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APPLICATION NUMBER:

21-497

21-498/S-001

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

Office of Clinical Pharmacology and Biopharmaceutics Review

NDA	21-497
Generic	Nitazoxanide 500 mg tablets
(Brand[®])	Alinia [®]
Submission Date	January 30, 2004
Applicant	Romark Laboratories, L.C.
Clinical Division	DSPIDP (HFD-590)
OCPB Division	DPE3 (HFD-880)
Type of Submission	NDA Resubmission
Reviewer	Dakshina Chilukuri, Ph.D.
Team Leader	Philip Colangelo, Pharm. D., Ph.D.
Review Date	July 14, 2004

1. EXECUTIVE SUMMARY

The applicant is seeking approval of Nitazoxanide 500 mg Tablets in NDA 21-497. The proposed indication is the treatment of chronic diarrhea in adult patients due to *Giardia lamblia* (*G.lamblia*). The proposed dosage regimen is 500 mg tablets administered twice a day for 3 days.

Nitazoxanide is a salicylamide acetate ester, which has demonstrated in vitro activity against the intracellular parasite *C. parvum*. The pharmacokinetics of the drug has been characterized in healthy subjects and in AIDS patients. Pharmacokinetic studies in humans and experimental animals have failed to detect parent nitazoxanide in plasma, urine, or fecal samples. Nitazoxanide is rapidly desacetylated to tizoxanide (desacetylnitazoxanide) in biological fluids, most likely by a combination of nonspecific esterase activity and spontaneous hydrolysis. Thus, the plasma concentration-time curves of tizoxanide have been monitored in clinical and preclinical pharmacokinetic studies.

The applicant previously submitted NDA 21-497 and 21-498 in 2002 for the approval of nitazoxanide tablets and oral suspension, respectively. Following recommendations of the clinical division, the applicant was issued an approval letter for 21-498, the powder for oral suspension. An approvable letter indicating deficiencies in the application was issued for 21-497. The applicant has since submitted the new NDA 21-497 with additional efficacy and safety data.

To support the approval of nitazoxanide tablets, the applicant conducted study RM02-1014; a food effect study of the nitazoxanide suspension. Additional in vitro studies to evaluate the potential for interaction of tizoxanide with cytochrome P450 enzymes were performed.

Based on the results of efficacy and safety, HFD-590 recommends approval for the nitazoxanide tablets.

2. Recommendations

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation III has reviewed the information included in the resubmission of NDA 21-497 for Nitazoxanide tablets and has deemed the information to be acceptable. The Human Pharmacokinetics and Bioavailability Section of NDA 21-497 has met the requirements of the 21 CFR 320 and the clinical pharmacology labeling requirements of 21 CFR 201.56.

In vitro drug metabolism study: Tizoxanide did not show potential to inhibit cytochrome (CYP) P450 enzymes. A weak inhibitory effect was observed for CYP 2C9 and 3A4 enzymes.

Dissolution: Based on the review of the submitted dissolution data, OCPB considers that the proposed dissolution method for the tablet (i.e., USP Apparatus 2, rotation speed of _____ rpm, and dissolution medium of _____), is acceptable. The acceptance criteria should be as follows:

- For the tablet, NLT _____ (Q= _____) of the labeled amount dissolved as combined nitazoxanide and tizoxanide at 60 minutes

Labeling: The proposed label for the combined tablet and suspension formulations is attached in Appendix-A.

Phase IV commitments: There are no Phase IV commitments.

/s/

Dakshina Chilukuri, Ph.D.
Division of Pharmaceutical Evaluation III
Office of Clinical Pharmacology and Biopharmaceutics

/s/

Initialed by Philip Colangelo, Pharm. D., Ph.D.
cc: NDA 21-497, HFD-590, HFD-880 and CDR (Biopharm).

3. Summary of Clinical Pharmacology Findings

Food effect study

The applicant studied the effect of high-fat, high-calorie meal on the pharmacokinetics of tizoxanide and tizoxanide glucuronide. The results show that the bioavailability of tizoxanide and tizoxanide glucuronide were 48% and 44% higher, respectively, for the suspension formulation when administered with a high-fat, high-calorie meal. Food delayed the time to maximum concentration for both tizoxanide and tizoxanide glucuronide. Since the oral suspension and tablets were administered with food in the pivotal clinical trials with acceptable safety and efficacy, both formulations will be recommended to be administered with food in the labeling

In vitro drug metabolism studies

The applicant determined the interaction of tizoxanide with the cytochrome P450 family of enzymes, specifically those most involved in drug metabolism: CYP1A2, CYP2D6, CYP3A, CYP2C9 and CYP2C19. Among the different enzymes, CYP2C9 and 3A4 displayed the minimal inhibitory potency, with an IC_{50} value of $>100\mu\text{M}$ and $2600\mu\text{M}$, respectively. These concentrations are higher than the clinical concentrations derived after single oral dose of 500 mg, for which the determined C_{max} was $40\mu\text{M}$.

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11 **Draft Labeling Page(s) Withheld**

Appendix-B: Individual Study Reports

Study RM02-1014: Food effect bioavailability study on nitazoxanide 100 mg/5 mL suspension in 24 healthy male and female volunteers

Objectives: To assess the effect of food on the rate and extent of absorption of nitazoxanide 100 mg/5 mL suspension.

Investigator: —

Formulations:

1. Treatment A: oral administration of 100 mg/5 mL oral suspension, batch no. 20708 with 215 mL water in **fasted** conditions
2. Treatment B: oral administration of 100 mg/5 mL oral suspension, batch no. 20708 with 215 mL water in **fed** conditions

Subjects: A total of 24 healthy subjects (12 males and 12 females) entered and completed the study. Subjects ranged from 18 to 55 years of age.

Study design:

A total of 24 healthy subjects (12 males and 12 females) were selected according to the inclusion and exclusion criteria listed in Section 9.3.

It was an open, randomised, single-dose, two-treatment (fed vs. fasting), two-period, two-sequence crossover design study, with a 7-day wash-out period.

On each of the 2 study periods :

- x the subjects were confined in the clinical centre on the evening before administration until 12h postdose.
- x the subjects were administered a single oral dose of 500 mg nitazoxanide (25 mL of 100 mg/5 mL suspension with 215 mL of water) either in fasted conditions or in fed conditions (30 min after the start of high-fat and high-calorie standardised breakfast).

For female subjects: a pregnancy test on urine was performed at screening.

Plasma samples were collected at different times before and up to 12 hours after the administration. The assay of nitazoxanide and nitazoxanide glucuronide was performed in all available samples using a validated method.

Meals: Subjects randomized to the 'fed' treatment received a high-fat, high-calorie breakfast after an overnight fast of at least 10 hours. The composition of the breakfast was: 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 125 g hash brown potatoes and 240 mL whole milk.

