

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**Application Number 74-771**

**BIOEQUIVALENCE REVIEW(S)**

OFFICE OF GENERIC DRUGS  
DIVISION OF BIOEQUIVALENCE

ANDA/AADA # 74-771

SPONSOR: Baker Norton

DRUG & DOSAGE FORM: Cholestyramine for Oral Suspension

STRENGTH(s): 4gm/9gm Packet (Scoopful)

TYPE OF STUDY: *In-vitro*

STUDY SITE: Baker Norton

STUDY SUMMARY:

Point estimates (test mean/reference mean) and 90% confidence intervals for differences in affinity constants ( $k_1$ ) and capacity constants ( $k_2$ ) for total salts between cholestyramine regular (Test) and Questran Regular (Reference) obtained from an equilibrium study without acid pretreatment of the resin. Nonlinear equation was used for calculating  $k$ 's.

Affinity Constant ( $k_1$ )				Capacity Constant ( $k_2$ )			
Test	Ref	T/R	(90% CI)	Test	Ref	T/R	(90% CI)
0.88±0.13	0.94±0.34	0.93	NA	51.0±0.90	49.7±0.56	1.03	87.3-119.1

Units:  $k_1$ , L/mMole;  $k_2$ , mMoles/10mg resin.

Results of equilibrium study without acid pretreatment of cholestyramine. Mean millimoles total bile acid salts bound per 10 mg resin (x/m) (N=6).

C <sub>i</sub>	Cholestyramine		Questran <sup>®</sup>		T/R
	x/m	%CV	x/m	%CV	
0.1	0.68	1.8	0.80	2.2	0.85
0.3	1.95	1.9	2.37	1.6	0.82
1	6.16	1.7	6.95	1.3	0.89
3	19.9	1.8	20.1	2.0	0.99
7	44.5	12.9	38.3	22.8	1.16
10	42.5	5.6	44.1	1.3	0.96
20	40.4	5.0	43.3	2.5	0.93
30	51.2	37.4	46.0	23.3	1.11

Units: C<sub>i</sub>, mM;

DISSOLUTION: NA

The test drug is bioequivalent to the reference drug.

PRIMARY REVIEWER : James Chonen BRANCH: I

INITIAL : JS DATE: 5/30/97

BRANCH CHIEF : JS BRANCH : I

INITIAL : JS DATE : 5/30/97

DIRECTOR NICHOLAS FLEISCHER  
DIVISION OF BIOEQUIVALENCE

INITIAL : NSF DATE : 5/30/97

DIRECTOR OFFICE OF GENERIC DRUGS

INITIAL : \_\_\_\_\_ DATE : \_\_\_\_\_

*Sub*



cc:

**Letter Out, Bio Acceptable**

Endorsements:

L. Sanchez

*LS 5/28*

DRAFTED:

STM 5/28/97

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MAY 27 1997

Cholestyramine for Oral Suspension, USP  
4gm/9gm Packet (Scoopful)  
ANDA # 74-771  
Reviewer: James Chaney  
WP #74771A.N96

Baker Norton Pharmaceuticals, Inc.  
Miami, Florida  
Submission Date:  
November 27, 1996

## REVIEW OF AN AMENDMENT TO AN *IN VITRO* BIOEQUIVALENCE STUDY

This is an amendment to Baker Norton Pharmaceuticals, Inc. ANDA 74-771 for Cholestyramine for Oral Suspension, USP dated October 20, 1995. BNP has amended the application by responding to the numbered deficiencies of the OGD correspondence dated May 20, 1996 and provided supportive documentation. In this review deficiency comments by the FDA Division of Bioequivalence in the May 20, 1996 letter to the firm are shown in **bold**.

1. **For the equilibrium binding studies you are advised to calculate the affinity ( $k_1$ ) and capacity ( $k_2$ ) for the total bile acid salts (GCA + GCDA + TDCA) using both the best fit Langmuir nonlinear equation (Equation 1 of the Guidance) and the linear equation (Equation 2 of the Guidance) for the test and the reference products. The 90% confidence intervals should be calculated for the  $k_2$  values derived from the equilibrium studies without acid pretreatment of the resin.**

### Firm's Response

The affinity ( $k_1$ ) and capacity ( $k_2$ ) for the total bile acid salts have been calculated using both the best fit Langmuir nonlinear equation (Equation 1 of the Guidance) and the linear equation (Equation 2 of the Guidance) for the test and reference products. The 90% confidence intervals have been calculated for the  $k_2$  values of total bile acid salt derived from the equilibrium studies without acid pretreatment of the resin. The data are presented in Exhibit 1.

### Reviewer's Comment on Response

The firm submitted in Exhibit 1 that for the equilibrium studies without acid pretreatment of the resin Langmuir equations 1 and 2 of the Guidance yielded the estimates for the affinity constants ( $k_1$ ) and capacity constants ( $k_2$ ) for the test cholestyramine and the reference Questran Regular shown in Table 1 of this review. However, it is not clear from the submission whether these results are derived from the revised data using the new LOQ or from the original data. Sigma Plot software was used. The test to reference ratios are acceptable and the 90% confidence intervals are contained within the 80 to 120 % interval.

The firm presents the satisfactory  $k_1$  and  $k_2$  parameters in Exhibit 4 which were obviously obtained using equation 2 with the new LOQ based data from Exhibit 4.

The reviewer calculated the  $k_1$  and  $k_2$  parameters, and T/R ratios from data derived from the

revised LOQ data from Exhibit 4 using equation 1 and Excel (Table 1). The values for  $k_1$  and  $k_2$  should be derived from using  $x/m$  values with units of  $\mu\text{Moles}/\text{mg}$  in the Langmuir equations. Obviously the firm used units of  $\mu\text{Moles}/10 \text{ mg}$ . Using the latter approach the reviewer obtained close agreement with the firm's calculation of  $k_2$ . The reviewer calculated test to reference ratios for  $k_1$  and  $k_2$  are acceptable, and the 90% confidence intervals for  $k_2$  are contained within the 80 to 120% interval although the reviewer calculated confidence intervals are not in agreement with the firms reported confidence intervals.

<b>Table 1. Point estimates (test mean/reference mean) and 90% confidence intervals for differences in capacity constants between cholestyramine regular (Test) and Questran Regular (Reference) obtained from an equilibrium study without acid pretreatment of the resin</b>								
Equation Used	Affinity Constant ( $k_1$ )				Capacity Constant ( $k_2$ )			
	Test	Ref	T/R	(90% CI)	Test	Ref	T/R	(90% CI)
<b>From information in Tables 1-5 of Exhibit 1, pp 100003-5</b>								
Eqn 1	0.8738	0.9455	0.9242	----	50.58	48.61	1.041	92.7-115.4
Eqn 2	0.6044	1.0450	0.5784	----	48.02	45.38	1.058	97.4-114.2
<b>From information in Exhibit 4, p 100213</b>								
Eqn 2	0.560	0.960	0.583	-----	48.78	45.87	1.063	----
<b>Reviewer calculated from new LOQ data using Excel for <math>k_s</math> and SAS for CI</b>								
Eqn 1	0.8766	0.9406	0.93	-----	51.03	49.70	1.03	87.3-119.1

- Only mean ( $\pm\text{SD}$ ) data was presented from the equilibrium and kinetics studies on the test and reference drug products. You should also submit the six individual % bound, and millimoles bound per 10 mg resin values for the individual and combined bile acid salts, in addition to the mean ( $\pm\text{SD}$ ) of the six values. This data should be submitted as a hard copy and on a 3.5 inch diskette.

Firm's Response

Individual observations for the six samples of the test and reference drug products are provided in Exhibit 2 and on diskette (enclosed). These data have been revised in order to report all  $C_{eq}$  values below LOQ as zero.

Reviewer's Comment on Response

The firm sent hard copies of this requested data. However, the data included on the

diskette was incomplete. The data on the total salts from the equilibrium study on the test product with no acid pretreatment of resin was not submitted on diskette. For recalculation of the reviewer did the data entry from the hard copy.

**3. All standard curves and associated data should be submitted.**

Firm's Response

Single-point calibration, suitable for the analysis of drug substances and products, was validated and used rather than repeated standard curves.

The chromatographic system was developed for its intended purpose and validated, with definition of the linear range of the system prior to use. The procedure specifies three operational details which insure that the entire range of concentrations in a binding equilibrium study are well within the defined linear range.

1. Samples are grouped by starting bile salt concentration so that the range of concentrations in an analytical run is known.
2. Two injection volumes are specified, dependent upon initial bile salt concentration. Twenty  $\mu\text{L}$  are injected of samples from experiments using concentrations below 3 mM, while 10  $\mu\text{L}$  are injected from those concentrations above 3 mM.
3. The reference used in determining the extent of bile acid binding at each level is the initial bile salt solution. These solutions are prepared as standards and are the highest concentration in the run.

Reviewer's Comment on Response

The firm's response is acceptable.

4. **In the kinetics studies, AUC values for binding of all three acids (GCA, TDCA, and GCDCA in molar ratios of 3:1:3) were determined. Please be advised that the use of this AUC parameter is inappropriate for establishing equivalence between the test and reference drug product.**

Firm's Response

Baker Norton acknowledges the agency's comments.

Reviewer's Comment on Response

The firm's response is acceptable.

5. **Chromatograms of the analysis of individual observations and chromatograms of blanks containing cholestyramine with no bile acid salt should be submitted for one-fifth of the runs chosen at random from each of the two equilibrium and two kinetics studies.**

**Chromatograms of bile acid salts injected separately should be submitted to show that there were no significant bile acid salt contaminants interfering with the analysis of other bile salts.**

Firm's Response

The requested chromatograms are presented in Exhibit 3 in the following manner:

- Section A: One-fifth, or more, of the chromatograms for the 0.3 mM kinetic study;
- Section B: One-fifth, or more, of the chromatograms for the 3.0 mM kinetic study;
- Section C: One-fifth, or more, of the chromatograms for the equilibrium study without acid pre-treatment;
- Section D: One-fifth, or more, of the chromatograms for the equilibrium study with acid pre-treatment;
- Section E: Chromatograms of bile acid salts injected separately to demonstrate that there were no significant bile acid salt contaminants interfering with the analysis of other bile salts.

