol™ chewable bar is comparable to Welchol™ Tablets in the equilibrium study of bile salt binding in simulated intestinal fluid (pH 6.8 phosphate buffer) without acid pretreatment (pivotal measure) and with acid pretreatment.

2.3.1.2. Kinetic binding

The kinetic testing for the Welchol™ chewable bars and the Welchol™ tablets were performed using methods M12602 and M4176, respectively. The sponsor followed recommendations outlined in the product

specific draft guidance for Colesevelam HCl (revised on Jan 2016). One lot of each flavor for the registration stability lots of Welchol™ chewable bars and one approved commercial lot of Welchol™ tablets were used for the study. The kinetics reaction took place in a dissolution apparatus at 37±0.5 °C using the USP Apparatus 2, paddle stirrer operated at 200 rpm. Aliquots of the test solutions for the chewable bars and tablet (initial concentrations of either 0.3 or 3 mM) were collected and filtered at several time intervals over a 24 hour period and are analyzed by HPLC.

Table 24: Comparison of kinetic data of binding 0.3 mM bile acid salts

Time (min)	GC (Bound, mg)				GCDC (Bound, mg)				TDC (Bound, mg)			
	Choco.	Straw.	Caram.	Tab.	Choco.	Straw.	Caram.	Tab.	Choco.	Straw.	Caram.	Tab.
3	29.3	31.6	31.2	26.2	33.3	33.3	33.6	30.9	12.8	13.0	12.7	11.8
6	31.2	31.9	32.2	29.2	33.9	33.2	33.7	32.6	12.9	13.0	12.7	12.5
9	32.6	32.9	33.2	31.0	34.4	33.6	34.0	33.6	13.1	13.1	12.9	13.3
12	34.5	34.5	34.9	32.0	35.6	34.6	35.0	34.3	13.6	13.5	13.3	13.5
15	34.0	33.8	34.3	32.7	35.4	34.4	34.8	34.7	13.5	13.4	13.2	13.6
30	34.8	34.3	34.7	33.8	35.5	34.4	34.9	35.1	13.6	13.4	13.3	13.6
60	35.6	35.0	35.5	34.5	36.3	35.2	35.6	35.5	13.7	13.6	13.4	13.6
120	35.7	35.1	35.6	34.9	36.3	34.9	35.5	35.7	13.6	13.6	13.4	13.6
240	35.9	35.2	35.7	35.0	36.3	34.9	35.5	35.8	13.7	13.5	13.4	13.6
480	36.0	35.4	35.9	35.1	36.3	35.3	35.7	35.9	13.7	13.6	13.6	13.6
960	35.9	35.2	35.7	35.1	36.3	35.1	35.6	36.0	13.7	13.6	13.5	13.6
1440	35.9	35.2	35.8	35.1	36.3	35.0	35.6	36.0	13.6	13.6	13.5	13.6

Choco = Chocolate; Straw = Strawberry; Caram = Caramel; Tab = Tablet; Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

Table 25: Comparison of kinetic data of binding 3.0 mM bile acid salts

Time (min)	GC (Bound, mg)				GCDC (Bound, mg)				TDC (Bound, mg)			
	Choco.	Straw.	Caram.	Tab.	Choco.	Straw.	Caram.	Tab.	Choco.	Straw.	Caram.	Tab.
3	138.9	193.6	166.5	152.3	231.5	295.7	264.2	237.5	102.1	119.2	113.8	99.7
6	163.3	203.6	190.8	185.0	266.5	306.9	288.5	279.6	113.3	122.4	120.5	114.8
9	181.3	209.2	205.7	204.8	286.3	312.9	301.2	300.1	119.0	123.8	123.7	121.3
12	195.3	213.6	214.3	216.2	301.2	319.2	309.2	309.7	123.3	125.4	126.0	124.2
15	201.4	214.0	216.7	222.8	305.2	319.5	312.7	314.8	124.2	125.6	126.7	125.6
30	214.0	217.1	220.2	232.0	314.0	323.9	316.8	322.3	126.5	126.6	127.7	127.6
60	219.1	217.8	223.2	236.2	321.7	328.1	322.5	327.8	128.4	127.9	129.2	129.4
120	218.0	217.1	222.4	234.6	323.1	329.2	323.6	329.4	128.8	128.1	129.5	129.9
240	216.7	216.1	221.2	233.4	324.0	329.2	324.2	330.6	129.1	128.4	129.6	130.2
480	214.6	214.6	218.5	232.3	324.6	331.2	325.2	331.1	129.4	129.7	130.0	130.4
960	211.3	212.4	214.7	230.7	324.1	331.0	324.3	331.2	129.2	128.7	129.9	130.5
1440	208.4	210.9	211.5	229.0	323.5	331.0	324.0	331.2	129.2	128.7	129.8	130.5