Sampling: On each of the two study periods, 14 blood samples of 5 mL were collected by venipuncture in the forearm into evacuated tubes containing heparin and centrifuged within 30 minutes after collection. An indwelling catheter was used for the purpose of sampling. The blood samples were obtained at the following times: 0 hours (predose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10 and 12 hours after dosing. Plasma samples were stored at -20°C until analysis was performed.

Assay: Nitazoxanide metabolites were determined in all available plasma samples using a validated LC/MS-MS method. The lower limit of quantitation was \rightarrow for tizoxanide and \rightarrow for tizoxanide glucuronide. The CV (%) of the QC were < 10% for both tizoxanide and tizoxanide glucuronide.

Pharmacokinetic Analysis: The pharmacokinetic analysis was performed by the noncompartmental method using WinNonlin 2.1.

The following parameters were calculated from the individual plasma concentration versus time profiles of tizoxanide and tizoxanide glucuronide:

- ∞ C_{max} and t_{max} : The value and time of the maximum plasma concentration was obtained directly from the experimental data of plasma concentration vs. time curves, without interpolation.
- ∞ AUC_t : The area under the plasma concentration vs. time curve observed from time 0 up to the last measurable data point was computed using the linear trapezoidal rule (Gibaldi and Perrier, 1982).
- ∞ AUC_∞ : The area under the curve extrapolated to infinity, calculated as the sum of AUC_t and a residual part extrapolated to infinite time. The residual area from the last concentration data point to infinite time was calculated using the approximation :

$$\int_t^\infty C_t dt = C_t / \lambda_z$$

where λ_z was the first order terminal rate constant, computed by log-linear regression of the terminal log-linear segment of the plasma concentration (C) vs. time curve, and C_t was the last quantifiable concentration data point.

- ∞ $t_{1/2}$: The elimination half-life associated with negative terminal slope ($-\lambda_z$), calculated as $0.693/\lambda_z$.

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Statistical Analysis: Statistical analyses were formed at the _____, using the SAS software Release 8.2, on Windows/PC (SAS Inc., Cary, NC).

The pharmacokinetic parameters of tizoxanide and tizoxanide glucuronide were compared for treatment differences, using validated methods.

Continuous variables were statistically evaluated according to a univariate model of analysis of variance, adapted to crossover experimental designs (Fleiss, 1986). The factors of the model were the sequence, the subject (nested to the sequence), the treatment and the period of administration. The data were log-transformed prior to statistical testing, following the usual recommendations (Steinijans and Hauschke 1990). The t_{max} values were compared using a non-parametric method : Wilcoxon – Mann – Whitney rank sum test as adapted by Koch to crossover designs (Koch, 1972; Fleiss, 1986).

The relative bioavailability of the formulations was evaluated regarding the different metrics of extent and rate of absorption (AUC, C_{max}): a standard 90 % confidence interval (90%CI) for the ratio test/reference was derived for each parameter of interest. The standard 90%CI was derived from the ANOVA for the continuous variables (Steinijans and Hauschke, 1990; Chow and Liu, 1992). For t_{max} , a non-parametric 90%CI was calculated according to the method of Moses (Hauschke et al., 1990).

As described in the FDA guideline (Food-Effect Bioavailability and Fed Bioequivalence Studies, December 2002), an equivalence approach is recommended for food-effect bioavailability studies to make a claim of no food effects.

The principal parameters for assessing the food-effect are AUC_{inf} (or AUC_t , if problems are met in extrapolation) and C_{max} : the absence of food-effect can be established if the 90%CI is entirely included within 80-125% limits for AUC and C_{max} .

Results:

The pharmacokinetic parameters derived from the individual tizoxanide plasma profiles are summarized in Table 1. Also presented are the 90% confidence intervals for the test/reference ratios.

Table 1. PK parameters of tizoxanide derived from the individual tizoxanide plasma profiles

PK parameter	Fasted	Fed	p-value	Point estimate	90% CI
C_{max} ($\mu\text{g/mL}$)	5.37 \pm 1.92	5.49 \pm 2.06	0.74	102	91.1-115
T_{max} (h)	1.50	2.50	0.039	-1.00	-1.52 - -0.04
AUC_t ($\mu\text{g-h/mL}$)	21.1 \pm 10.3	28.9 \pm 11.2	<0.0001	145	128-165
AUC_{inf} ($\mu\text{g-h/mL}$)	21.3 \pm 10.5	30.2 \pm 12.3	<0.0001	148	130-168
$T_{1/2}$ (h)	1.43 \pm 0.496	1.60 \pm 0.729	0.38	109	92.8-127

Values are medians for t_{max} and $t_{1/2}$

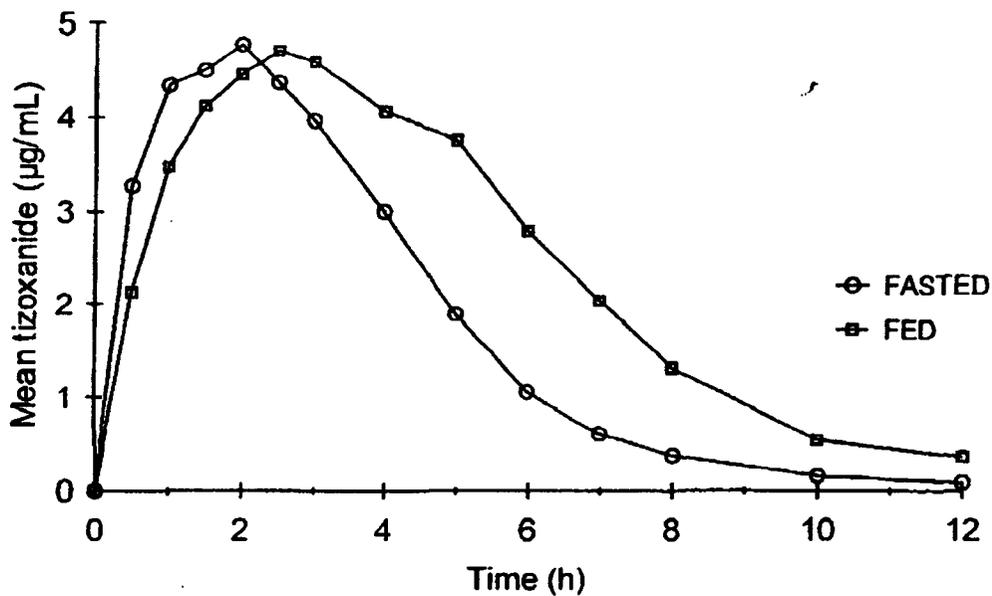
1: probability associated with the hypothesis of no difference between formulations (Koch's test for t_{max} . ANOVA for the other parameters)

2: expected geometric means test/reference ratio (%) and standard 90% CI, derived from ANOVA, except for t_{max} : non-parametric