Reviewer's Comment on Response

As requested, chromatograms of the analysis of individual observations and chromatograms of blanks containing cholestyramine with no bile acid salt were submitted for one-fifth of the runs chosen at random from each of the two equilibrium and two kinetics studies.

Also, chromatograms of bile acid salts injected showed that there were no significant bile acid salt contaminants interfering with the analysis of other bile salts.

6. **Mean individual bile acid salt concentrations were reported which are less than your declared limits of quantitation (LOQ), e.g., TDCA and GCDCA concentration levels in the equilibrium studies (with acid pretreatment and no acid pretreatment) and the kinetics studies at the total bile acid salt concentration of 0.3 mM. If any of the non-zero values used in determining the mean values were less than the LOQ, the firm has the following choices: establishment of a lower LOQ; or recalculation of the reported mean values which were derived from nonzero individual values less than the LOQ. (The recalculation should treat these lower than LOQ values as zero). The chromatograms for the LOQ concentrations of each analyte should be provided.**

Firm's Response

The reported mean values and individual values were recalculated by treating all responses below LOQ as zero. The new  $k_1$ ,  $k_2$  and  $r^2$  for both equilibrium studies are reported in Tables 1 and 2 in Exhibit 4.

Reviewer's Comment on Response

The original LOQ of 0.05 mM was replaced by a new LOQ established as 0.01 mM for GCA, TDCA and GCDCA solutions. The reported mean values and individual values were recalculated by treating all responses below LOQ as zero. The firm's response to this deficiency is acceptable.

7. **Satisfactory linearity, and accuracy and precision data should be submitted for concentrations down to the appropriate LOQ.**

Firm's Response

The new LOQ established for the *in vitro* study is 0.01 mM for all three bile acid salts. The linearity, accuracy and precision data that establishes this new LOQ are presented in Exhibit 5. The method is linear over the range of 0.01mM (LOQ) to 3.0 mM for each of the three bile acid salts with  $r^2$  values of 0.9999 to 1.000. The proximity of the individual points to the linear regression demonstrates the accuracy. The standard deviation at each point demonstrates the precision of the method down to the LOQ level.

Reviewer's Comment on Response

The firm's response is adequate.

8. **No stability data was submitted on the bile acid salt solutions. Stability testing should be done on filtrates of the three analytes to show they are stable under the time frame and conditions of the analytical methodology.**

Firm's Response

As demonstrated by the following table the three bile acid salts are stable for 24 hours at 37°C, the conditions to which the bile acids were subjected in the *in vitro* study. This data was generated by preparing the sample solutions at the specified bile salt concentrations in SIF pH 6.8 and 0.1 M NaCl from a common stock bile salt solution and placing in a water bath for 24 hours at 37°C. After 24 hours elapsed, the samples were removed, filtered and analyzed on the chromatograph along with standard bile salt solutions prepared at the same concentrations. The standard solutions were freshly prepared at the time of analysis from the same stock solution as the sample solutions. The percent recovery was calculated by comparing the responses from the sample solution to that of the standard solutions.

Sample Preparation	Percent Recovery after 24 hours at 37°C		
	GCA	TDCA	GCDCA
1.0 mM in SIF pH 6.8	98.64	98.32	98.68
10.0 mM in SIF pH 6.8	99.32	99.23	99.48
1.0 mM in 0.1 M NaCl	100.46	102.10	100.74
10.0 mM in 0.1 M NaCl	99.92	99.32	100.03

Reviewer's Comment on Response

The firm adequately demonstrated that the three bile acid salts are stable for 24 hours at 37°C, the time frame and conditions to which the bile acids were subjected in the *in vitro* study.

9. **No content uniformity or assay data, or expiration date was reported on the reference product used in the study. This information should be supplied.**

Firm's Response

Reference Product: Questran®  
 Lot Number: LAJ56B  
 Expiration Date: 11/97  
 Potency: 98.4% LC  
 Content Uniformity: 92.0%, 95.0%, 97.5%, 89.9%, 94.1%, 95.9%, 97.9%, 94.8%, 91.5%, 93.3%  
 Average (n=10) Content Uniformity, 94.2% LC; RSD, 2.74%; Range, 89.9%-97.9%

Reviewer's Comment on Response

Satisfactory content uniformity and the expiration date were supplied reported on the reference product.

10. **The test batch size should be a size intended to produce a net yield of at least 10 percent of the number of finished-dosage units proposed in the maximum-size production batch for which authorization is sought or 100,000 finished-dosage units, whichever is greater. Please refer to The Office of Generic Drugs, Policy and Procedure Guide #22-90, *Bio-Batch Requirements Revised 9-13-90*, for further information.**

Please explain why the batch size of \_\_\_\_\_ was chosen while the above Guidance designates a minimum of 100,000 units. The number of finished-dosage units proposed in the maximum-size production batch for which authorization is sought

should supply.

Firm's Response

The proposed Production batch size will be identical in size to the test batch size of kilograms. This is the maximum amount that will be used for the future manufacture of Cholestyramine for Oral Suspension USP, Powder. Since each unit dose contains 9 grams of finished product, the maximum number of finished dosage units that can be produced is \_\_\_\_\_ units.

Reviewer's Comment on Response

The firm has explained that \_\_\_\_\_ units is the maximum number of finished dosage units that can be produced. The explanation is acceptable.

**COMMENTS :**

1. For the equilibrium study without acid pretreatment of the cholestyramine resin the  $k_1$  and  $k_2$  parameters obtained using Langmuir's nonlinear equation (equation 1) exhibited Test/Reference ratio of means within  $\pm 20\%$  of each other. Also, the required 90% confidence interval criterion was met for  $k_2$ .
2. The results of the equilibrium studies involving the acid treated resin and the kinetics studies are shown in the original review of May 10, 1996. No further action is required regarding these studies.
3. The assay for the study was appropriately validated.
4. The firm has satisfactorily addressed the deficiencies listed in the May 10, 1996 review. From the bioequivalence point of view the firm has met the *in vitro* bioequivalence requirements and ANDA 74-771 is acceptable.

**RECOMMENDATION:**

1. The bioequivalence studies conducted by Baker Norton on its Cholestyramine 4 gm, powder for oral suspension, lot # RD94077-03 comparing it to Questran<sup>R</sup> powder for oral suspensions, has been found acceptable by the Division of Bioequivalence. The study demonstrates that Baker Norton's Cholestyramine, 4 gm powder for oral suspension is bioequivalent to the reference drug product Questran<sup>R</sup>, 4 gm powder for oral suspension from Bristol-Myers Squibb.

/S/

James E. Chaney, Ph.D.  
Division of Bioequivalence  
Review Branch I

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FT INITIALED YHUANG

/S/

5/22/97

for Concur: \_\_\_\_\_  
Nichalos M. Fleischer, Ph.D.  
Director, Division of Bioequivalence

/S/

ch

Date: 5/27/1997

JEC/051997/WP#74771A.N96



acid salt should be submitted for one-fifth of the runs chosen at random from each of the two equilibrium and two kinetics studies.

Chromatograms of bile acid salts injected separately should be submitted to show that there were no significant bile acid salt contaminants interfering with the analysis of other bile salts.

6. Mean individual bile acid salt concentrations were reported which are less than your declared limits of quantitation (LOQ), e.g., TDCA and GCDCA concentration levels in the equilibrium studies (with acid pretreatment and no acid pretreatment) and the kinetics studies at the total bile acid salt concentration of 0.3 mM. If any of the non-zero values used in determining the mean values were less than the LOQ, you have the following choices: establishment of a lower LOQ; or recalculation of the reported mean values which were derived from nonzero individual values less than the LOQ. (The recalculation should treat these lower than LOQ values as zero). The chromatograms for the LOQ concentrations of each analyte should be provided.
7. Satisfactory linearity, accuracy, and precision data should be submitted for concentrations down to the appropriate LOQ.
8. No stability data was submitted on the bile acid salt solutions. Stability testing should be done on filtrates of the three analytes to show they are stable under the time frame and conditions of the analytical methodology.
9. No content uniformity or assay data, or expiration date was reported on the reference product used in the study. This information should be supplied.
10. The test batch size should be a size intended to produce a net yield of at least 10 percent of the number of finished-dosage units proposed in the maximum-size production batch for which authorization is sought or 100,000 finished-dosage units, whichever is greater. Please refer to The Office of Generic Drugs, Policy and Procedure Guide #22-90, Bio-Batch Requirements Revised 9-13-90, for further information.

Please explain why the batch size of \_\_\_\_\_ was chosen while the above Guidance designates a minimum of 100,000 units. The number of finished-dosage units proposed in the maximum-size production batch for which authorization is sought should be supplied.

As described under 21 CFR 314.96 an action which will amend this application is required. The amendment will be required to address all of the comments presented in this letter. Should you have any questions, please call Jason A. Gross, Pharm.D., at (301) 594-2290. In future correspondence regarding this issue, please include a copy of this letter.

Sincerely yours,

/s/

✓Keith K. Chan, Ph.D.  
Director, Division of Bioequivalence  
Office of Generic Drugs  
Center for Drug Evaluation  
and Research



acid salt should be submitted for one-fifth of the runs chosen at random from each of the two equilibrium and two kinetics studies.

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As described under 21 CFR 314.96 an action which will amend this application is required. The amendment will be required to address all of the comments presented in this letter. Should you have any questions, please call Jason A. Gross, Pharm.D., at (301) 594-2290. In future correspondence regarding this issue, please include a copy of this letter.

