INTERIM GUIDANCE[1]

CHOLESTYRAMINE POWDER

IN VITRO BIOEQUIVALENCE

I. INTRODUCTION

A. Clinical Usage/Pharmacology

Cholestyramine resin is a bile acid sequestering antilipemic agent. It is used as an adjunct to dietary therapy to reduce elevated serum total and low-density lipoprotein (LDL) cholesterol levels in patients with primary hypercholesterolemia when diet alone is not adequately effective. Cholestyramine may lower plasma cholesterol levels in patients with hypertriglyceridemia. The drug is not indicated for treatment of hypertriglyceridemia where elevated triglyceride levels are of primary concern. Cholestyramine resin is also prescribed for the relief of pruritus associated with partial cholestasis associated with biliary obstruction (1).

In humans, cholesterol is oxidized to bile acids and converted into salts of glycine and taurine conjugates in the liver. These conjugates are then stored in the gall bladder. In the gastrointestinal lumen, bile salts emulsify ingested fats and thereby promote digestion. During the absorptive phase of digestion, approximately 90 per cent of bile salts are reabsorbed. Thus, bile salts produced from cholesterol in the liver pass to the intestine and are then returned to the liver. This process of enterohepatic circulation facilitates the excretion of sterols and the digestion of dietary lipids (1,2).

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[1]This guidance, prepared by the Division of Bioequivalence in the Office of Generic Drugs in the Center for Drug Evaluation and Research at FDA, is an informal communication under 21 CFR 10.90(b)(9) that represents the best judgment of the Division at this time. This statement does not necessarily represent the formal position of the Center, and does not bind or otherwise obligate the Center to the views expressed. For further information about this guidance, contact the Division of Bioequivalence, Office of Generic Drugs, 7500 Standish Place, Metro Park North, Rockville, MD 20855 (Phone: 301-295-8290; Fax: 301-295-8183).

Following oral administration, cholestyramine resin releases chloride ions and adsorbs anions of bile acid conjugates in the intestine, forming nonabsorbable complexes. The complexes are excreted with unchanged resin in the feces, a process that results in partial removal of bile acids from the enterohepatic circulation. By interrupting the enterohepatic circulation of bile salts, cholestyramine resin produces a two to fifteen fold increase in fecal excretion of bile acids and triggers a compensatory increase in the oxidation of systemic cholesterol to bile acids and in the liver. Despite increased production of cholesterol by the liver, plasma cholesterol and LDL concentrations fall in patients with primary type II hyperlipoproteinemia (1,2).

The dosage of cholestyramine is expressed in terms of amount of dry resin. The recommended starting dose for an adult is 4 gm of cholestyramine resin once or twice a day. The maximum recommended dose is 24 gm of resin daily in divided doses. Increases in daily dose should be gradual with periodic assessment of serum cholesterol (LDL, HDL, VLDL, and total) levels in patients. Cholestyramine resin is recommended to be administered with meals (1).

The most common adverse reaction associated with cholestyramine resin is constipation. Less frequent adverse reactions include: abdominal discomfort and/or pain, nausea, flatulence, vomiting, and diarrhea (1,2).

Cholestyramine is currently marketed as a powder for oral suspension containing 4 gm cholestyramine resin/9 gm powder (Questran ^{R}) and 4 gm resin/5 gm powder (Questran Light ^{R}) by Bristol Laboratories. Parke/Davis Division of Warner-Lambert Company markets a cholestyramine chewable bar, 4 gm/bar under the trade-name Cholybar (3).

B. Chemistry

Cholestyramine resin is the chloride form of a basic quaternary ammonium anion-exchange resin which is hydrophilic, insoluble in water, and unabsorbed in the gastrointestinal tract. The strongly basic synthetic resin contains trimethylbenzylammonium groups attached to a large styrene-divinylbenzene copolymer backbone. The resin occurs as a white to buff-colored, fine,

hygroscopic powder (1,2). The anion binding properties of the resin depend on its physical characteristics, such as cross-linkage, particle size, hydrophilicity, and the environment in which the resin exists, such as type of bile salts present, relative concentration of these bile salts, presence of anions such as chloride and bicarbonate, and possibly its location in the GI tract. *In vitro* studies have shown that the binding of bile salts to cholestyramine is not influenced by pH and temperature (4-8).