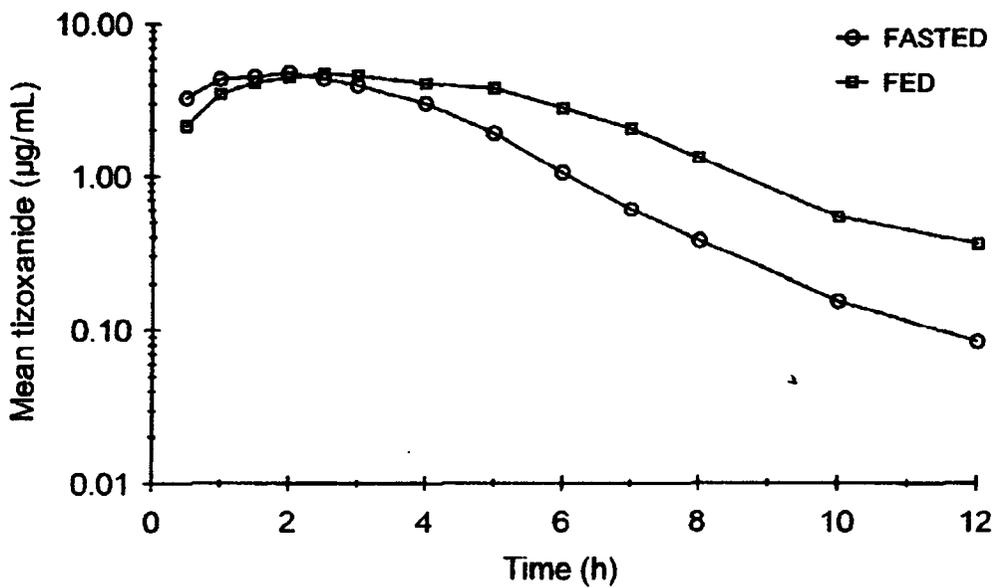
90% CI of the treatment difference.

The Cmax was slightly higher (2%) when nitazoxanide suspension was administered with food compared to fasting conditions. The Tmax was significantly delayed following administration with food. AUC was significantly higher (45%) following administration with food compared to fasting conditions. The apparent elimination half-life was slightly longer following administration with food.

Figure 1: Average tizoxanide plasma concentration versus time, by treatment. Top: linear scale, bottom: semi-logarithmic scale



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Table 2 shows the pharmacokinetic parameters derived from the individual tizoxanide glucuronide plasma profiles:

Table 2. PK parameters of tizoxanide glucuronide derived from the individual tizoxanide plasma profiles

PK parameter	Fasted	Fed	p-value	Point estimate	90% CI
C _{max} (µg/mL)	2.93 ± 1.19	3.21 ± 1.05	0.12	110	99.4-123
T _{max} (h)	2.50	4.00	0.0007	-1.50	-2.01 - -0.77
AUC _t (µg-h/mL)	15.6 ± 7.38	20.6 ± 5.87	0.0002	139	122-158
AUC _{inf} (µg-h/mL)	16.6 ± 7.57	22.8 ± 6.49	<0.0001	144	126-164
T _{1/2} (h)	1.90 ± 0.523	2.41 ± 1.21	0.022	120	106-137

Values are medians for t_{max} and t_{1/2}

1: probability associated with the hypothesis of no difference between formulations (Koch's test for t_{max}. ANOVA for the other parameters)

2: expected geometric means test/reference ratio (%) and standard 90% CI, derived from ANOVA, except for t_{max}: non-parametric 90% CI of the treatment difference.

The C_{max} was slightly higher (10%) when nitazoxanide suspension was administered with food compared to fasting conditions. The T_{max} was significantly delayed following administration with food. AUC was significantly higher (44%) following administration with food compared to fasting conditions. The apparent elimination half-life was slightly longer following administration with food.

Summary and Conclusions:

The results show that the bioavailability of tizoxanide was 48% higher for the suspension formulation when administered with a high-fat, high-calorie meal. The bioavailability of tizoxanide glucuronide was 44% higher when administered with a high-fat, high-calorie meal. Food delayed the time to maximum concentration for both tizoxanide and tizoxanide glucuronide.

Reviewer's Comments:

The sponsor's conclusions are acceptable.

Report RM-99.203: Interaction of Nitazoxanide with Human Cytochrome (CYP) P450 enzymes

Purpose: To test tizoxanide in various cytochrome P450 inhibition assays.

Results: The IC₅₀ of all the CYP enzymes are given in Table 3.

Table 3. Potency of Tizoxanide as an inhibitor of CYP P450 enzymes

Enzyme	IC ₅₀ (M)
2C9	$> 1 \times 10^{-4}$
3A4	2.6×10^{-4}

Summary and Conclusions: The potential for interaction of tizoxanide with different human cytochrome P450 enzymes was investigated. Tizoxanide at 10 µM and 100 µM did not display a significant inhibition ($\leq 25\%$ inhibition) of CYP 1A2, 2B6, 2C19, 2D6, 2E1 and 3A5 enzyme activities. Among the different enzymes, 2C9 and 3A4 isoforms displayed the slight inhibitory potency with an IC₅₀ values of $>100\mu\text{M}$ and $2600\mu\text{M}$, respectively. These concentrations are higher than the clinical concentrations derived after single oral dose of 500 mg, for which the determined C_{max} was $40\mu\text{M}$.

Reviewer's Comments: The *in vitro* metabolism results indicate that the IC₅₀ values for tizoxanide against 2C9 and 3A4 are higher than the clinical concentrations seen after administration of single oral dose of Nitazoxanide tablets. Hence tizoxanide is not expected to cause clinically significant inhibition of the CYP enzymes and no *in vivo* drug interaction studies are needed.

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Dissolution

Background: An initial dissolution method for Alinia tablets (500 mg) was developed using _____ as the dissolution medium. Following FDA's recommendations, the company successfully developed a method using _____ as the dissolution medium. Following is the method and proposed dissolution specifications by Romark.

Table 7.17 Proposed Product Dissolution Method and Specifications

1. <u>Dosage Form:</u>	Film-coated tablets
2. <u>Strength:</u>	500 mg
3. <u>Apparatus Type:</u>	Paddle Apparatus
4. <u>Media:</u>	/
5. <u>Volume:</u>	/
6. <u>Speed of Rotation:</u>	/
7. <u>Sampling Time:</u>	/
8. <u>Brief Description of Dissolution Analytical Method:</u>	HPLC
9. <u>Recommended Dissolution Specification:</u>	Q=NLT combined sum of nitazoxanide and tizoxanide after

Unit #	Lot 97E06A	Lot 97E07A	Lot 97E13A	Lot 97E06A	Lot 97E07A	Lot 97E13A	Lot 97E06A	Lot 97E07A	Lot 97E13A
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Mean	34.776	50.596	42.201	55.976	64.942	61.169	64.141	69.584	67.146
STDEV	6.724	4.003	3.571	3.428	1.068	1.273	0.990	1.641	1.616
% CV	19.335	7.911	8.462	6.124	1.645	2.082	1.544	2.358	2.406

Unit #	Lot 97E06A	Lot 97E07A	Lot 97E13A	Lot 97E06A	Lot 97E07A	Lot 97E13A	Lot 97E06A	Lot 97E07A	Lot 97E13A
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									1
Mean	68.482	72.172	70.368	73.277	75.985	74.674	75.649	77.637	76.892
STDEV	0.477	1.765	1.920	0.549	1.865	1.754	0.802	2.302	1.656
% CV	0.696	2.446	2.728	0.749	2.454	2.349	1.060	2.965	2.154

Reviewer's Comments: Based on review of the individual tablet dissolution data provided for Lot # 97E06A, 97E07A and 97E13A, the proposed dissolution acceptance criteria should be revised to "NLT - combined nitazoxanide and tizoxanide (Q= - , at 60 minutes".