Sincerely yours,

~~/S/~~  
~~/Keith K. Chan, Ph.D.~~  
~~Director, Division of Bioequivalence~~  
~~Office of Generic Drugs~~  
~~Center for Drug Evaluation~~  
~~and Research~~

cc: Date

**BIO-LETTER INCOMPLETE**

**Endorsements:**

*Sir* J. Chaney  
Y.C. Huang  
J. Gross

/S/

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Cholestyramine for Oral Suspension, USP  
4gm/9gm Packet (Scoopful)  
ANDA # 74-771  
Reviewer: James Chaney  
WP #74771s.O95

Baker Norton Pharmaceuticals, Inc.  
Miami, Florida  
Submission Date:  
October 20, 1995

### Review of an in-vitro Bioequivalence Study

Cholestyramine resin is a bile acid sequestering antilipemic agent, 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. 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 cholesterol to bile acids.

The dosage of cholestyramine is expressed in terms of amount of dry resin. The recommended starting dose for an adult is 4 g of cholestyramine resin once or twice a day. The maximum recommended dose is 24 g of resin daily in divided doses. Cholestyramine resin is recommended to be administered with meals. The most common adverse reactions associated with cholestyramine resin is constipation, abdominal discomfort and/or pain, nausea, flatulence, vomiting, and diarrhea.

Cholestyramine as a powder for oral suspension containing 4 g cholestyramine resin/9 g powder (Questran<sup>R</sup>) and 4 g resin/5 g powder (Questran Light<sup>R</sup>) is currently marketed by Bristol Laboratories.

Because the drug is not absorbed into the systemic circulation, pharmacokinetics information is not available. The Division of Bioequivalence has concluded that *in vivo* studies are not necessary to document the bioequivalence of cholestyramine resin formulations. (Reference: Interim Guidance Cholestyramine Powder *In Vitro* Bioequivalence prepared by the Division of Bioequivalence, July 15, 1993). According to the Interim Guidance, equilibrium and kinetic *in vitro* bile acid salt binding studies are recommended to document bioequivalence between generic and innovator formulations of cholestyramine.

The equilibrium binding study should be conducted under conditions of constant time and varying concentrations of bile acid salts. The equilibrium studies should be conducted with and without acid pretreatment of the drug product. For the equilibrium studies, six individual observations with mean  $\pm$  SD for the following parameters should be reported for both the test and reference products: (i) percent binding of bile acid salt to 10 mg of resin at each concentration in tabular and graphical forms; (ii) micromoles of bile acid salts bound to 10 mg of resin at each concentration in tabular and graphical forms; (iii) affinity constant,  $k_1$ ; (iv) capacity constant,  $k_2$ ; and (v) coefficient of correlation,  $r^2$ , when linear regression is used to determine  $k_1$  and  $k_2$ .

A kinetic binding study should be conducted with constant concentration of bile acid salts and varying times of observation. The kinetic studies should be conducted at two concentrations of bile salts (0.3 and 3 mM) in the presence of added sodium chloride (0.1 M).

For the kinetic studies, six individual observations with mean  $\pm$  SD for the following parameters should be reported for both the test and reference products: (i) percent binding of bile acid salt to 10 mg of resin at each time point in tabular and graphical forms; and (ii) micromoles of bile acid salt bound to 10 mg of resin at each time point in tabular and graphical forms.

The firm has sent the results of *in vitro* bioequivalence studies on its cholestyramine for oral suspension, USP Powder, 4g/9g packet. The in-vitro studies and the limited statistical analyses were carried out by Baker Norton Pharmaceuticals, Inc.

**I. IN VITRO BIOEQUIVALENCE STUDIES:**

**A. Product Information:**

Test Product: Baker Norton Pharmaceuticals, Inc.'s Cholestyramine for Oral Suspension, USP Powder, Lot No. RD94077-03, Batch size: \_\_\_\_\_ Content Uniformity: reported to be within USP specifications; Assay, 100.7%.

Reference Product: Bristol-Myers Squibb's Questran<sup>R</sup>, Lot No. L4J56B. No content uniformity, assay or expiration date was reported. The composition of Baker Norton's Cholestyramine for Oral Suspension, USP Powder is shown in Table 1.

Table 1. Composition of Baker Norton's Cholestyramine for Oral Suspension, USP Powder.		
INGREDIENTS	Weight Per Dose (Packet)	% Weight
Cholestyramine 1	4.444*	49.38
Sucrose,		
Acacia, 1		
Lemon Flavor		
Xanthum Gum,		
Citric Acid, 1	0.000	
<b>TOTAL</b>	<b>9.000</b>	<b>100.00</b>

\* This amount corresponds to approximately 4.00 grams of anhydrous cholestyramine, since Cholestyramine ;

**B. Equilibrium Study of Binding of Bile Acid Salts to Resin in SIF Without Acid Pretreatment:**

**(i) Procedure:**

**(ii) Analytical Method:**

The system consisted of a \_\_\_\_\_ and a UV detector set at \_\_\_\_\_. The three bile salts, sodium glycocholic acid (GCA), sodium taurodeoxycholic acid (TDCA) and sodium glycochenodeoxycholic acid (GCDCA), were analyzed simultaneously without internal standard.

**(iii) Pre-study Method Validation:**

Linearity:

Method linearity is a validation scheme that assures that the diluter is capable of dispensing the required amounts of bile acids across a linear range. The results in Table 2 show that the solutions can be dispensed and analyzed within the two linear ranges.

Type of Linearity	$\mu\text{L}$ Injected, System #	Total Salts Range (mM)	GCA		TDCA		GCDCA	
			Range (mM)	$r^2$	Range (mM)	$r^2$	Range (mM)	$r^2$
Method Linearity N=5	20, 36	0.1-7.0	0.0428-2.9995	0.9940	0.0143-0.9996	0.9937	0.0428-2.9995	0.9941
	10, 36	3.0-30.0	1.2855-12.855	0.9985	0.4284-4.2840	0.9992	1.2855-12.855	0.9987
HPLC Linearity N=10	20, --	0.0781-40.0	0.0335-17.140	0.9998	0.0112-5.7120	0.9964	0.0335-17.140	0.9994
	10, --	0.0195-40.0	0.0084-17.140	0.9998	0.0028-5.7120	0.9996	0.0084-17.140	0.9998

Chromatographic linearity is a validation scheme which assures that the bile acids salts can be analyzed within a linear range of the chromatograph. The results show that the

concentrations of the bile salts are within the linear range of the chromatographs (Table 2).

The firm has demonstrated that the method is rugged from day to day and system to system.

The method calls for an injection volume of 20.0  $\mu$ L for the bile salts solutions ranging from 0.1 to 7.0 mM and an injection volume of 10.0  $\mu$ L for the bile salts solutions ranging from 10.0 to 30.0 mM. So, the validation ranges consist of 20  $\mu$ L injections of the 0.1 to 7.0 mM salt solutions and 10  $\mu$ L injections of the 10.0 mM to 30.0 mM salt solutions.

**Accuracy and Precision:**

The accuracy and precision results are satisfactory (Table 3). Precision and accuracy were assessed by the ability to accurately dispense and analyze the requisite concentration of bile salts. Concentrations of the bile acid salts were selected throughout the appropriate ranges. Six solutions were prepared at each concentration. The first sample was referred to as the standard to which the subsequent 5 samples were compared. Accuracy was measured as the percent recovery of the last 5 samples relative to the standard. Precision was measured as the %RSD.

Table 3. Accuracy and precision for equilibrium studies on bile acid salts (SIF pH 6.8), N=5.

Total Bile Salts Conc.	GCA			TDCA			GCDCA		
	Concentration	Accuracy	Precision	Concentration	Accuracy	Precision	Concentration	Accuracy	Precision
0.1 mM	0.0428	100.7	0.5	0.0143	100.6	0.7	0.0428	100.4	0.9
1.0 mM	0.4285	99.2	0.4	0.1428	99	0.5	0.4285	99.1	0.3
3.0 mM	1.2855	100.4	0.3	0.4284	99.7	1.1	1.2855	100.3	0.3
7.0 mM	2.9995	100.4	0.7	0.9996	102.6	0.9	2.9995	100.6	0.8
30.0 mM	12.855	100.1	0.8	4.284	99.5	1.5	12.855	100.4	1.4

**Sensitivity:**

The LOQ (N=5) was determined at the 0.05 mM total bile salt level in SIF pH 6.8 as specified in the Guidance (Table 4). The sensitivity limits are 0.0214 mM for GCA, 0.0214 mM for GCDCA and 0.0072 mM for TDCA.

Table 4. Limit of quantitation for equilibrium studies at total salt concentration of 0.05 mM.

HPLC System	GCA		TDCA		GCDCA	
	Concentration	%CV	Concentration	%CV	Concentration	%CV
System #36	0.0214	4.2	0.0072	5.0	0.0214	5.9
System #23	0.0214	0.8	0.0072	6.2	0.0214	2.3

**Assay Specificity:**

The chromatographic separation between the three bile acid salts GCA, TDCA and GCDCA, is

acceptable. The firm claims that no significant interfering peaks with those of the above analytes were seen when filtrates of the cholestyramine suspensions were chromatographed.

**Stability:**

No stability data were given.

**(iv) Results:**

The binding of the test and reference products to glycolic acid (GCA), glycochenodeoxycholic acid (GCDCA) and taurodeoxycholic acid (TDCA) in a 3:3:1 molar ratio was determined in six replicate experiments. As shown in Table 5, the percent binding of the three combined bile salts per 10 mg of resin over the concentration range of 0.1 mM to 30 mM was similar between the test and reference products. The test and reference % bound values for each of the eight concentrations were within  $\pm 20\%$  of each other except for the 0.1 mM concentration which had a T/R ratio of 0.79.