Choco = Chocolate; Straw = Strawberry; Caram = Caramel; Tab = Tablet; Source: NDA 210895, Module 5.3.1.2. In-vitro Bioequivalence Study Report

In general, the binding at the initial 0.3 mM and 3.0 mM concentration was rapid for all three flavors of the chewable bars and tablets; almost no bile acid salts were left after the first few minutes of the study. The kinetic binding of 0.3 mM and 3.0 mM of each bile salt for both dosage forms were comparable.

2.3.2. What are the key findings of the chewing study?

To address the Agency's concern regarding the impact of chewing on the in vivo performance of colesevelam HCl, the sponsor conducted an in vitro study evaluating the bile acid binding capacity for three different sizes of Welchol™ chewable bar pieces using the caramel flavored formulation as a representative drug product for all three flavors. This approach was used due to lack of availability of a standardized method for studying the impact of chewing on products designed to be completely ingested. The rationale

for the various sizes was based on the variable nature of human chewing and the possibility that different sized pieces of the bars could be swallowed post-chewing. To prepare the samples, a 1 cm bar sample was manually cut in half, quarters and eighths resulting in 2, 4, or 8 pieces of the chewable bar. The 1 cm piece represented the non-masticated condition. The largest piece measured 1 cm x 2.7 cm x 1 cm while the smallest pieces measured approximately 0.5 cm x 0.675 cm x 1 cm. The study design of the in vitro chewing experiment is provided in Table 26. The two bile acids, GC and GCDC, were analyzed for bile acid unbound (mg) and capacity (g/g) for each size and extraction time. The analysis was conducted using the Bile Acid Binding Capacity HPLC Method (M12600) used for release and stability testing; refer to the CMC review for additional details on this analytical method. The data show that the bile acid binding of GC (Table 27) and GCDC (Table 28) was essentially same across mastication by the size of the pieces of chewable bar and extraction time.

Table 26: In vitro chewing study design

Type of Mastication	Number of Cuts	Number of Resulting Pieces	Extraction Time (hours)
No Mastication	-	1	2, 3, 4
Mastication Variable 1	2	2	2, 3, 4
Mastication Variable 2	4	4	2, 3, 4
Mastication Variable 3	8	8	2, 3, 4

Sample sizes
No mastication: 1 cm x 2.7 cm x 1 cm
Variable 1: 1 cm x 1.35 cm x 1 cm
Variable 2: ~0.5 cm x 1.35 cm x 1 cm
Variable 3: ~0.5 cm x 0.675 cm x 1 cm

Source: NDA 210895, Module 3.2.P.2. Pharmaceutical Development/Drug Product

Table 27: Bile acid binding: Comparison of mastication variables on GC

Time (hours)	Replicate	No Mastication		Mastication Variable 1		Mastication Variable 2		Mastication Variable 3	
		Unbound (mg)	Capacity (g/g)	Unbound (mg)	Capacity (g/g)	Unbound (mg)	Capacity (g/g)	Unbound (mg)	Capacity (g/g)
2	1	1.3	0.6	1.4	0.6	1.3	0.6	1.3	0.6
	2	1.3	0.6	1.3	0.6	1.3	0.6	1.4	0.6
	Mean	1.3	0.6	1.3	0.6	1.3	0.6	1.3	0.6
3	1	1.2	0.6	1.2	0.6	1.2	0.6	1.2	0.6
	2	1.3	0.6	1.4	0.6	1.3	0.6	1.2	0.6
	Mean	1.2	0.6	1.3	0.6	1.2	0.6	1.2	0.6
4	1	1.2	0.6	1.2	0.6	1.2	0.6	1.3	0.6
	2	1.2	0.6	1.3	0.6	1.2	0.6	1.3	0.6
	Mean	1.2	0.6	1.2	0.6	1.2	0.6	1.3	0.6