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Office of Clinical Pharmacology and Biopharmaceutics Review
Division of Pharmaceutical Evaluation III

NDA:	21-497 and 21-498
Generic	Nitazoxanide
(Brand[®])	Cryptaz [®]
Submission Date:	May 28, 2002
Applicant:	Romark Laboratories, L.C.
Clinical Division	DSPIDP (HFD-590)
OCPB Division	DPE3 (HFD-880)
Type of Submission:	NDA
Reviewer:	Dakshina Chilukuri, Ph.D.
Team Leader	Barbara Davit, Ph.D.
Review Date	October 4, 2002

1. EXECUTIVE SUMMARY

The applicant is seeking approval of Nitazoxanide 500 mg Tablets and Nitazoxanide Powder for Oral Suspension in NDAs 21-497 and 21-498, respectively. The proposed indications are treatment of chronic diarrhea in — pediatric patients due to *Cryptosporidium parvum* (*C. parvum*) and *Giardia lamblia* (*G. lamblia*). Since patients took the drug with food in the pivotal clinical trials, the label specifies that the drug is to be taken with food.

Nitazoxanide is a salicylamide acetate ester, which has demonstrated *in vitro* activity against the intracellular parasite *C. parvum*. The pharmacokinetics of the drug has been characterized in healthy normal subjects and in AIDS patients. Pharmacokinetic studies in humans and experimental animals have failed to detect parent nitazoxanide in plasma, urine, or fecal samples. Nitazoxanide is rapidly desacetylated to tizoxanide (desacetylnitazoxanide) in biological fluids, most likely by a combination of nonspecific esterase activity and spontaneous hydrolysis. Thus, the plasma concentration-time curves of tizoxanide have been monitored in clinical and preclinical pharmacokinetic studies.

The applicant previously submitted an NDA 20-871 in 1997 for the approval of nitazoxanide tablets to treat AIDS patients with chronic diarrhea due to *C. parvum*. Following recommendations of the Anti-Infectives Advisory Committee, the applicant was issued a non-approvable letter indicating deficiencies in the application. The applicant has since submitted these new NDAs 21-497 and 21-498 for nitazoxanide powder for oral suspension and tablet, respectively, with additional data. The tablet formulation in NDA 21-497 is targeted for adult patients. The powder for suspension in NDA 21-498 is targeted for pediatric patients of ages 12 months to 11 years.

To support the approval of nitazoxanide tablets and powder for oral suspension, the applicant conducted studies B099597, RM01/02-1015 and 198.637; a bioequivalence study of a suspension and tablet formulations of nitazoxanide, a PK study in pediatric patients and a multiple dose study in healthy volunteers. Additional *in vitro* studies to evaluate intestinal permeability and the potential for interaction of nitazoxanide with cytochrome P450 enzymes were performed.

HFD-590 recommends approval for the nitazoxanide powder for oral suspension, NDA 21-498. HFD-590 recommends an action of approvable for NDA 21-497 based on the results of the efficacy studies. This decision is due to lack of clear efficacy in adults with chronic diarrhea receiving the tablet. Thus, the nitazoxanide powder for oral suspension will be approved for use in children.

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2. Recommendations

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation III has reviewed the information included in original NDA 21-497 and 21-498 for Nitazoxanide tablets and powder for oral suspension. The Human Pharmacokinetics and Bioavailability Section of NDA 21-497 and 21-498 has met the requirements of the 21 CFR 320 and the clinical pharmacology labeling requirements of 21 CFR 201.56.

Administration with food: The bioequivalence data, when viewed together with the food-effect data and the efficacy data suggest that the food effect should be studied further. Food increases systemic bioavailability, but it may be that it is more advantageous to achieve high local concentrations in the gastrointestinal tract where the site of action is presumed to reside. The sponsor is advised to investigate the effect of food on tizoxanide availability from the powder for oral suspension formulation.

Drug Interaction Studies: Nitazoxanide showed potential to inhibit cytochrome P450 2C9. However, since only tizoxanide can be determined in the systemic circulation, the clinical relevance of this study is not clear. It is recommended that the applicant repeat the in vitro drug-drug interaction studies with tizoxanide.

In Vitro Transfer across the Epithelial Barrier: It is recommended that the applicant investigate in vitro transfer of tizoxanide across the digestive epithelium. This is because it is not known to what extent conversion of nitazoxanide to tizoxanide occurs prior to absorption through the gut wall.

Dissolution: Based on the review of the submitted dissolution data, OCPB considers that the proposed dissolution method for the suspension (i.e., USP Apparatus 2, rotation speed of 100 rpm, and dissolution medium of phosphate buffer at pH 7.5 with 6% hexadecyltrimethyl ammonium bromide), is acceptable. Specification should be as follows:

- For the suspension, NLT \geq (Q) of the labeled amount dissolved as nitazoxanide and tizoxanide combined at 30 minutes

The applicant is asked to develop a dissolution method for nitazoxanide tablets, 500 mg, by varying the rotation speeds at the following conditions:

Labeling: The proposed label for the powder for oral suspension is attached. Labeling language for the tablet formulation will not be proposed at this time.

Phase IV commitments: The applicant is asked to address the Phase IV commitments by conducting the following studies:

- In vivo study of the effect of food on pharmacokinetics following oral administration of Nitazoxanide for Oral Suspension
- In vitro study of the effect of nitazoxanide metabolites (tizoxanide and tizoxanide glucuronide) on cytochrome P450 enzymes
- Study of the in vitro transfer of tizoxanide across the epithelial barrier

Please convey the recommendations and comments as appropriate to the applicant.

/S/

Dakshina Chilukuri, Ph.D.
Division of Pharmaceutical Evaluation III
Office of Clinical Pharmacology and Biopharmaceutics

/S/

Initialed by Barbara Davit, Ph.D.
Briefing Day 10/11/02
cc: NDA 21-497, HFD-590, HFD-880 and CDR (Biopharm).