Cholestyramine for Oral Suspension				Questran <sup>R</sup>			T/R
C <sub>i</sub> (mM)	x/m	%CV	Average % Bound	x/m	%CV	Average % Bound	
0.1	0.6393	1.8	63.93	0.8043	2.2	80.43	0.79
0.3	1.9191	2.0	63.97	2.292	1.5	76.40	0.84
1	6.1598	1.7	61.6	6.8839	1.2	68.84	0.89
3	19.885	1.8	66.28	20.1488	2.0	67.16	0.99
7	44.506	12.9	63.58	38.2817	22.8	54.62	1.16
10	42.527	5.6	42.53	44.1457	1.3	44.82	0.96
20	40.384	5.0	20.19	43.2747	2.5	21.64	0.93
30	51.173	37.4	17.17	46.0396	23.3	15.35	1.11

The binding of the individual bile acids, GCA, TDCA, and GCDCA are shown in Tables 4A, 5A, 7A, 8A, 10A, and 11A of the attachments. In general, the amount bound to the test and reference product was similar, particularly at concentrations >3 mM. The biggest difference between the two products was evident for GCA at low concentrations (<3 mM).

The mean affinity (k<sub>1</sub>) and mean capacity (k<sub>2</sub>) values for each product are shown in Table 6. The reported capacity (k<sub>2</sub>) values for TDCA, GCDCA and total bile acids are similar between the two products. Affinity (k<sub>1</sub>) on the other hand differed between the two products for the 3

salts by 43% for the total salts.

Table 6. Mean affinity ( $k_1$ ), mean capacity ( $k_2$ ) and linear regression ( $r^2$ ) values for GCA, TDCA, GCDCA, and total bile salts from the equilibrium studies without acid pretreatment of cholestyramine.								
Product:	Cholestyramine for Oral Suspension, USP			Questran <sup>R</sup> (Reference)			T/R	
	$k_1$	$k_2$	$r^2$	$k_1$	$k_2$	$r^2$	$k_1$	$k_2$
Bile Salt								
GCA	0.866	7.75	0.9108	-6.20	5.91	0.9545	-0.14	1.31
TDCA	0.012	14.68	0.9961	0.021	13.89	0.9990	0.57	1.06
GCDCA	0.005	23.81	0.9968	0.012	23.64	0.9988	0.42	1.01
GCA+TDCA+GCDCA	0.524	49.02	0.9865	0.912	46.08	0.9982	0.57	1.06

**C. Equilibrium Study of Binding of Bile Acid Salts to Resin in SIF With Acid Pretreatment:**

**(i) Procedure:**

The equilibrium study with acid pretreatment was similar to the equilibrium study without acid pretreatment except that cholestyramine test and reference products had been incubated in 0.1N HCL at 37°C for 1 hour and centrifuged. The supernatant was aspirated, the pellet was resuspended with SIF until a pH of 6.8 was obtained prior to the overnight soak in SIF pH 6.8. The data treatment was the same as that for the study without acid pretreatment.

**(ii) Analytical Method:**

Same as in Part B.

**(iii) Method Validation:**

Linearity:

See Table 2 under B.

Accuracy and Precision:

See Table 3 under B.

Sensitivity:

See Table 4 under B.

Assay Specificity:

See Part B.

Stability:

As in Part B no stability data were given.

**(iv) Results:**

Under equilibrium conditions following acid pretreatment of the resin, the binding of the combined acid salts was slightly higher for the reference product than for the test product (Table 7). The test and reference % bound values for each of the eight concentrations were within  $\pm 20\%$  of each other except for the 20 mM and 30 mM concentrations which had T/R ratios of 0.79 and 0.75, respectively. Binding of the individual bile acids are shown in Tables 4B, 5B, 7B, 8B, 10B, and 11B of the review attachments.

Cholestyramine for Oral Suspension, USP, Powder (BNP)				Questran <sup>R</sup> (BMS)			T/R
C <sub>i</sub> (mM)	x/m	%CV	Average % Bound	x/m	%CV	Average % Bound	
0.1	0.6360	1.95	63.60	0.7675	1.49	76.74	0.83
0.3	1.7417	1.85	58.06	2.1149	3.19	70.50	0.82
1	5.5146	2.25	55.15	6.2509	2.44	62.51	0.88
3	16.9568	2.38	56.52	18.9628	1.42	63.21	0.89
7	27.4043	6.33	39.15	33.1762	3.40	47.39	0.83
10	29.3755	4.53	29.38	33.7961	3.32	33.80	0.87
20	28.0749	11.62	14.04	35.6184	4.14	17.81	0.79
30	30.1895	4.22	10.06	40.4686	15.85	13.49	0.75

The comparison of the results of binding of total bile acid salts using cholestyramine pretreated with acid and cholestyramine without acid pretreatment is shown in Table 8. The % binding for the test and reference products were consistently lower following acid pretreatment than without acid pretreatment. All the with acid pretreatment/without acid pretreatment ratios over the entire concentration range for the reference product were within 0.82 to 0.95 except for the 10 mM concentration (ratio = 0.75). The with acid pretreatment/without acid pretreatment ratios were within 0.85-0.99 for the test product from 0.1 mM through 3 mM bile salt concentrations. However, the ratios ranged from 0.59 to 0.70 for the test product over the concentration range of 7 mM to 30 mM.

**Table 8. Effect of acid treatment of cholestyramine on the mean % binding of total bile salts.**

C <sub>i</sub> (mM)	Test			Reference		
	Without Acid Pre-Treatment	With Acid Pre-Treatment	With Acid/Without Acid Ratio	Without Acid Pre-Treatment	With Acid Pre-Treatment	With Acid/Without Acid Ratio
0.1	63.93	63.60	0.99	80.43	76.74	0.95
0.3	63.97	58.06	0.91	76.40	70.50	0.92
1	61.6	55.15	0.90	68.84	62.51	0.91
3	66.28	56.52	0.85	67.16	63.21	0.94
7	63.58	39.15	<b>0.62</b>	54.62	47.39	0.87
10	42.53	29.38	<b>0.69</b>	44.82	33.80	<b>0.75</b>
20	20.19	14.04	<b>0.70</b>	21.64	17.81	0.82
30	17.17	10.06	<b>0.59</b>	15.35	13.49	0.88

The calculated affinity ( $k_1$ ) values for GCA, TDCA and total acids were very similar between the two products (Table 9). However, capacity ( $k_2$ ), the more important parameter, was greater for the reference product for the three salts, individually and combined. The capacity for the combined salts using the test cholestyramine was 23% less than that of the reference product. However, the equilibrium study using acid pretreated cholestyramine is not considered as a pivotal study but a confirmatory test to show that acid treatment does not change the binding characteristics of the resin.

**Table 9. Reported mean values of affinity ( $k_1$ ), capacity ( $k_2$ ) and linear regression ( $r^2$ ) for GCA, TDCA, GCDCA, and total bile salts from the equilibrium studies with acid pretreatment of cholestyramine.**

Product:	Cholestyramine for Oral Suspension, USP			Questran <sup>R</sup> (Reference)			T/R	
Bile Salt	$k_1$	$k_2$	$r^2$	$k_1$	$k_2$	$r^2$	$k_1$	$k_2$
GCA	0.680	7.58	0.9905	0.690	10.57	0.9712	0.99	0.72
TDCA	0.013	8.73	0.9970	0.015	10.28	0.9972	0.87	0.85
GCDCA	4.53	14.71	0.9966	6.19	19.23	0.9985	0.73	0.76
GCA+TDCA+GCDCA	0.685	31.65	0.9950	0.703	40.98	0.9966	0.97	0.77

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

**(i) Procedure:**

The kinetics studies in a 0.3 mM concentration of combined bile salts solutions involved soaking the equivalent of 10 mg of resin of each of the test and reference products in 0.1 M NaCl overnight at room temperature. The requisite amounts of a stock bile salt combination solution and 0.1 M NaCl were then added to the incubation flask containing the samples for a total volume of 10 mL. Blanks and standard bile acid solutions were also prepared for this test. There were eight incubation mixtures with the test product and the reference product. Each of the incubation mixtures containing the test or reference drug product was 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. The experiment was repeated for a total of six observations. The following data for the six individual observations (mean±SD) for the test and reference products were determined: percent binding of bile acid salt to 10 mg of resin at each time point and micromoles of bile acid salts bound to 10 mg of resin at each time point.

**(ii) Analytical Method:**

Same as in Part B.

**(iii) Method Validation:**

Linearity:

The data in Table 10 show that the solutions can be dispensed and analyzed within the two linear ranges, and the concentrations of the bile salts are within the linear range of the chromatographs.

Type of Linearity	µL Injected, System #	Total Salts Range (mM)	GCA		TDCA		GCDCA	
			Range (mM)	r <sup>2</sup>	Range (mM)	r <sup>2</sup>	Range (mM)	r <sup>2</sup>
Method Linearity	20, 36	0.1-7.0	0.0428-2.9995	1.000	0.0143-0.9996	1.000	0.0428-2.9995	1.000
---	20, --	0.0195-40.0	0.0084-17.140	0.9995	0.0028-5.7120	0.9991	0.0084-17.140	0.9997

**Accuracy and Precision:**

Precision and accuracy were assessed by the ability to accurately dispense and analyze the requisite concentration of bile salts. Concentrations of the bile acid salts were selected throughout the appropriate ranges for the 0.1M NaCl media. Six solutions were prepared at each concentration. The first sample was referred to as the standard to which the subsequent 5 samples were compared. Accuracy was measured as the percent recovery of the last 5 samples relative to the standard, and precision was measured as the %RSD (Table 11).

Concentration of Total Bile Salts	GCA			TDCA			GCDCA		
	Concentration	Accuracy	Precision	Concentration	Accuracy	Precision	Concentration	Accuracy	Precision
0.1 mM	0.0428	100.1	0.6	0.0143	101.5	0.4	0.0428	102.0	0.6
1.0 mM	0.4285	100.4	0.8	0.1428	100.6	0.9	0.4285	100.1	0.7
3.0 mM	1.2855	100.1	0.5	0.4284	99.8	0.7	1.2855	100.2	0.5

**Sensitivity:**

The LOQ (N=5) was determined at the 0.05 mM total bile salt level in 0.1 M NaCl as specified in the Guidance (Table 12).