Source: NDA 210895, Module 3.2.P.2. Pharmaceutical Development/Drug Product

Table 28: Bile acid binding: Comparison of mastication variables on GCDC

Time (hours)	Replicate	No Mastication		Mastication Variable 1		Mastication Variable 2		Mastication Variable 3	
		Unbound (mg)	Capacity (g/g)	Unbound (mg)	Capacity (g/g)	Unbound (mg)	Capacity (g/g)	Unbound (mg)	Capacity (g/g)
2	1	0.5	1.5	0.5	1.6	0.5	1.5	0.5	1.5
	2	0.5	1.5	0.5	1.5	0.5	1.5	0.5	1.6
	Mean	0.5	1.5	0.5	1.5	0.5	1.5	0.5	1.5
3	1	0.4	1.4	0.4	1.5	0.4	1.5	0.4	1.5
	2	0.4	1.5	0.5	1.6	0.4	1.5	0.4	1.5
	Mean	0.4	1.5	0.4	1.5	0.4	1.5	0.4	1.5
4	1	0.4	1.4	0.4	1.5	0.4	1.5	0.4	1.5
	2	0.4	1.4	0.4	1.5	0.4	1.5	0.5	1.5
	Mean	0.4	1.4	0.4	1.5	0.4	1.5	0.5	1.5

Source: NDA 210895, Module 3.2.P.2. Pharmaceutical Development/Drug Product

In the discipline review letter dated 03/19/2018, the Agency raised concern that the in vitro chewing study is not adequate to address the impact of chewing as this study simulated a condition where a patient may simply bite off small chunks of the chewable bar and swallow with minimal or no chewing. However, in a clinical scenario, patients may chew the bar in a way that can form a bolus which will then be swallowed. There is no evidence that the swallowed bolus would disintegrate in the same manner as the small piece of intact bar used in the in vitro chewing study.

To address this concern, the sponsor conducted an open-label one-treatment study in healthy volunteers to assess the effect of chewing on the in vitro disintegration of the Welchol™ bar (i.e., bolus) using USP <711> Dissolution Apparatus 3 reciprocating cylinder under acidic condition (Study WEL-A-U120). A total of 12 subjects were enrolled in the study; 6 subjects were studied at one time, i.e., 2 cohorts of 6 subjects each. Each subject took 6 bite-sized pieces of the bar (strawberry flavor) and chewed each piece for up to 45 seconds or until there is an urge to swallow. At either of these two times for each bite, the subject was to fully expectorate the bolus into a separate sample tube (1 for each subject). The process was repeated until the entire bar was completely chewed and expectorated. A visual inspection of each subject's mouth was performed to verify that residual pieces and material had been expectorated into the sample collection tube.

After the entire bar had been chewed and the cumulative expectorates (bolus) placed in the sample tube for all the subjects, the respective tubes were placed in the USP<711> Dissolution Apparatus 3 dissolution vessels; all vessels were filled with 200 mL of simulated gastric fluid (0.1N hydrochloric acid without enzymes), and the disintegration test was conducted (Refer to the CMC review for the disintegration study procedure). The mean (SD) time to complete disintegration of the bolus in the simulated gastric fluid for evaluable subjects (n=10) was 24.0 (13.7) min with individual time ranging from 5 min to 45 min (Table 29). Samples from 2 subjects were not considered evaluable due to technical malfunctions.[3] The time to complete disintegration of the chewed bolus appears to be in the range of the reported gastric emptying time Hellmig S *et al*., J Gastroenterol Hepatol. 2006; Vasavid P *et al.* J Neurogastroenterol Motil, 2014). Hence, the chewed mass is likely to be disintegrated in stomach prior to reaching the intestine – the site of action. Therefore, considering the totality of evidence, chewing does not appear to have any considerable impact on the therapeutic performance of the drug product.

[3] For these two samples, the mesh that was to allow flow of simulated gastric fluid at the bottom of the disintegration chambers into the sample tube became clogged.

Table 29: Assessment of time to complete disintegration of the bolus in the dissolution apparatus

Statistic	Time (min) to complete disintegration
	With exclusion[a]
n	10
Mean	24.0
SD	13.7
Median	22.5
Range	5 – 45

[a]During the disintegration study, sample tube was clogged preventing normal flow of gastric fluid thus prolonging disintegration. The descriptive statistics without values from Subjects (b) (6) and (b) (6) are shown. Source: NDA 210895, Module 5.3.1.1. CSR WEL-A-U120

2.3.3. How is the proposed to-be-marketed formulation linked to the clinical formulation?

The product lots used in the pivotal equilibrium and kinetic binding study represent the to-be-marketed chewable bar formulations.