3. Summary of Clinical Pharmacology Findings

Bioequivalence of the tablet and suspension formulations

The applicant studied the bioequivalence between nitazoxanide powder for oral suspension diluted to 100 mg/5 mL and nitazoxanide 500 mg tablet. The pharmacokinetic parameters determined were: maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), terminal elimination half-life ($t_{1/2}$), area under the plasma concentration versus time (AUC_t) curve and area under the plasma concentration curve versus infinite time (AUC_{inf}). The results showed that the bioavailability of the nitazoxanide active metabolite tizoxanide was 41% lower for the suspension formulation compared to the tablet formulation and the bioavailability of tizoxanide glucuronide was 30% lower for the suspension compared to the tablet. The 90% confidence intervals of the test/reference ratios were shifted towards lower values and were outside the acceptable limits of 0.8-1.2.

Pharmacokinetics in pediatric patients

The applicant determined the time-course of plasma concentrations of major nitazoxanide metabolites: tizoxanide and tizoxanide glucuronide in healthy pediatric volunteers. The pharmacokinetic parameters determined were the maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), terminal elimination half-life ($t_{1/2}$), and area under the plasma concentration versus time (AUC_t) curve and area under the plasma concentration curve versus infinite time (AUC_{inf}). The plasma concentrations of tizoxanide and tizoxanide glucuronide observed for the adolescents administered nitazoxanide were similar to those previously observed in healthy volunteers. Plasma concentration of the two metabolites was almost identical for the 12-47 month age group (100 mg dose) and the 4-11 year age group (200 mg dose). Plasma concentrations and pharmacokinetic parameters in children receiving the suspension dosage form were approximately one-third of those observed in adolescents receiving tablets. It is not clear if this is due to age-related differences in nitazoxanide absorption, or due to the fact that the tablet is better absorbed than the suspension. The C_{max} and AUC_t observed for tizoxanide in children receiving the 500 mg tablet were calculated to be 28% and 25% lower, respectively, than those observed in healthy adult volunteers (Study B099597). The T_{max} appears to be comparable between children and adults

Multiple Dose Pharmacokinetics

The applicant evaluated the safety and tolerability of nitazoxanide in healthy subjects after multiple dose of 0.5 g b.i.d. and 1 g b.i.d. in fed conditions for 7 days and to determine the time course of plasma concentration of nitazoxanide major metabolites, tizoxanide (T) and tizoxanide glucuronide (TG) after the first and the last dose, and to determine whether accumulation is likely to occur. The pharmacokinetic parameters determined were maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), terminal elimination half-life ($t_{1/2}$), area under the plasma concentration versus time (AUC_t) curve from 0 until the last measurable time point, area under the plasma concentration versus time (AUC_t) curve from 0-12 hours and area under the plasma concentration curve versus infinite time (AUC_{inf}), Minimum (or trough) plasma concentration during the dosing interval (C_{min}), Peak to trough fluctuation calculated as $PTF = (C_{max} - C_{min})/C_{av}$. The pharmacokinetics of both tizoxanide and tizoxanide glucuronide were both affected by repeated administration of 1g b.i.d. nitazoxanide and the pharmacokinetics of tizoxanide glucuronide was affected by repeated administration of 0.5g b.i.d. nitazoxanide. The PK of tizoxanide was not affected by repeated administration of 0.5g b.i.d. nitazoxanide. This indicates that accumulation occurred upon repeated administration of nitazoxanide.

In vitro drug metabolism studies

The applicant determined the interaction of nitazoxanide with the cytochrome P450 family of enzymes, specifically those most involved in drug metabolism: CYP1A2, CYP2D6, CYP3A, CYP2C9 and CYP2C19. The potential for interaction of nitazoxanide with different human cytochrome P450 isoforms was investigated. Among the different isoforms, CYP2C9 displayed the highest inhibitory potency, which was 10-fold higher than the other isoforms. The clinical significance of these studies is not clear. Nitazoxanide has never been detected in the systemic circulation. It is more appropriate to conduct in vitro

metabolism studies using the tizoxanide, which is present in measurable amounts in plasma after nitazoxanide dosing.

In vitro drug transport studies

An *in vitro* investigation was to study the absorption of nitazoxanide across the epithelial barrier according to the mode (mucosal or serosal) of administration of the drug with respect to its intracellular absorption. Nitazoxanide was found to pass through the digestive epithelium *in vitro* and the overall magnitude of this passage is similar after apical and basolateral administration. Both transcellular and paracellular mechanisms appear to be involved in the transport of nitazoxanide. However, nitazoxanide is rapidly metabolized to tizoxanide and the concentrations of nitazoxanide cannot be estimated in plasma. It is not known to what extent this conversion occurs before gut absorption. It is recommended that the applicant conduct similar studies with tizoxanide.

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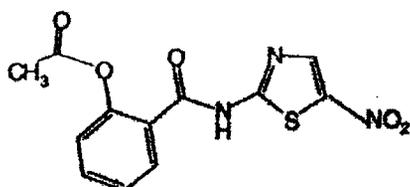
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4. Question Based Review

4.1. General Attributes

What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product?

Nitazoxanide 500 mg tablets and powder for oral suspension contain nitazoxanide, which is a N-(nitrothiazoly) salicylamide compound and has a chemical name of 2-acetoxy-N-(5-nitro-thiazol-2-yl) benzamide ($C_{12}H_9N_2O_5S$). It is insoluble in water, ethanol, chloroform and acetone and soluble in DMSO and pyridine. It has a pKa of 5.81 measured in ethanol/water (1:1 v/v) and is also insoluble in aqueous solutions of low pH and soluble in aqueous solutions of high pH. Nitazoxanide has the following structural formula:



There are two proposed commercial formulations of the tablets: a yellow, film-coated tablet of 500 mg strength and Powder for Oral Suspension (100 mg in 5 mL). The composition of the commercial tablet formulation is as follows:

Ingredient	Amount (mg/tablet)
<u>Tablet core</u>	
Nitazoxanide	500
Maize Starch	
Pregelatinized corn starch	
Hydroxypropylmethylcellulose	—
Sucrose	
Sodium starch glycollate	
Talc	
Magnesium Stearate	
—	
<u>Tablet coating</u>	
/	
Total Tablet Weight	

The composition of the powder for oral suspension formulation is as follows:

Ingredient	Unit Formula (g/bottle)
Nitazoxanide	1.2
Sodium benzoate	
Sucrose	
Xanthan gum	
Microcrystalline cellulose & carboxymethylcellulose sodium	
Citric acid anhydrous	
Sodium citrate dihydrate	
— strawberry powder	

4.2. Clinical Pharmacology

4.2.1. Dosage and Administration

What is the proposed dosage and route of administration?

The proposed dosage is one tablet (500 mg) orally twice-daily with food. For younger patients, the dosage (oral suspension to be administered with food) is as follows:

Age 4-11 years: 10 mL (200 mg nitazoxanide) every 12 hours for 3 days.