System	GCA		TDCA		GCDCA	
	Concentration	%CV	Concentration	%CV	Concentration	%CV
System #36	0.0214	0.9	0.0072	2.3	0.0214	1.2
System #23	0.0214	1.1	0.0072	2.8	0.0214	1.2

**Assay Specificity:**

The separation between the three analytes, GCA, TDCA and GCDCA, is acceptable. No significant interference peaks are seen for blank bile salt samples.

**Stability:**

No stability data were given.

**(iv) Results:**

The binding of the test and reference products to glycolic acid (GCA), glycochenodeoxycholic acid (GCDCA) and taurodeoxycholic acid (TDCA) in a 3:3:1 molar ratio was determined in six replicate experiments. In the kinetics studies at 0.3 mM maximal binding

of each of the individual bile salts for both the test and reference products occurred by 2 hours and remained unchanged for the duration of the experiment up to 24 hours (Tables 3C to 8C of the review attachment). At 24 hours the percent binding for TDCA and for GCDCA to both resin products differed  $\leq 6\%$ , but for GCA it differed by 24%, with higher amounts bound to the reference product than the test product for each of the three bile salts. The total binding of the combined acids to the test product was less than for the reference product at each time point, but the differences were all within less than 20% (Table 13).

Table 13. Results of the kinetics study of the binding of the total bile acid salts (GCA + TDCA + GCDCA) to cholestyramine in 0.3 mM aqueous bile salt solution in the presence of added 0.1 M NaCl. Average millimoles combined bile acid salts bound per 10 mg resin (x/m) and average % bound (N=6).

Cholestyramine for Oral Suspension, USP				Questran <sup>R</sup> (BMS)			T/R x/m
Time (Hr)	Avg x/m	%CV	Average % Bound	Avg x/m	%CV	Average % Bound	
0.25	1.8267	2.01	60.89	2.168	1.18	72.27	0.84
0.5	1.8986	3.74	63.29	2.1599	1.10	72.00	0.88
1	1.9165	3.94	63.88	2.1844	1.03	72.81	0.88
2	1.9714	2.10	65.71	2.2022	0.67	73.41	0.90
4	1.9471	2.20	64.90	2.1749	0.66	72.50	0.90
8	1.9732	2.75	65.77	2.19	0.89	73.00	0.90
16	1.9817	2.13	66.06	2.194	1.12	73.13	0.90
24	1.9334	2.89	64.45	2.1702	0.79	72.34	0.89

The firm assessed bioequivalence using the results of the kinetics studies. The variable used was millimoles of bound bile salts per 10 mg resin at each assessment time of the 24-hour experiment. The area under the curve (AUC) from 0-24 hours was calculated by the trapezoidal rule. The six AUCs were then used to determine the mean for the two products and to calculate 90% confidence limits on the difference between products. The mean estimates and confidence limits were expressed as the percentage of the reference product. The AUCs and confidence intervals are shown in Table 14. The upper and lower limits for the ratios of test/reference products were within 80-125% of the reference.

Table 14. AUC summary from the kinetics studies in 0.3 mM aqueous bile salt solution in the presence of added 0.1 M NaCl.					
Mean AUC (mmole hour/10 mg resin), N=6			Test as % Reference		
Concentration	Reference	Test	Point Estimate	90% LL	90% UL
0.3 mM	9.04	8.06	89.1%	87.1%	91.1%

LL = Lower limit; UL = Upper limit;

**E. Kinetics Study of Binding of Bile Acid Salts in 3.0 mM Aqueous Solution in the Presence of Added Sodium Chloride (0.1M):**

**(i) Procedure:**

The procedure was the same as presented in Part D except that 3.0 mM bile acid salt solutions are incubated with the resin instead of 0.3 mM bile acid salt solutions.

**(ii) Analytical Method:**

Same as in Part B.

**(iii) Method Validation:**

Same as for the 0.3 mM aqueous bile acid salt solution (Part D).

**(iv) Results:**

At a total concentration of 3.0 mM for the combination of GCA, TDCA, and GCDCA in molar ratios of 3:1:3, maximal binding for each of these bile acid salts occurred by two hours and remained constant for the duration of the experiment up to 24 hours. The percent bound for each bile acid salt was similar for both products from about 1-2 hours to 24 hours. The mean values for binding of the total bile acid salts are listed in Table 15, and the values for the individual bile acid salts are shown in Table 11C-16C of the review attachments.

Table 15. Results of the kinetics study of the binding of bile acid salts (GCA + TDCA + GCDCA) to cholestyramine in 3.0 mM aqueous bile salt solution in the presence of added 0.1 M NaCl. Average millimoles total bile acid salts bound per 10 mg resin (x/m) and average % bound (N=6).

Cholestyramine for Oral Suspension, USP, Powder (BNP)				Questran <sup>R</sup> (BMS)			T/R
Time (Hr)	Avg x/m	%CV	Average % Bound	Avg x/m	%CV	Average % Bound	
0.25	16.6269	2.46	55.4230	18.3103	0.87	61.0344	0.91
0.5	17.3454	2.04	57.8181	18.5092	1.24	61.6973	0.94
1	18.0779	1.33	60.2595	18.7655	1.20	62.5515	0.96
2	18.8121	1.33	62.7072	18.8793	0.29	62.9310	1.00
4	18.7162	1.76	62.3873	18.8949	1.68	62.9829	0.99
8	18.2669	2.47	60.8895	18.8336	1.11	62.7786	0.97
16	18.5701	2.29	61.9002	19.1969	1.31	63.9897	0.97
24	18.4934	3.49	61.6463	18.8748	1.14	62.9161	0.98

The firm assessed bioequivalence using the results of the kinetics studies following the method used for the 0.3 mM combined bile salt concentration. The AUC values and confidence intervals are shown in Table 16. The upper and lower limits for the ratios of test/reference products were within 80-125% of the reference.

Table 16. AUC summary from the kinetics studies in 3.0 mM aqueous bile salt solution in the presence of added 0.1 M NaCl.

Mean AUC (mmole hour/10 mg resin), N=6			Test as % Reference		
Concentration	Reference	Test	Point Estimate	90% LL	90% UL
3.0 mM	7.87	7.71	98.0%	95.3%	100.6%

LL = Lower limit; UL = Upper limit;

## II. COMMENTS:

1. In the equilibrium study without acid pretreatment the test and reference mean percent bound values for GCA + TDCA + GCDCA at each of the eight concentrations were

within  $\pm 20\%$  of each other except for the 0.1 mM concentration which had a T/R ratio of 0.79.

2. In the equilibrium study without acid pretreatment of the resins the reported mean capacity ( $k_2$ ) values for TDCA, GCDCA and total bile acids are similar between the two products. However, the mean affinity ( $k_1$ ) values differed significantly between the two products. The ( $k_1$ ) value for the combined salts using the test cholestyramine was 43% less than that of the reference product.
3. Under equilibrium conditions following acid pretreatment of the resin, the binding of the combined acid salts was slightly higher for the reference product than for the test product (Table 7). The test and reference % bound values for each of the eight concentrations were within  $\pm 20\%$  of each other except for the 20 mM and 30 mM concentrations which had T/R ratios of 0.79 and 0.75, respectively.
4. In the equilibrium study following acid pretreatment of the resin the calculated affinity ( $k_1$ ) values for GCA, TDCA and total bile acids were very similar between the two products. The mean capacity ( $k_2$ ) for the combined salts using the test cholestyramine was 23% less than that of the reference product. However, this equilibrium study using acid pretreated cholestyramine is not considered as a pivotal study but a confirmatory test to show that acid treatment does not change the binding characteristics of the resin.
5. For the equilibrium binding studies the firm is advised to calculate  $k_1$  and  $k_2$  using both the Langmuir linear equation (Equation 2 of the Guidance) and the best fit nonlinear equation (Equation 1 of the Guidance) for the total bile acid salts (GCA + TDCA + GCDCA).
6. In the kinetics studies using total concentrations of 0.3 mM and 3.0 mM for the combination of GCA, TDCA, and GCDCA in molar ratios of 3:1:3, maximal binding to the reference and test cholestyramine products for each of these bile acid salts occurred by two hours and remained constant for the duration of the experiment up to 24 hours.
7. In the kinetics study on the 0.3 mM bile acid salt solution the total binding of the combined bile acid salts for the test product was less than for the reference product at each time point, but the differences were all within less than 20%. Also, for this concentration the mean percent binding at 24 hours for TDCA and for GCDCA to both resin products differed  $\leq 6\%$ , but for GCA it differed by 24% with higher amounts bound to the reference product than the test product for each of the three bile salts.
8. The mean % binding for the test and reference products were consistently lower following acid pretreatment than without acid pretreatment. All the with acid pretreatment/without acid pretreatment ratios over the entire concentration range for the reference product were within 0.82 to 0.95 except for the 10 mM concentration (ratio = 0.75). The with acid pretreatment/without acid pretreatment ratios were within 0.85-0.99

for the test product from 0.1 mM through 3 mM bile salt concentrations. However, the ratios ranged from 0.59 to 0.70 for the test product over the concentration range of 7 mM to 30 mM.

The firm offered the following explanation for the difference effects of acid pretreatment:

"Following acid pretreatment and centrifugation of the products, the pellet from Questran was easily resuspended, whereas it was not easily resuspended from the test product. Therefore, when the bile salts were added to the suspension, the binding sites may have been less available from the Baker Norton product than from the reference product. However, this process is somewhat artificial since gut contents are not centrifuged *in vivo*. With acid pretreatment *in vivo* the normal agitation of the gut contents should allow comparable binding of the bile acids to both products."

Although the acid pretreatment study is not pivotal, and the firm does not intend to pretreat its drug product (to be marketed) with 0.1 M HCl, the reviewing chemist should be made aware of the differences in resuspendability and binding for the test and reference products following acid pretreatment.