C. Pharmacokinetics

Because the drug is not absorbed into the systemic circulation, pharmacokinetic information is not available.

II. *IN VIVO* BIOEQUIVALENCE STUDIES

The Division of Bioequivalence has concluded that *in vivo* studies are not necessary to document the bioequivalence of cholestyramine resin formulations.

III. *IN VITRO* BIOEQUIVALENCE STUDIES

Equilibrium and kinetic *in vitro* bile acid salts binding studies are recommended to document bioequivalence between generic and innovator formulations of cholestyramine.

An equilibrium binding study is conducted under conditions of constant time and varying concentrations of bile acid salts. The equilibrium studies described in Sections III/B and III/C of this *Interim Guidance* should be conducted with and without acid pretreatment of the drug product.

A kinetic binding study is conducted under constant concentration of bile acid salts with varying times of observation. The kinetic studies described in Sections III/D and III/E of this *Interim Guidance* should be conducted at two concentrations of bile salts (0.3 and 3 mM).

A. Product Information

FDA Designated Reference Product: Questran ^{R}, 4 gm Resin/Packet (Bristol Myers) and Questran Light ^{R}, 4 gm Resin/Packet (Bristol Myers).

Generic Formulation: The test batch or lot of the generic formulation must be manufactured under production conditions and must be of a size at least 10% that of the largest lot planned for full production or a minimum of 100,000 units, whichever is larger.

B. Protocol for Equilibrium Study of Binding of Bile Acid Salts to Resin in SIF Without Acid Pre-treatment

Objective:

To compare the affinity and capacity binding constants of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulation under identical experimental conditions.

Materials:

1. Simulated intestinal fluid (SIF): 0.05 M potassium phosphate buffer solution without enzyme, pH 6.8 (9).

2. Stock solution of bile acid salts in SIF: Prepare in SIF a 40 mM solution containing the sodium salts of the following bile acids in the molar proportion 3:3:1: glycocholic acid (GCA), 17.14 mM, glycochenodeoxycholic acid (GCDA), 17.14 mM, and taurodeoxycholic acid (TDCA), 5.72 mM.

3. Cholestyramine powder: generic formulation and reference drug product (Questran ^{R} or Questran Light^{R}).

Procedures:

1. Incubation Mixtures for Test and Reference Drug Products

   Set up eight incubation flasks of the test product and eight of the reference product, each containing the equivalent of 10 mg resin. Add 2 ml of SIF and soak at room temperature overnight. The following day, add to each container the requisite volumes of SIF and 40 mM bile acid salts solution in SIF to make the final volume of the solvent mixture 10 ml with the target concentrations of bile acid salts covering the ranges of 0.1 - 30 mM (Table 1).

TABLE 1: COMPOSITION OF INCUBATION MIXTURES AND CONCENTRATIONS OF INDIVIDUAL BILE ACID SALTS AT VARIOUS TARGET CONCENTRATIONS IN EQUILIBRIUM STUDY (ML STOCK SOLUTION + ML SIF + 2 ML SIF = 10 ML)

TARGET (mM)	STOCK (mL)	SIF (mL)	GCA (mM)	GCDCA (mM)	TDCA (mM)
0.1	0.025	7.975	0.0428	0.0428	0.0143
0.3	0.075	7.925	0.1286	0.01286	0.0429
1.0	0.250	7.750	0.4285	0.4285	0.1428
3.0	0.750	7.250	1.2855	1.2855	0.4284
7.0	1.750	6.250	2.9995	2.9995	0.9996
10.0	2.500	5.500	4.2850	4.2850	1.4280
20.0	5.000	3.000	8.5700	8.5700	2.8560
30.0	7.500	0.500	12.855	12.855	4.2840

2. Blank Incubation Mixture

   Prepare four blank incubation flasks each containing the drug product equivalent to 10 mg resin in 2 ml of SIF and soak at room temperature overnight. The next day add 8 ml of SIF to each blank. Two blanks will be used for the test and two blanks will be used for the reference product.