Age 12-47 months: 5 mL (100 mg nitazoxanide) every 12 hours for 3 days.

What efficacy and safety information contributes to the assessment of clinical pharmacology and biopharmaceutics data?

Efficacy and safety information was collected from the following five clinical studies using the proposed three-day regimen:

RM-NTZ-98-001	A double-blind placebo-controlled study in adults with diarrhea caused by <i>G.lamblia</i> or <i>E.histolytica</i>
RM-NTZ-98-002	A double-blind placebo-controlled study in adults and children with diarrhea caused by <i>C.parvum</i>
RM-NTZ-98-010	A double-blind metronidazole-controlled study in children with diarrhea caused by <i>G.lamblia</i>
RM02-3007	A double-blind placebo-controlled study in HIV-seronegative children with diarrhea caused by <i>C.parvum</i>
RM02-3007	A double-blind placebo-controlled study in HIV-seropositive children with diarrhea caused by <i>C.parvum</i>

One of the above-mentioned studies (RM-NTZ-98-002) evaluated the efficacy of both the 500-mg tablet and the pediatric suspension and another study (RM-NTZ-98-001) evaluated the efficacy of tablets and the remaining studies evaluated the efficacy of the pediatric suspension.

The doses of nitazoxanide administered for each of the above-mentioned studies were the same and are given below:

Adults and adolescents (≥12 years):	One nitazoxanide 500 mg tablet every 12 hours for 3 days with a meal
Children age 4 to 11 years:	10 mL of nitazoxanide suspension every 12 hours

Children age 12 months to 47 months: for three days with a meal
5 mL of nitazoxanide suspension every 12 hours for three days with a meal

The above-mentioned studies were all conducted in foreign countries where the infections are endemic. The studies were monitored by the applicant to ascertain the quality.

In Table 1 are presented the results of efficacy and safety from Study RM-NTZ-98-002

Table 1 **RM-NTZ-98-002: Summary of Efficacy Data**

	Nitazoxanide	Placebo	P ^a
Clinical response			
All subjects	39/49 (80%)	20/49 (41%)	<.0001
All children	21/24 (88%)	9/24 (38%)	.0004
Age 1-3	10/11 (91%)	4/11 (36%)	.01187
Age 4-11	11/13 (85%)	5/13 (38%)	.0207
Adults and adolescents	18/25 (72%)	11/25 (44%)	.0423
Parasitological response			
All subjects	33/49 (67%)	11/50 (22%)	<.0001
All children	18/24 (75%)	5/25 (20%)	.0001
Age 1-3	8/11 (73%)	3/11 (27%)	.0431
Age 4-11	10/13 (77%)	2/14 (14%)	.0016
Adults and adolescents	15/25 (60%)	6/25 (24%)	.0104
Median time from initiation of treatment to passage of last unformed stool			
All subjects	3 days	>6 days	.0006
All children	3.5 days	>6 days	.0001
Age 1-3	4 days	>6 days	
Age 4-11	3 days	>6 days	
Adults and adolescents	3 days	>6 days	.0493

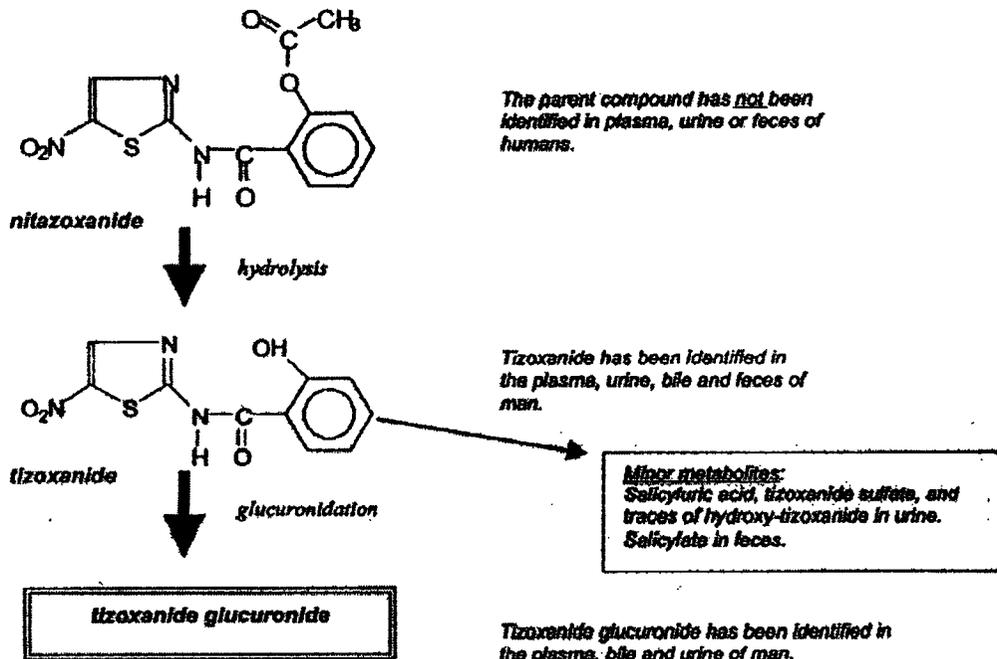
^a Fisher's exact test, one-sided

Are the active moieties in serum appropriately identified and measured to assess pharmacokinetic parameters and exposure/response relationships?

Following oral administration in humans, nitazoxanide is rapidly hydrolyzed in plasma to an active metabolite, tizoxanide (desacetyl-nitazoxanide), which possesses antimicrobial activity comparable to that of nitazoxanide. Tizoxanide then undergoes conjugation by glucuronidation. Tizoxanide glucuronide, also an active metabolite (but less active than nitazoxanide and tizoxanide) is excreted in urine and bile, and tizoxanide is excreted in the urine, bile and feces.

The following schematic shows the metabolic pathway of nitazoxanide.

Fig. 4.6.4 Metabolism of nitazoxanide



The pharmacokinetic parameters of tizoxanide and tizoxanide glucuronide following administration of the tablet and suspension dosage forms are given below in Table 4.6.6

Table 4.6.6 Pharmacokinetic parameters of tizoxanide in plasma

Population	Dose (mg)	Dosage form	C _{max} (µg/ml)	AUC _t (µg·h/ml)	T _{max} (h)	Reference
Adults	500	tablet	10.4	41.8	3.0	Study 198.637
12-17 yrs	500	tablet	9.12	39.5	4.0	Study RM01/02-1015
4-11 yrs	200	suspension	3.0	13.5	2.0	Study RM01/02-1015
12-47 months	100	suspension	3.11	11.7	3.5	Study RM01/02-1015

Table 4.6.7 Pharmacokinetic parameters of tizoxanide glucuronide in plasma

Population	Dose (mg)	Dosage form	C _{max} (µg/ml)	AUC _t (µg·h/ml)	T _{max} (h)	Reference
Adults	500	tablet	10.4	64.7	4.5	Study 198.637
12-17 yrs	500	tablet	7.27	46.5	4.0	Study RM01/02-1015
4-11 yrs	200	suspension	2.84	16.9	4.0	Study RM01/02-1015
12-47 months	100	suspension	3.64	19.0	4.0	Study RM01/02-1015

4.3. Intrinsic Factors

Are there any gender differences observed for nitazoxanide?