### **III. DEFICIENCIES:**

1. For the equilibrium binding studies the firm is advised to calculate the affinity ( $k_1$ ) and capacity ( $k_2$ ) for the total bile acid salts (GCA + GCDCA + TDCA) using both the best fit Langmuir nonlinear equation (Equation 1 of the Guidance) and the linear equation (Equation 2 of the Guidance) for the test and the reference products. The 90% confidence intervals should be calculated for the  $k_2$  values derived from the equilibrium studies without acid pretreatment of the resin.
2. The firm has presented only mean ( $\pm$ SD) data from the equilibrium and kinetics studies on its test and reference drug products. The firm should submit the six individual values, in addition to the mean ( $\pm$ SD) of the six values. This data should be on submitted as a hard copy and on a 3.5 inch diskette.
3. The sponsor should submit all standard curves and associated data.
4. In the kinetics studies, AUC values for binding of all three acids (GCA, TDCA, and GCDCA in molar ratios of 3:1:3) were determined. The firm should be advised that the use of this AUC parameter is inappropriate for establishing equivalence between the test and reference drug product.
5. Chromatograms of the analysis of individual observations and chromatograms of blanks containing cholestyramine with no bile acid salt should be submitted for one-fifth of the runs chosen at random from each of the two equilibrium and two kinetics studies.

Chromatograms of bile acid salts injected separately should be submitted to show that there were no significant bile acid salt contaminants interfering with the analysis of other bile salts.

6. The firm has reported mean individual bile acid salt concentrations which are less than its declared limits of quantitation (LOQ), e.g., TDCA and GCDCA concentration levels in the equilibrium studies (with acid pretreatment and no acid pretreatment) and the kinetics studies at the total bile acid salt concentration of 0.3 mM. If any of the non-zero values used in determining the mean values were less than the LOQ, the firm has the following choices: establishment of a lower LOQ; or recalculation of the reported mean values which were derived from nonzero individual values less than the LOQ. (The recalculation should treat these lower than LOQ values as zero).

Also, the firm should provide chromatograms for the LOQ concentrations of each analyte.

7. Satisfactory linearity, and accuracy and precision data should be submitted for concentrations down to the appropriate LOQ.
8. No stability data was submitted on the bile acid salt solutions. The firm should do stability testing on filtrates of the three analytes to show they are stable under the time frame and conditions of the analytical methodology.
9. No content uniformity or assay data, or expiration date was reported on the reference product used in the study. This information should be supplied.
10. The test batch size should be a size intended to produce a net yield of at least 10 percent of the number of finished-dosage units proposed in the maximum-size production batch for which authorization is sought or 100,000 finished-dosage units, whichever is greater. The firm is referred to The Office of Generic Drugs, Policy and Procedure Guide #22-90, *Bio-Batch Requirements Revised 9-13-90*, for further information.

The firm should explain why it chose the batch size of \_\_\_\_\_ while the above Guidance designates a minimum of 100,000 units. Also, the firm should supply information on the number of finished-dosage units proposed in the maximum-size production batch for which authorization is sought.

#### **IV. RECOMMENDATION:**

The *in-vitro* bioequivalence studies conducted by Baker Norton Pharmaceuticals, Inc. on its Cholestyramine for Oral Suspension, USP, lot # RD-94077-03, comparing it with Questran<sup>R</sup> for Oral Suspension have been found incomplete for the reasons cited in the Deficiencies 1-10.

The firm should be advised of the recommendation and deficiencies 1-10.

13/13 5/8/96

James E. Chaney, Ph.D.  
Division of Bioequivalence  
Review Branch I

RD INITIALED YHUANG  
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Date:

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Attachments: 11 pages

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BAKER NORTON PHARMACEUTICALS, INC.

Table 3A. Equilibrium Study Without Acid Pretreatment. Total  $C_{eq}/(x/m)$  Versus Total  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{n-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{n-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{n-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{n-1}$ )
0.0342	0.0006	0.0535	0.0010	0.0175	0.0003	0.0218	0.0005
0.1050	0.0020	0.0547	0.0018	0.0665	0.0014	0.0290	0.0005
0.3792	0.0133	0.0616	0.0027	0.2771	0.0054	0.0403	0.0008
0.9695	0.0186	0.0488	0.0014	0.9072	0.0157	0.0450	0.0004
2.4670	0.5898	0.0574	0.0187	3.0448	0.8935	0.0923	0.0674
5.5753	0.2591	0.1317	0.0137	5.4550	0.0779	0.1236	0.0033
15.8212	0.3010	0.3929	0.0279	15.5350	0.1420	0.3592	0.0119
24.7020	2.1310	0.6103	0.5290	25.1989	1.1494	0.5760	0.1533

Table 4A. Equilibrium Study Without Acid Pretreatment (GCA). Percent Bound GCA per 10 mg Resin Versus Initial Concentration ( $C_i$ , mM).

$C_i$ (mM)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )
0.1	28.8708	0.8482	57.5765	1.2475
0.3	33.5512	1.2522	53.8670	0.7446
1.0	31.5635	1.3575	44.5690	0.5436
3.0	38.3916	0.9872	40.3534	0.4446
7.0	36.5008	14.3867	23.7680	16.3956
10.0	14.7763	1.5969	15.9807	0.6117
20.0	6.2163	0.8572	7.0314	0.4367
30.0	8.3246	6.5470	6.1452	3.3953

Table 5A. Equilibrium Study Without Acid Pretreatment (GCA). mmoles GCA Bound per 10 mg Resin ( $x/m$ ) Versus  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{n-1}$ )	$x/m$	SD ( $\sigma_{n-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{n-1}$ )	$x/m$	SD ( $\sigma_{n-1}$ )
0.0301	0.0006	0.1236	0.0036	0.0175	0.0003	0.2464	0.0053
0.0848	0.0014	0.4315	0.0161	0.0580	0.0010	0.6927	0.0096
0.2921	0.0077	1.3525	0.0582	0.2279	0.0032	1.9098	0.0233
0.7815	0.0116	4.9452	0.1446	0.7466	0.0107	5.1874	0.0572
1.8841	0.4388	10.9485	4.3153	2.2630	0.5124	7.1292	4.9178
3.6260	0.0750	6.3317	0.6843	3.5799	0.0305	6.8478	0.2621
8.0181	0.0868	5.3274	0.7346	7.9482	0.0409	6.0259	0.3742
11.7504	0.8861	10.7013	8.4162	12.0303	0.4639	7.8996	4.3647

**Table 6A. Equilibrium Study Without Acid Pretreatment (GCA).  $C_{eq}/(x/m)$  Versus  $C_{eq}$  (mM).**

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{p-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{p-1}$ )
0.0301	0.0006	0.2437	0.0116	0.0175	0.0003	0.0712	0.0019
0.0848	0.0014	0.1968	0.0100	0.0580	0.0010	0.0838	0.0018
0.2921	0.0077	0.2165	0.0141	0.2279	0.0032	0.1194	0.0026
0.7815	0.0116	0.1585	0.0060	0.7466	0.0107	0.1439	0.0018
1.8841	0.4388	0.2011	0.0904	2.2630	0.5124	-0.0232	0.6346
3.6260	0.0750	0.5794	0.0746	3.5799	0.0305	0.5236	0.0239
8.0181	0.0868	1.5321	0.2382	7.9482	0.0409	1.3235	0.0866
11.7504	0.8861	1.4734	0.5964	12.0303	0.4639	2.0868	1.5365

**Table 7A. Equilibrium Study Without Acid Pretreatment (TDCA). Percent Bound TDCA per 10 mg Resin Versus Initial Concentration ( $C_i$ , mM).**

$C_i$ (mM)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )
0.0143	96.6816	2.6511	97.5368	2.2145
0.0429	90.8403	1.5497	97.8138	1.6154
0.1428	89.9004	1.6751	90.3715	1.3856
0.4284	92.3667	1.5375	91.4267	2.3405
0.9996	91.4270	1.9159	86.5015	6.1733
1.4280	77.6851	2.5770	78.2783	0.5860
2.8560	45.7309	1.1287	46.1133	0.7473
4.2840	35.1802	6.4605	32.6319	3.6820

**Table 8A. Equilibrium Study Without Acid Pretreatment (TDCA). mmoles TDCA Bound per 10 mg Resin ( $x/m$ ) Versus  $C_{eq}$  (mM).**

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$x/m$	SD ( $\sigma_{p-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$x/m$	SD ( $\sigma_{p-1}$ )
0.0000	0.0000	0.1390	0.0034	0.0000	0.0000	0.1395	0.0032
0.0033	0.0001	0.3897	0.0066	0.0002	0.0004	0.4196	0.0069
0.0134	0.0010	1.2838	0.0239	0.0073	0.0003	1.2905	0.0198
0.0244	0.0020	3.9570	0.0658	0.0216	0.0014	3.9167	0.1003
0.0689	0.0207	9.1391	0.1916	0.1048	0.0582	8.6467	0.6171
0.2740	0.0350	11.0934	0.3680	0.2772	0.0094	11.1781	0.0837
1.5052	0.0610	13.0607	0.3224	1.4972	0.0317	13.1700	0.2134
2.7369	0.3044	15.0712	2.7677	2.8268	0.1713	13.9795	1.5773

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Table 9A. Equilibrium Study Without Acid Pretreatment (TDCA).  $C_{eq}/(x/m)$  Versus  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{B-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{B-1}$ )
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0033	0.0001	0.0085	0.0004	0.0002	0.0004	0.0004	0.0009
0.0134	0.0010	0.0105	0.0007	0.0073	0.0003	0.0057	0.0002
0.0244	0.0020	0.0061	0.0005	0.0216	0.0014	0.0055	0.0003
0.0689	0.0207	0.0076	0.0024	0.1048	0.0582	0.0126	0.0085
0.2740	0.0350	0.0248	0.0038	0.2772	0.0094	0.0248	0.0009
1.5052	0.0610	0.1154	0.0074	1.4972	0.0317	0.1137	0.0039
2.7369	0.3044	0.1881	0.0422	2.8268	0.1713	0.2055	0.0346

Table 10A. Equilibrium Study Without Acid Pretreatment (GCDCA). Percent Bound GCDCA per 10 mg Resin Versus Initial Concentration ( $C_i$ , mM).