2.4. Analytical

2.4.1 How the bile acid concentrations were measured in the in vitro equilibrium and kinetic binding study?

The equilibrium binding study for the Welchol™ chewable bar and Welchol™ tablets were performed using methods M12601 and M13400, respectively, and the kinetic binding study was conducted using methods M12602 and M4176, respectively. In response to an information request during filing, the sponsor provided a side-by-side comparison of the equilibrium binding and kinetic binding methods used for the chewable bar and tablet, as summarized in Tables 30 and 31, respectively. The sponsor mentioned that the analytical method developed for tablet could not be used for the chewable bar presentations as separate method was necessary to achieve suitable separation due to significant differences in formulation components between the tablet the chewable bar matrix. It was noted that the analytical methods for the chewable bar and tablet differ in regards to HPLC condition (isocratic vs gradient), mobile phase, the column use, in addition to the sampling procedure. Further, per the sponsor, the sample concentration for tablet and the chewable bars was also different – 1 mg/mL and 0.8 mg/mL for the tablet and the bars, respectively. In the discipline review letter dated 03/19/2018, the Agency advised the sponsor to repeat the in vitro equilibrium and in vitro kinetic binding study using the same analytical method for the tablet and the chewable bars. The sponsor responded that since both methods were independently validated, repetition of the study is not warranted. The Agency, however, did not agree, and recommended that a cross-validation study, at minimum, should be performed for the two methods to address any possible bias in the study results.

Table 30: Comparison of HPLC bile acid binding rate equilibrium method used for Welchol™ Tablets and Chewable bars

Method Parameter	Tablet Method (M13400)	Chewable Bar Method (M12601)
Reference Standards	Same	Same
Reagents	Same	Same
Bile Acid Standards	Same	Same
Sample Preparation	Weight of crushed tablets equiv. to 80 mg API in 80 mL (1 mg/mL)	1600 mg of bar (200 mg active) in 250 mL (0.8 mg/mL)
Without Acid Pre-Treatment	Overnight soak at 37°C with 0.05M potassium phosphate buffer pH 6.8	Overnight soak at 37°C with 0.05M potassium phosphate buffer pH 6.8[a]
Acid Pre-Treatment	Same	Same
Bile Acid Sample Concentration / Sample Treatment	Same	Same
HPLC Conditions	Isocratic	Gradient
Mobile Phases	20 mM TBAP Buffer (pH 7.5) : Acetonitrile (58:42)	A) 20 mM TBAP to Acetonitrile pH 7.5 B) Acetonitrile
Flow Rate	Same	Same
Column	Phenomenex Luna C18, 5 µm, 4.6 x 150 mm	Phenomenex Luna C18, 5 µm, 4.0 x 125 mm
Guard Column	Same	Same
Column Temp.	Same	Same
Autosampler Temp.	Same	Same
Injector Volume	Same	Same
Detector Wavelength	Same	Same
Gradient Sequence	Not applicable	Applied over 30 min.
Injection Sequence	Same	Same
System Suitability	Same	Same
Calculations	Same, except includes calculation for mMol/g of each bile acid	Same, except includes calculation for mMol/g of bile acids combined

Source: NDA 210895, Module 1.11.3. Clinical Pharm Response 07-Dec-2017

Table 31: Comparison of HPLC bile acid kinetic binding rate method used for Welchol™ Tablets and Chewable bars

Method Parameter	Tablet Method (M4176)	Chewable Bar Method (M12602)
Reference Standards	Same	Same
Reagents	Same	Same
Bile Acid Standards	Same	Same
Sample Preparation	1 tablet containing 625 mg (587 mg anhydrous) API. Remaining parts of sample and blank preparation are the same	4.75 g sample equivalent to 623 mg (594 mg anhydrous) API. Remaining parts of sample and blank preparation are the same
Dissolution Apparatus/Conditions	Same	Same
HPLC Conditions	Gradient	Gradient
Mobile Phases	A) 20 mM TBAP to Acetonitrile (58:42) B) 20 mM TBAP to Acetonitrile (55:45)	A) 20mM TBAP B) Acetonitrile
Flow Rate	Same	Same
Column	Alltima C18, 5 um, 4.6 x 150 mm	Phenomenex Luna C18, 5 µm, 4.0 x 125 mm
Guard Column	Alltima C18, 5 um, 7.5 x 4.6 mm	Phenomenex Security Guard C18, 4.0 x 3 mm ID
Column Temp.	20°C	Ambient
Autosampler Temp.	Same	Same
Injector Volume	Same	Same
Detector Wavelength	Same	Same
Gradient Sequence	Method Specific	Method Specific
Injection Sequence	Same	Same
System Suitability	Same	Same
Calculations	Same but includes additional mMol/g bile acid bound calculation.	Same