No significant differences between men and women were observed for the combination tablet.

Are there any age differences observed for nitazoxanide?

Regression analysis on the effect of age and body weight on clearance was performed and as shown below in Figures 2 and 3, no effect of age and body weight on clearance was observed. Table 2 shows the slope, intercept, R^2 and p-value of the regression analysis. As seen in the table, the p-value indicated a significant effect of age on clearance of tizoxanide. However, with due consideration to the R^2 and the fact that increase of clearance is only two-fold in the age range studied, there does not appear to be a clinically meaningful effect of age on clearance.

Figure 2: Relationship of age and clearance.

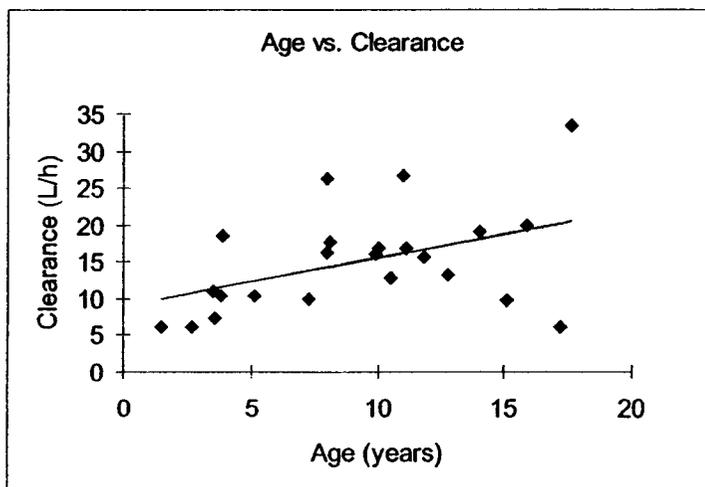


Figure 3: Relationship of body weight and clearance.

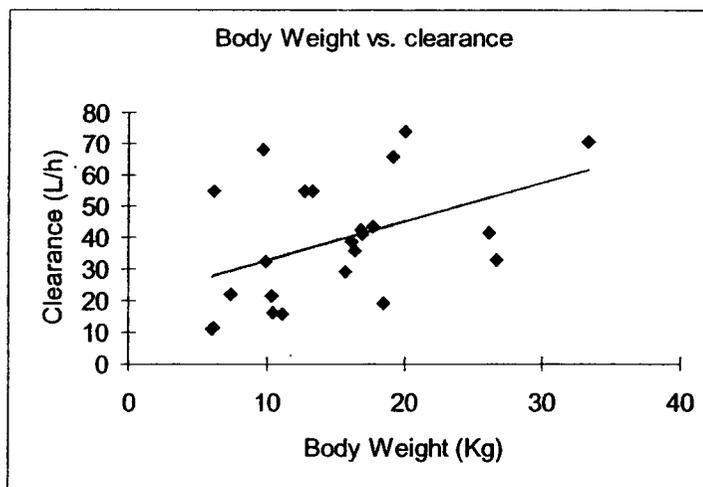


Table 2. Slope, Intercept and R² of the regression analysis

	Age vs. clearance	Body Weight vs. clearance
Slope	0.65	1.23
Intercept	9.08	20.41
R ²	0.2	0.2
p-value	0.0327	0.0336

4.4. Extrinsic Factors

Does food affect the bioavailability of nitazoxanide?

Food prolonged the rate of appearance of nitazoxanide in plasma and increased the extent of systemic exposure. Administration of nitazoxanide tablets following a high-fat (48% of kcal as fat) meal compared with the fasted state resulted in a $116 \pm 83\%$ (range 6% to 289%) increase in AUC and $73 \pm 81\%$ (range -26% to 248%) increase in C_{max}. The median T_{max} was greater when nitazoxanide was given with food (3.25 vs 2 hr).

Desacetyl-NTZ pharmacokinetic parameters in 18 fed and fasted healthy male subjects receiving a single 1000 mg dose of NTZ						
Parameter	C _{max} (µg/mL)	T _{max} (hr)	AUC _t (µg*hr/mL)	AUC _∞ (µg*hr/mL)	T _{1/2} (hr)	MRT (hr)
Fasted	8.57±2.50	2	31.7±9.81	32.7±11.6	2.83±2.13	3.94±1.23
Fed	13.8±4.53	3.25	61.4±21	58.3±21.8	2.06±0.98	4.72±1.18

Confidence intervals and point estimates were:

90% confidence intervals and point estimates, comparison of fed(test):fasted(reference) for AUC _t and C _{max}		
	AUC _t	C _{max}
Point estimate	1.86	1.57
90% CI	1.56,2.25	1.33,1.89

Based on the above results, it is clear that administration of nitazoxanide with food results in higher exposure. Results from the efficacy studies indicated that nitazoxanide, which was administered in children as a suspension was more effective in treating the diarrhea compared to adults who were administered tablets. A comparison of the exposure of nitazoxanide and nitazoxanide glucuronide between the suspension and tablet dosage forms indicated a greater exposure (41%) for tablets compared to suspension. When this data is viewed in conjunction with the food-effect study, it suggests that food increases the absorption of the metabolites of nitazoxanide, but the increased exposure may actually result in a decreased efficacy due to removal of the drug from the site of action, which is the gastrointestinal tract. Stated otherwise, the increased exposure of nitazoxanide and nitazoxanide glucuronide with food may actually result in reduced efficacy, since the site of action of the drug is the gastrointestinal tract. Thus, a recommendation is being made to further study the effects of food on efficacy of nitazoxanide.

Is there an in vitro basis to suspect in vivo drug-drug interactions?

The potential for interaction of nitazoxanide with different human cytochrome P450 enzymes was investigated. Among the different isoforms, CYP2C9 displayed the highest inhibitory potency, which was 10-fold higher than the other isoforms. The clinical relevance of these findings is not clear, since nitazoxanide has never been detected in the systemic circulation.

Is the drug an inhibitor and/or an inducer of CYP enzymes?

Nitazoxanide showed little potential to inhibit other CYP 450 enzymes.

4.5. Biopharmaceutics

Are the proposed dissolution methodology and specifications acceptable?

In the previous submission (NDA 20-871), the applicant evaluated stability of the 500 mg to-be-marketed nitazoxanide tablets with a dissolution method using 20% DMSO/80% phosphate buffer pH 7.5 as the media. During development, the applicant tested various media with a variety of surfactants, and found that, with the exception of the DMSO/phosphate solution, nitazoxanide solubility was low and degradation to tizoxanide was rapid. The applicant requested that: (1) the requirement of dissolution testing be waived; and (2) tablet disintegration be used to support product stability. However, the company's request for a waiver for dissolution testing was not granted.