$C_i$ (mM)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{B-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{B-1}$ )
0.1	89.4767	4.0511	97.5368	2.2145
0.3	85.4561	1.4676	91.8076	1.6919
1.0	82.1820	1.2922	85.9212	1.1827
3.0	85.4670	1.5709	85.8722	2.0517
7.0	81.3638	4.2605	74.8365	10.6967
10.0	58.5357	3.3104	60.9110	0.6815
20.0	25.6207	1.1920	28.0514	0.6491
30.0	19.9769	6.8328	18.7499	3.7233

Table 11A. Equilibrium Study Without Acid Pretreatment (GCDCA). mmoles GCDCA Bound per 10 mg Resin ( $x/m$ ) Versus  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$x/m$	SD ( $\sigma_{B-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$x/m$	SD ( $\sigma_{B-1}$ )
0.0041	0.0003	0.3758	0.0099	0.0000	0.0000	0.4175	0.0095
0.0170	0.0009	1.0990	0.0189	0.0083	0.0003	1.1807	0.0218
0.0736	0.0047	3.5215	0.0554	0.0419	0.0019	3.6817	0.0507
0.1637	0.0058	10.9868	0.2019	0.1390	0.0044	11.0389	0.2637
0.5140	0.1361	24.4051	1.2779	0.6563	0.3236	22.4472	3.2085
1.6754	0.1515	25.0825	1.4185	1.5979	0.0395	26.1004	0.2920
6.2979	0.1595	21.9569	1.0216	6.0896	0.0760	24.0401	0.5563
10.2148	0.9416	25.6803	8.7835	10.3418	0.5145	24.1029	4.7864

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Table 3B. Equilibrium Study With Acid Pretreatment. Total  $C_{eq}/(x/m)$  Versus Total  $C_{eq}$  (mM).

	Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
	$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{p-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{p-1}$ )
	0.0361	0.0005	0.0568	0.0016	0.0212	0.0015	0.0277	0.0020
	0.1251	0.0036	0.0719	0.0022	0.0838	0.0058	0.0397	0.0035
	0.4483	0.0163	0.0814	0.0042	0.3424	0.0126	0.0553	0.0030
	1.3330	0.0214	0.0777	0.0030	1.0680	0.0391	0.0563	0.0017
	4.2107	0.1508	0.1544	0.0141	3.6219	0.1603	0.1093	0.0065
	6.9855	0.1167	0.2384	0.0149	6.5232	0.1319	0.1933	0.0098
	17.1026	0.3547	0.6162	0.0727	16.3457	0.1671	0.4597	0.0237
	26.8930	0.1703	0.8923	0.0412	25.8222	0.6863	0.6514	0.1014

Table 4B. Equilibrium Study With Acid Pretreatment (GCA). Percent Bound GCA per 10 mg Resin Versus Initial Concentration ( $C_i$ , mM).

$C_i$ (mM)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )
0.1	31.8690	0.8317	49.9888	1.2876
0.3	29.8514	0.8834	46.7393	2.7502
1.0	26.2914	1.3521	37.4554	1.2692
3.0	28.4375	0.8603	36.5266	0.6855
7.0	17.4525	0.9530	21.0269	0.7853
10.0	12.0980	0.6481	14.7675	0.5185
20.0	7.3327	1.5667	9.2694	0.3087
30.0	5.3581	0.2398	8.3034	2.0527

Table 5B. Equilibrium Study With Acid Pretreatment (GCA). mmoles GCA Bound per 10 mg Resin ( $x/m$ ) Versus  $C_{eq}$  (mM).

	Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
	$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$x/m$	SD ( $\sigma_{p-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$x/m$	SD ( $\sigma_{p-1}$ )
	0.0291	0.0002	0.1364	0.0035	0.0208	0.0007	0.2140	0.0055
	0.0901	0.0016	0.3839	0.0114	0.0671	0.0038	0.6011	0.0354
	0.3157	0.0073	1.1266	0.0579	0.2603	0.0057	1.6050	0.0544
	0.9225	0.0121	3.6557	0.1106	0.8069	0.0195	4.6955	0.0881
	2.4666	0.0268	5.2349	0.2858	2.3573	0.0235	6.3070	0.2356
	3.7528	0.0308	5.1840	0.2777	3.6506	0.0259	6.3279	0.2222
	7.9213	0.1432	6.2842	1.3426	7.7550	0.0320	7.9438	0.2646
	12.1462	0.0247	6.8878	0.3083	11.7528	0.2785	10.6741	2.6387

Table 6B. Equilibrium Study With Acid Pretreatment (GCA).  $C_{eq}/(x/m)$  Versus  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)					Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{p-1}$ )		$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{p-1}$ )
0.0291	0.0002	0.2134	0.0071		0.0208	0.0007	0.0975	0.0051
0.0901	0.0016	0.2348	0.0105		0.0671	0.0038	0.1123	0.0127
0.3157	0.0073	0.2811	0.0206		0.2603	0.0057	0.1624	0.0087
0.9225	0.0121	0.2526	0.0100		0.8069	0.0195	0.1719	0.0069
2.4666	0.0268	0.4225	0.1217		2.3573	0.0235	0.3743	0.0169
3.7528	0.0308	0.7260	0.0454		3.6506	0.0259	0.5750	0.0240
7.9213	0.1432	1.3021	0.2349		7.7550	0.0320	0.9772	0.0364
12.1462	0.0247	1.7665	0.0817		11.7528	0.2785	1.1507	0.2433

Table 7B. Equilibrium Study With Acid Pretreatment (TDCA). Percent Bound TDCA per 10 mg Resin Versus Initial Concentration ( $C_i$ , mM).

$C_i$ (mM)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )
0.0143	99.5146	1.5694	97.5463	2.2678
0.0429	85.9579	1.8452	92.1530	2.5252
0.1428	85.3212	1.7349	86.7282	1.7550
0.4284	88.3141	2.0627	90.1100	2.2989
0.9996	69.8787	3.7255	79.8461	2.0815
1.4280	56.8557	2.7314	61.7772	1.8084
2.8560	27.6052	1.6450	33.4949	1.3628
4.2840	20.2910	0.9543	24.2214	2.1761

Table 8B. Equilibrium Study With Acid Pretreatment (TDCA). mmoles TDCA Bound per 10 mg Resin ( $x/m$ ) Versus  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)					Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$x/m$	SD ( $\sigma_{p-1}$ )		$C_{eq}$ (mM)	SD ( $\sigma_{p-1}$ )	$x/m$	SD ( $\sigma_{p-1}$ )
0.0000	0.0000	0.1423	0.0023		0.0000	0.0000	0.1397	0.0030
0.0059	0.0005	0.3688	0.0079		0.0025	0.0004	0.3953	0.0108
0.0214	0.0025	1.2184	0.0248		0.0130	0.0011	1.2385	0.0251
0.0528	0.0034	3.7834	0.0883		0.0352	0.0033	3.8603	0.0985
0.2886	0.0306	6.9851	0.3724		0.1869	0.0179	7.9814	0.2081
0.5949	0.0321	8.1190	0.3901		0.5205	0.0283	8.8218	0.2582
2.0425	0.0535	7.8840	0.4698		1.8239	0.1403	9.5662	0.3892
3.3894	0.0347	8.6926	0.4088		3.2130	0.1032	10.3765	0.9322

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Table 9B. Equilibrium Study With Acid Pretreatment (TDCA).  $C_{eq}/(x/m)$  Versus  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{B-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$C_{eq}/(x/m)$	SD ( $\sigma_{B-1}$ )
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0059	0.0005	0.0160	0.0011	0.0025	0.0004	0.0063	0.0010
0.0214	0.0025	0.0172	0.0014	0.0130	0.0011	0.0105	0.0011
0.0528	0.0034	0.0140	0.0009	0.0352	0.0033	0.0091	0.0007
0.2886	0.0306	0.0416	0.0062	0.1869	0.0179	0.0235	0.0027
0.5949	0.0321	0.0736	0.0074	0.5205	0.0283	0.0591	0.0046
2.0425	0.0535	0.2601	0.0205	1.8239	0.1403	0.1964	0.0125
3.3894	0.0347	0.3908	0.0223	3.2130	0.1032	0.3122	0.0344

Table 10B. Equilibrium Study With Acid Pretreatment (GCDCA). Percent Bound GCDCA per 10 mg Resin Versus Initial Concentration ( $C_i$ , mM).

$C_i$ (mM)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{B-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{B-1}$ )
0.1	83.2446	1.6498	96.5375	1.3999
0.3	76.9856	1.4610	87.0546	2.3763
1.0	73.9229	1.5342	79.4755	1.9043
3.0	73.9922	1.7637	80.9110	1.3832
7.0	50.5771	3.7255	62.9239	2.4129
10.0	37.4633	1.9552	43.4702	1.5905
20.0	16.1819	1.7012	21.0844	0.9853
30.0	11.3191	0.4512	15.0602	2.2159

Table 11B. Equilibrium Study With Acid Pretreatment (GCDCA). mmoles GCDCA Bound per 10 mg Resin ( $x/m$ ) Versus  $C_{eq}$  (mM).