3. Standard Solutions of Bile Acid Salts

   Dilute the requisite volumes of 40 mM stock solution of bile acid salts with SIF to yield 10 ml standard solutions of the following concentrations: 0.1, 0.3, 1, 3, 7, 10, 20, and 30 mM (n = 8).

Incubation flasks for one set of experiments will thus

include: 1) eight of the test product; 2) eight of the reference product; 3) four blanks; and 4) eight standards.

Incubate all 28 flasks at 37 °C for 24 hours. Filter to collect the filtrate and assay the filtrate to determine concentrations of the bile acid salts. After incubation, the 0.1 mM standard solution filtrate is diluted with SIF to obtain the ninth standard with a concentration 0.05 mM.

The experiment should be repeated six times under the conditions described above to obtain six sets of data.

Data Treatment and Analysis

The amount of bile acid salt bound to cholestyramine resin 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 adsorbate (bile acid salt) molecules from solution, at constant temperature, on to an adsorbent (cholestyramine resin) is described by the following Langmuir-type equation (5), Equation 1:

$$\frac{x}{m} = \frac{k_1 k_2 C_{eq}}{1 + k_1 C_{eq}}$$

Upon rearranging, Equation 2 is obtained:

$$\frac{C_{eq}}{x/m} = \frac{1}{k_1 k_2} + \frac{C_{eq}}{k_2}$$

where:

$\mathbf{C_{eq}}$ = concentration of the adsorbate (bile acid salt) remaining in the solution at equilibrium;

x = the amount of adsorbate bound to the adsorbent (cholestyramine resin); and

m = the amount of adsorbent 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 adsorbate that can be adsorbed per unit weight of adsorbent.

From the concentration of bile acid salt in the solution at equilibrium, the amount of bile acid salt, expressed in micromoles and as a percentage, bound to 10 mg of cholestyramine resin may be calculated. From this the amount of bile acid salt bound per mg of resin, the relationship x/m is calculated. A plot of $C_{eq}/(x/m)$ versus C_{eq} on rectilinear coordinates should yield a straight line. Application of regression analysis will yield a slope (a) and intercept (b) of the line. The affinity constant, k_1, and capacity constant, k_2, are calculated from the slope and intercept as follows:

$$k_1 = a/b$$

$$k_2 = 1/a$$

Statistical packages with nonlinear regression programs are available that yield k_1 and k_2 values directly.

Parameters To Be Reported:

Six observations with mean $\pm$ SD for the following parameters should be obtained and reported for both the test and reference products:

1. Percent binding of bile acid salt to 10 mg of resin at each concentration (tabular and graphical forms);

2. Micromoles of bile acid salts bound to 10 mg of resin at each concentration (in tabular and graphical forms);

3. Affinity constant, k_1;

4. Capacity constant, k_2;

5. Coefficient of determination, r^2, when linear regression is used to determine k_1 and k_2.

C. Protocol for Equilibrium Study of Binding of Bile Acid Salts to Resin in SIF With Acid Pre-treatment

Objective:

To compare the affinity and capacity constants of binding of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulation after acid pre-treatment of both the products.

Materials:

1. 0.1 N hydrochloric acid

2. Other materials as in Section B

Procedures:

Soak the test and reference drug products equivalent to 10 mg of resin in 10 ml 0.1N hydrochloric acid at 37 °C for 1 hour. Centrifuge and aspirate the supernate. Wash the drug product with SIF until pH 6.8 is attained. Soak the acid pre-treated resin product in 2 ml SIF at room temperature overnight. Conduct the remainder of the experiment as described in Section B.

D. Protocol for Study of Kinetics of Binding of Bile Acid Salts in 0.3mM Aqueous Solution in the Presence of Added Sodium Chloride (0.1 M)

Objective:

To compare the kinetics of binding of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulations (Questran® and Questran Light®) under identical experimental conditions.