Source: NDA 210895, Module 1.11.3. Clinical Pharm Response 07-Dec-2017

In response, the sponsor conducted a cross-validation study and submitted the results on 05/31/2018. In the cross-validation study, the sponsor investigated bile acid binding of the tablets using the analytical methods for equilibrium binding and kinetic binding studies used for the chewable bar preparation, and compared that with the bile acid binding observed from the original analytical method used for tablet. Data demonstrate that the extent of bile acid binding is comparable between the two methods (Tables 32 and 33). The sponsor also confirmed that the drug substance sample concentration was incorrectly stated as 1 mg/mL and 0.8 mg/mL for the tablet and chewable bar method, respectively. The actual sample concentration was 1 mg/mL for both methods.

Table 32: Welchol™ tablets tested (lot#FG0001003) by tablet method vs chewable bar method equilibrium test with acid treatment

Bile acid	Parameter	%Bile acid unbound	
		Tablets by bar method (M12601)	Tablets by Tablet Method (M13400)
GC	Average	52.3	50.1
	Range	51.7 – 52.9	50.6 – 51.9
GCDC	Average	9.2	9.8
	Range	8.9 – 9.5	9.4 – 10.0
TDC	Average	5.2	5.8
	Range	4.8 – 5.6	5.6 – 6.0

Source: NDA 210895, Module 1.11.1. Clin Pharm and CMC Response to IR

Table 33: Welchol™ tablets tested (lot #FG0001003) by tablet method vs chewable bar method 0.3 mM concentration with acid treatment

Bile acid	Parameter	%Bile acid unbound	
		Tablets by bar method (M12602)	Tablets by Tablet Method (M4176)
GC	Average	30.3	29.0
	Range	28.1 – 32.6	25.0 – 32.9
GCDC	Average	16.2	15.0
	Range	15.3 – 17.9	12.6 – 18.8
TDC	Average	14.5	13.4
	Range	13.7 – 15.7	11.7 – 17.2

Source: NDA 210895, Module 1.11.1. Clin Pharm and CMC Response to IR

Table 34: Welchol™ tablets tested (lot #FG0001003) by tablet method vs chewable bar method 3.0 mM concentration with acid treatment

Bile acid	Parameter	%Bile acid unbound	
		Tablets by bar method (M12602)	Tablets by Tablet Method (M4176)
GC	Average	60.6	59.0
	Range	59.2 – 62.2	56.8 – 60.7
GCDC	Average	35.9	32.8
	Range	33.7 – 39.0	31.1 – 34.7
TDC	Average	29.3	27.2
	Range	26.7 – 32.5	25.9 – 28.2

Source: NDA 210895, Module 1.11.1. Clin Pharm and CMC Response to IR

2.4.2 What are the findings from the OSIS inspection?

The Office of Study Integrity and Surveillance (OSIS) inspection was requested for the analytical sites of the in vitro equilibrium and kinetic binding studies. At the time of this review, the OSIS inspection report is pending.

3. LABEL RECOMMENDATIONS

The sponsor did not propose any new clinical pharmacology related labeling edits to the existing labels of Welchol™ tablet and powder for suspension products. The proposed edit in Section 2 regarding the dose of Welchol™ chewable bar corresponds to the approved doses of the tablet and powder for suspension products, and hence, is acceptable from a clinical pharmacology perspective.

(b) (4)

APPENDIX A

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 210895

DISCIPLINE REVIEW LETTER

Daiichi Sankyo Inc.
Attention: Scott Greenfeder, PhD
Senior Director, Regulatory Affairs
211 Mount Airy Road
Basking Ridge, NJ 07920-2311

Dear Dr. Greenfeder:

Please refer to your New Drug Application (NDA) dated and received October 30, 2017, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Welchol (colesevelam) Chewable.