Cholestyramine for Oral Suspension, USP Powder (BNP)				Questran® (BMS)			
$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$x/m$	SD ( $\sigma_{B-1}$ )	$C_{eq}$ (mM)	SD ( $\sigma_{B-1}$ )	$x/m$	SD ( $\sigma_{B-1}$ )
0.0070	0.0002	0.3563	0.0071	0.0000	0.0009	0.4132	0.0060
0.0292	0.0016	0.9900	0.0188	0.0142	0.0017	1.1195	0.0306
0.1116	0.0076	3.2604	0.2863	0.0717	0.0058	3.4055	0.0816
0.3411	0.0163	9.5617	0.2618	0.2259	0.0181	10.4011	0.1778
1.4555	0.0986	15.1706	1.1175	1.0927	0.0689	18.8740	0.7237
2.6378	0.0731	16.0531	0.8378	2.3687	0.0776	18.6270	0.6815
7.1388	0.1595	13.8679	1.4579	6.7161	0.0939	18.0694	0.8443
11.3574	0.0512	11.3191	0.4512	10.8564	0.3061	19.3599	2.8485

**Table 3C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 0.3 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. Percent Bound Bile Salt (GCA) per 10 mg Resin Versus Time (Hours).**

GCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)		
	Time (Hours)	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )
	0.25	35.5858	0.8564	48.3931	0.4529
	0.5	36.1947	1.1733	47.2958	0.6304
	1.0	37.6336	1.3945	48.1886	0.5781
	2.0	37.7462	0.7568	48.1263	0.2801
	4.0	36.8473	0.7114	47.6709	0.7146
	8.0	37.4565	0.9287	48.7149	0.5804
	16.0	37.8598	0.9605	48.6401	0.7666
	24.0	36.5207	0.5986	48.0872	0.8419

**Table 4C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 0.3 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. Percent Bound Bile Salt (TDCA) per 10 mg Resin Versus Time (Hours).**

TDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)		
	Time (Hours)	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )
	0.25	86.4602	1.8508	95.0242	1.5507
	0.5	89.8459	3.8259	95.0931	1.1426
	1.0	89.6301	3.5794	96.9699	1.1574
	2.0	92.7583	1.9491	98.4836	0.7992
	4.0	91.9903	2.4978	96.9267	0.6687
	8.0	92.6959	3.0560	96.3960	1.5409
	16.0	93.0412	1.9780	96.7832	1.4081
	24.0	90.8822	3.4233	95.9189	0.8410

**Table 5C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 0.3 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. Percent Bound Bile Salt (GCDCA) per 10 mg Resin Versus Time (Hours).**

GCDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)		
	Time (Hours)	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{p-1}$ )
	0.25	77.6971	1.5829	88.5688	1.2784
	0.5	81.5484	3.2608	89.0165	1.0369
	1.0	81.5716	3.3167	89.3928	0.9839
	2.0	84.6822	2.0714	90.3411	0.7697
	4.0	83.9475	1.8798	89.1946	0.7941
	8.0	85.1316	2.6519	89.4965	0.7047
	16.0	85.2749	1.8017	89.7572	0.8868
	24.0	83.5777	2.8533	88.7456	0.5084

**Table 6C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 0.3 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. mmoles Bile Salt (GCA) Bound per 10 mg Resin Versus Time (Hours).**

GCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Time (Hours)	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )	Average x/m
0.25	0.4546	0.0110	0.6223	0.0058
0.5	0.4655	0.0151	0.6082	0.0081
1.0	0.4840	0.0180	0.6197	0.0074
2.0	0.4854	0.0097	0.6189	0.0036
4.0	0.4739	0.0091	0.6131	0.0092
8.0	0.4817	0.0120	0.6265	0.0075
16.0	0.4869	0.0124	0.6255	0.0099
24.0	0.4697	0.0077	0.6184	0.0108

**Table 7C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 0.3 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. mmoles Bile Salt (TDCA) Bound per 10 mg Resin Versus Time (Hours).**

TDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Time (Hours)	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )	Average x/m
0.25	0.3709	0.0079	0.4077	0.0066
0.5	0.3854	0.0164	0.4079	0.0049
1.0	0.3845	0.0154	0.4160	0.0050
2.0	0.3980	0.0084	0.4225	0.0034
4.0	0.3946	0.0107	0.4158	0.0029
8.0	0.3977	0.0131	0.4135	0.0066
16.0	0.3992	0.0085	0.4152	0.0060
24.0	0.3899	0.0147	0.4115	0.0036

**Table 8C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 0.3 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. mmoles Bile Salt (GCDCA) Bound per 10 mg Resin Versus Time (Hours).**

GCDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Time (Hours)	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )	Average x/m
0.25	0.9992	0.0204	1.1390	0.0164
0.5	1.0487	0.0419	1.1448	0.0133
1.0	1.0490	0.0426	1.1496	0.0126
2.0	1.0890	0.0267	1.1618	0.0099
4.0	1.0796	0.0242	1.1470	0.0102
8.0	1.0948	0.0341	1.1509	0.0091
16.0	1.0966	0.0232	1.1543	0.0114
24.0	1.0748	0.0367	1.1413	0.0065

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Table 9C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. Total Percent Bound Bile Salt per 10 mg Resin Versus Time (Hours).

Time (Hours)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )
0.25	55.4230	1.3638	61.0344	0.5286
0.5	57.8181	1.1805	61.6973	0.7678
1.0	60.2595	0.8029	62.5515	0.7479
2.0	62.7072	0.8365	62.9310	0.1829
4.0	62.3873	1.0984	62.9829	1.0558
8.0	60.8895	1.5069	62.7786	0.6949
16.0	61.9002	1.4159	63.9897	0.8361
24.0	61.6463	2.1512	62.9161	0.7180

Table 10C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. mmoles Total Bile Salt Bound per 10 mg Resin Versus Time (Hours).

Time (Hours)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )
0.25	16.6269	0.4091	18.3103	0.1586
0.5	17.3454	0.3542	18.5092	0.2303
1.0	18.0779	0.2409	18.7655	0.2244
2.0	18.8121	0.2510	18.8793	0.0549
4.0	18.7162	0.3295	18.8949	0.3167
8.0	18.2669	0.4521	18.8336	0.2085
16.0	18.5701	0.4248	19.1969	0.2508
24.0	18.4934	0.6454	18.8748	0.2154

Table 11C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. Percent Bound Bile Salt (GCA) per 10 mg Resin Versus Time (Hours).

Time (Hours)	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )
0.25	30.8786	0.8407	34.5369	0.5591
0.5	31.3961	1.0462	34.1827	0.8094
1.0	32.3957	0.4710	34.8613	0.8463
2.0	33.5764	0.5470	34.7111	0.3257
4.0	33.1331	0.8283	33.9475	0.9569
8.0	32.2252	0.7155	33.9185	0.7447
16.0	32.5548	0.7361	34.9927	0.9281
24.0	32.8005	0.8912	34.0583	0.8089

Table 12C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. Percent Bound Bile Salt (TDCA) per 10 mg Resin Versus Time (Hours).

TDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )
Time (Hours)				
0.25	81.2292	2.0921	86.9004	0.8282
0.5	84.8593	1.3694	88.4954	0.8991
1.0	87.9405	1.3481	88.7840	0.6110
2.0	91.4976	1.2109	89.7875	0.6941
4.0	91.3822	2.2807	90.8009	1.7788
8.0	88.8946	2.4177	90.0245	1.2366
16.0	91.0878	2.1701	91.7461	0.8638
24.0	90.2764	3.6220	90.5417	0.8638

Table 13C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. Percent Bound Bile Salt (GCDCA) per 10 mg Resin Versus Time (Hours).

GCDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )	Average Percent Bound	Standard Deviation ( $\sigma_{n-1}$ )
Time (Hours)				
0.25	71.3482	1.7444	78.8953	0.7174
0.5	75.2101	1.3619	80.2644	0.8551
1.0	78.8812	1.1048	81.4839	0.7593
2.0	82.2262	1.0701	82.1851	0.4673
4.0	81.9618	1.6604	82.7318	1.4525
8.0	80.2044	2.1050	82.5429	0.9882
16.0	81.5016	1.9082	83.7207	1.0048
24.0	80.9341	2.9715	82.5515	0.8035

Table 14C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. mmoles Bile Salt (GCA) Bound per 10 mg Resin Versus Time (Hours).

GCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )
Time (Hours)				
0.25	3.9695	0.1081	4.4397	0.0719
0.5	4.0360	0.1345	4.3942	0.1041
1.0	4.1645	0.0605	4.4814	0.1088
2.0	4.3163	0.0703	4.4621	0.0419
4.0	4.2593	0.1065	4.3640	0.1230
8.0	4.1426	0.0920	4.3602	0.0957
16.0	4.1849	0.0946	4.4983	0.1193
24.0	4.2165	0.1145	4.3782	0.1040

Table 15C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. mmoles Bile Salt (TDCA) Bound per 10 mg Resin Versus Time (Hours).

TDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Time (Hours)	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )	Average x/m
0.25	3.4799	0.0896	3.7228	0.0355
0.5	3.6354	0.0587	3.7911	0.0385
1.0	3.7674	0.0578	3.8035	0.0262
2.0	3.9198	0.0519	3.8465	0.0297
4.0	3.9148	0.0977	3.8899	0.0762
8.0	3.8083	0.1036	3.8567	0.0530
16.0	3.9022	0.0930	3.9304	0.0370
24.0	3.8674	0.1551	3.8788	0.0370

Table 16C. Kinetics of the Binding of Bile Acid Salts to Cholestyramine in 3.0 mM Aqueous Bile Salt Solution in the Presence of Added 0.1 M NaCl. mmoles Bile Salt (GCDCA) Bound per 10 mg Resin Versus Time (Hours).

GCDCA	Cholestyramine for Oral Suspension, USP Powder (BNP)		Questran® (BMS)	
	Time (Hours)	Average x/m	Standard Deviation ( $\sigma_{n-1}$ )	Average x/m
0.25	9.1718	0.2243	10.1420	0.0922
0.5	9.6683	0.1750	10.3180	0.1099
1.0	10.1402	0.1420	10.4747	0.0976
2.0	10.5702	0.1375	10.5649	0.0601
4.0	10.5362	0.2135	10.6351	0.1868
8.0	10.3103	0.2706	10.6109	0.1270
16.0	10.4770	0.2453	10.7623	0.1292
24.0	10.4041	0.3820	10.6120	0.1033