Materials:

1. Stock solution of sodium salts of bile acids: prepare a 40 mM solution containing sodium salts of the following bile acids in the molar proportion 3:3:1 in water: glycocholic acid (GCA), 17.14 mM, glycochenodeoxycholic acid (GCDA), 17.14 mM, and taurodeoxycholic acid (TDCA), 5.72 mM.

2. 0.1 M sodium chloride in water.

3. 1.0 M solution of sodium chloride in water.

4. Cholestyramine powder: generic formulation and reference drug formulations (Questran ^{R} and Questran Light ^{R}).

Procedure:

1. Incubation Mixtures for the Test and Reference Drug Products

   Soak the drug product equivalent to 10 mg resin in 2 ml of 0.1M sodium chloride solution at room temperature overnight. To this add quickly 0.075 ml of 40 mM bile acid salts solution in water, 0.8 ml of 1M sodium chloride stock solution, and 7.125 ml water to obtain a final volume of 10 ml fluid with a bile acid salts concentration of 0.3 mM. Prepare eight replicates of the incubation mixture as described above for the generic product and eight replicates for the reference drug products (Table 2).

TABLE 2: COMPOSITION OF INCUBATION MIXTURES AND CONCENTRATIONS OF INDIVIDUAL BILE ACID SALTS AT TARGET CONCENTRATIONS OF 0.3 AND 3.0 mM IN KINETIC STUDY (ML STOCK SOLUTION + ML WATER + 0.8 ML 1M NACL + 2 ML 0.1M NACL = 10 ML)

TARGET (mM)	STOCK (mL)	WATER (mL)	1M NaCl (mL)	GCA (mM)	GCDCA (mM)	TDCA (mM)
0.3	0.075	7.125	0.80	0.1285	0.1285	0.043

3.0	0.750	6.450	0.80	1.286	1.286	0.428

2. Blank Incubation Mixtures

Soak the drug product equivalent to 10 mg of resin in 2 ml of 0.1 M solution of sodium chloride at room temperature overnight. Add 8 ml of 0.1 M sodium chloride solution, incubate for 24 hours at 37°C, filter, and collect the filtrate. At least two such blanks should be prepared for each drug product.

3. Standard Solutions of Bile Acid Salts

Prepare two standard solutions of bile acid salts of concentrations 0.1 and 0.3 mM in water by adding, to requisite volumes of 40 mM stock bile acid salts in water, 1 ml of 1.0 M stock sodium chloride solution and water to yield a final volume of 10 ml. Incubate the two standards at 37°C for 24 hours, filter, and collect the filtrate. Additional standards of concentrations 0.05 and 0.075 are obtained from the filtrate of 0.1 mM solution by dilution with 0.1 M sodium chloride solution. Additional standards of concentrations 0.15 and 0.21 mM are obtained from the filtrate of 0.3 mM solution by dilution with 0.1 M sodium chloride solution.

In one set of experiments, there will be eight incubation mixtures with the test product and eight with **each** of the reference products; six blank incubation mixtures; and two standard solutions of bile acid salts. Each of the incubation mixture containing the test or reference drug product is incubated at 37 °C for its designated time of incubation (0.25, 0.50, 1, 2, 4, 8, 16, or 24 hours), filtered, and the filtrate collected to determine the concentrations of the bile acid salts.

Data Treatment and Analysis:

The amount of bile acids salts bound to the resin is calculated from the initial concentrations of bile acid salts introduced into the system and the concentrations of bile acid salts present in the filtrate at the

designated time points. From these values, bile acid salt bound to 10 mg of resin, expressed as percent and micromoles, at each time point are calculated.

The experiment should be repeated six times under the conditions described above to obtain six sets of data.

Parameters To Be Reported:

Six individual observations with mean $\pm$ SD for the following parameters should be reported for both the test and reference products:

1. Percent binding of bile acid salt to 10 mg of resin at each time point in tabular and graphical forms; and

2. Micromoles of bile acid salt bound to 10 mg of resin at each time point in tabular and graphical forms.

E. Protocol for the Study of Kinetics of Binding of Bile Acid Salts in 3 mM Aqueous Bile Acid Salts Solution in the Presence of Added Sodium Chloride (0.1 M).