Based on our review of the Clinical Pharmacology section of your submission, we have identified the following deficiencies:

1. **In vitro equilibrium binding study and in vitro kinetic binding study:**
   For the pivotal in vitro equilibrium binding study and supportive in vitro kinetic binding study, you used different analytical methods for test (chewable bar, T) and reference (tablet, R) product (Methods M12601 and M12602 for the T product, and M13400 and M4176 for R product). Upon review of the experimental methods, we have significant concern regarding acceptance of the reported data based upon the differences between the two methods. Ideally, T and R products should be investigated using the same analytical method. You should repeat both the in vitro equilibrium binding study and the in vitro kinetic binding study using the same analytical method. Also, ensure that the experimental procedure is the same for T and R products, e.g., amount/concentration of colesevelam should be the same for T and R. Refer to the product-specific *Draft Guidance on Colesevelam Hydrochloride Tablet* (https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm083337.pdf) for Agency's recommendation. For detail study protocol, we recommend that you also follow the product-specific *Draft Guidance on Cholestyramine Powder* (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM273910.pdf).

   We also remind you that for the in vitro equilibrium binding study, twelve observations with mean±SD for the following parameters should be obtained and reported for both test and reference products:
   - Percent binding of bile acid salt to the resin at each concentration (tabular and graphical forms)

- Micromoles of bile acid salts bound to the resin at each concentration (in tabular and graphical forms)
- Affinity constant K_1
- Capacity constant K_2
- Coefficient of determination r^2, when linear regression is used to determine K_1 and K_2

For kinetic binding study, twelve individual observations with mean±SD for the following parameters should be reported for both T and R:

- Percent binding of bile acid salt to the resin at each time point in tabular and graphical forms
- Micro moles of bile acid salt bound to the resin at each time point in tabular and graphical forms

2. **Chewing study:**
The Agency previously expressed concern whether in vitro assessments are sufficient to conclude that an ingested chewable bar would have similar safety and effectiveness as the approved product. Your study simulates a condition where a patient may simply bite off small chunks of the chewable bar and swallow with minimal or no chewing. As we previously pointed out during the pre-NDA telecon, in a clinical scenario, the patient may chew the bar in a way that can form a bolus which will then be swallowed. However, there is no evidence that the swallowed bolus would disintegrate in the same manner as the small piece of intact bar used in the chewing study. Please provide justification that swallowed bolus will disintegrate in the same manner as small pieces.

We also previously recommended that you conduct in vitro equilibrium binding study to assess the effect of chewing on the product performance. We note that you used (b) (4) method for bile acid binding (M12600) which, according to you, is used for release and stability testing. Justify the sensitivity of this method in detecting differences in bile salts binding to assess impact of chewing. Alternatively, we recommend that you repeat the chewing study using in vitro equilibrium binding study.

3. **Stability study:**
You conducted in vitro equilibrium binding study to assess the impact of color change on product performance, and compared the capacity constant with the approved tablet product. Because of the differences in analytical method for the chewable bar and tablet product as outlined above, we recommend that you repeat the in vitro equilibrium binding study using the same analytical method. In addition to the T/R ratio, please also provide 90% confidence interval for each comparison for the capacity constant, K_2. Also, provide T/R ratio for affinity constant, K_1, as recommended in the draft guidance.

We are providing these comments to you before completing our review of your entire application to give you preliminary notice of issues that we have identified. In conformance with the prescription drug user fee reauthorization agreements, these comments do not reflect a final decision on the information reviewed and should not be construed to do so. These comments are preliminary and subject to change as we finalize our review of your application. In addition, we may identify other information that must be provided before we can approve this application. If

you respond to these issues during this review cycle, depending on the timing of your response, and in conformance with the user fee reauthorization agreements, we may or may not be able to consider your response before we take an action on your application during this review cycle.

If you have any questions, call Kati Johnson, Senior Regulatory Project Manager, at (301) 796-1234.

Sincerely,

{See appended electronic signature page}

Chandrahas G. Sahajwalla, PhD
Director
Division of Clinical Pharmacology 2
Office of Clinical Pharmacology
Office of Translational Science
Center for Drug Evaluation and Research

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MOHAMMAD S ABSAR
06/12/2018

JAYABHARATHI VAIDYANATHAN
06/12/2018