Objective:

To compare the kinetics of binding of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulations (Questran ^{R} and Questran Light ^{R}) under identical experimental conditions.

Materials:

1. Stock solution of sodium salts of bile acids in water: prepare as described in Section D.

2. A solution of 0.1 M sodium chloride in water.

3. A solution of 1.0 M sodium chloride in water.

4. Cholestyramine powder: generic formulation and reference drug products (Questran ^{R} and Questran Light^{R})

Procedures:

1. Incubation Mixtures for the Test and Reference Drug Products

   Soak the drug product equivalent to 10 mg resin in 2 ml of 0.1 M sodium chloride solution at room temperature overnight. To this add quickly 0.75 ml of 40 mM bile acid salts solution in water, 0.8 ml of 1 M of sodium chloride stock solution, and 6.45 ml water to obtain a final volume of 10 ml and bile acid salts concentration of 3.0 mM. Prepare eight replicates of the incubation mixture each for the generic product and the two reference products.

2. Blank Incubation Mixtures

   Prepare as described in section D above.

3. Standard Solutions of Bile Acid Salts

Prepare two standard solutions of bile acid salts of concentrations 0.1 and 3.0 mM in water by adding, to requisite volumes of 40 mM stock solution of bile acid salts in water, 1 ml of 1.0 M stock solution of sodium chloride and water to make the final volume 10 ml. Incubate the two standards at 37 °C for 24 hours. Additional standards of 0.05 and 0.075 mM are obtained from the filtrate of 0.1 mM solution. Additional standards of 0.3 and 1.0 mM are obtained from the filtrate of 3.0 mM solution by dilution with 0.1 M sodium chloride solution.

In one set of experiments, there will be eight incubation mixtures for the test product, eight with each of the reference products, six blank incubation mixtures, and two standard solutions of bile acid salts. Each of the incubation mixtures containing the test or the reference drug product is incubated at 37 °C for its designated time of incubation (0.25, 0.50, 1, 2, 4, 8, 16, or 24 hours), filtered, and the filtrate collected to determine the concentrations of bile acid salts.

The experiment should be repeated six times under the conditions described above to obtain six sets of data.

Data Treatment and Analysis:

As in Section D above.

G. Facilities

The analytical facility used for the study should be identified. The names, titles, and curriculum vitae of the scientific/analytical directors should be included in the study report.

H. Retention of Samples

The laboratory conducting the bioequivalence testing should retain an appropriately identified reserve sample of the test and reference drug products used in the *in vitro* testing. Each reserve sample should consist of at least 200 dosage units. For more information refer to CFR 21, 320.32.

IV. REFERENCES

1. Physician's Desk Reference (PDR). 47th Edition, Montvale, NJ: Medical Economics Company, 1993:732-4.

2. American Hospital Formulary Service (AHFS) Drug Information. Bethesda, MD: American Society of Hospital Pharmacists,Inc., 1990:883-7.

3. Approved Drug Products with Therapeutic Equivalence Evaluation (Orange Book). 12th Edition, Washington DC: US Dept of HHS, 1992:3-63.

4. Data obtained by the Division of Bioequivalence and data on file with the Divisions of Bioequivalence and Biopharmaceutics.

5. Johns WM , Bates TR. Quantification of the Binding Tendencies of Cholestyramine I: Effect of Structure and Added Electrolytes on the Binding of Unconjugated and Conjugated Bile-Salt Anions. J Pharmaceut Sci 1969;58:179-183.

6. Graham DY, Sackman JW, Giesing DH, Rusner DJ. *In vitro* adsorption of bile salts and aspirin to sucralfate. Digestive Diseases and Sciences 1984;29:402-6.

7. Kos R, White JL, Hem SL, Borin MT. Effect of anions on binding of bile salts by cholestyramine. Pharm Res 1991;8:238-41.

8. Luner PE, Amidon GL. Equilibrium and Kinetic Factors influencing bile sequestrant efficacy. Pharm Res 1992;9:670-6.

9. The United States Pharmacopeia (USP) XXII, USP convention, Inc. Rockville, MD. 1990:1798.

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