It was recommended¹ that the company develop a dissolution method using pH 6.8 borate buffer + laurylsulfate, since of the conventional surfactants tested with pH 6.8 borate buffer, the addition of 6% laurylsulfate appeared to result in the highest solubility of nitazoxanide. The applicant was asked to assay both nitazoxanide and tizoxanide and report the concentrations of each as well as the concentrations of the two combined over time. Further, it was recommended that the applicant develop a dissolution method using pH 7.5 phosphate buffer + 6% hexadecyltrimethyl ammonium bromide. Since the desacetylation of nitazoxanide is temperature dependent, it was also recommended that studies of nitazoxanide dissolution be conducted at 25°C rather than at 37°C.

In this submission, the applicant submitted dissolution data in the medium containing 6% hexadecyltrimethylammonium bromide for both the suspension and tablet dosage forms. The applicant's choice of medium, temperature, and apparatus is acceptable. The applicant was asked to evaluate lower paddle speeds. The proposed specification for the suspension is acceptable. For the tablet a specification will be suggested based on evaluation of additional dissolution data at the lower paddle speeds.

Apparatus:	Paddle (USP Apparatus 2)
Dissolution medium:	Phosphate Buffer pH 7.5 with 6% hexadecyltrimethyl ammonium bromide
Volume:	900 mL
Bath temperature:	25 ± 0.5 °C
Rotation speed:	100 rpm
Specifications:	NLT — (Q) of the labeled amount dissolved as nitazoxanide and tizoxanide combined at 30 minutes (powder for suspension)

¹B. Davit, Clinical Pharmacology/Biopharmaceutics Review, NDA 20-871

The following two tables illustrate the dissolution data obtained for nitazoxanide powder for suspension and tablets.

Table 7.15 Summary of the dissolution performance for the nitazoxanide 500 mg tablet

Dosage form	Test Conditions [Apparatus, Medium, Speed of Rotation]	Lot no.	Media temperature	Collection time	Units tested	Mean % dissolved for units tested		
						Nitazoxanide	Tizoxanide	Sum
500 mg tablet	paddle apparatus with peak vessels and covers to retard evaporation	97E06	37°C	30 minutes	6	52.75	18.28	71.03
		97E06	37°C	45 minutes	6	51.43	25.09	76.52
		97E06	37°C	60 minutes	6	48.56	31.01	79.57
		97E06	25°C	15 minutes	6	33.00	2.92	35.92
		97E06	25°C	30 minutes	6	55.12	3.51	58.63
		97E06	25°C	45 minutes	6	57.90	8.65	66.55
		97E06	25°C	60 minutes	6	56.88	13.96	70.84
		97E07	25°C	15 minutes	6	49.31	4.68	53.99
		97E07	25°C	30 minutes	6	65.69	4.45	70.14
	97E07	25°C	45 minutes	6	64.97	9.57	74.54	
	97E07	25°C	60 minutes	6	62.40	14.69	77.09	
	97E13	25°C	15 minutes	6	40.14	4.49	44.63	
	97E13	25°C	30 minutes	6	60.65	4.87	65.52	
	97E13	25°C	45 minutes	6	60.83	9.62	70.45	
	97E13	25°C	60 minutes	6	56.06	12.19	68.25	
	97E06	25°C	30 minutes	6	57.27	2.1*	59.44	
	97E07	25°C	30 minutes	6	65.69	2.53	68.22	
	97E13	25°C	30 minutes	6	64.77	2.34	67.15	

Table 7.17 Summary of the dissolution performance for the nitazoxanide powder for oral suspension

Dosage form	Test Conditions [Apparatus, Medium, Speed of Rotation]	Lot no.	Media temperature	Collection time	Units tested	Mean % dissolved for units tested		
						Nitazoxanide	Tizoxanide	Sum
100 mg/5 ml powder for oral suspension	paddle apparatus with peak vessels and covers to retard evaporation	26726 ¹	25°C	5 minutes	1*	56.7	1.6	60.3
		26726	25°C	10 minutes	1*	74.2	5.0	79.2
		26726	25°C	15 minutes	1*	75.6	7.9	83.5
		26726	25°C	30 minutes	1*	74.9	10.9	85.8
		26726	25°C	45 minutes	1*	72.1	12.5	85.6
	Medium pH buffer 7.5 + 6% hexadecyltrimethylammonium bromide	26751	25°C	15 minutes	4 ^b	80.1	2.2	82.3
		26752	25°C	15 minutes	4 ^c	75.4	4.3	79.7
	Speed of Rotation 100 rpm	121201 ^d	25°C	15 minutes	4 ^b	79.4	1.5	80.9
		03117 ^e	25°C	15 minutes	11	??	??	??
	200 mg dispersible tablet	Same as above	112590 ^f	25°C	15 minutes	11	??	??

*Tests conducted December 6, 2001; ^bTests conducted January 16, 2002; ^cTests conducted January 17, 2002

¹ Lot no. 26726 was used in pharmacokinetic study RM01/02-1015 and is identical to the lots used in the adequate and well-controlled studies.

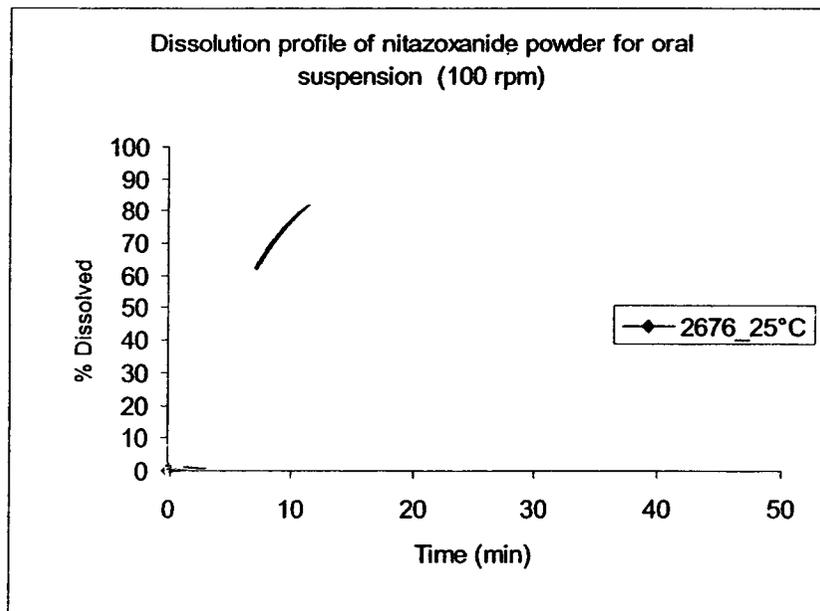
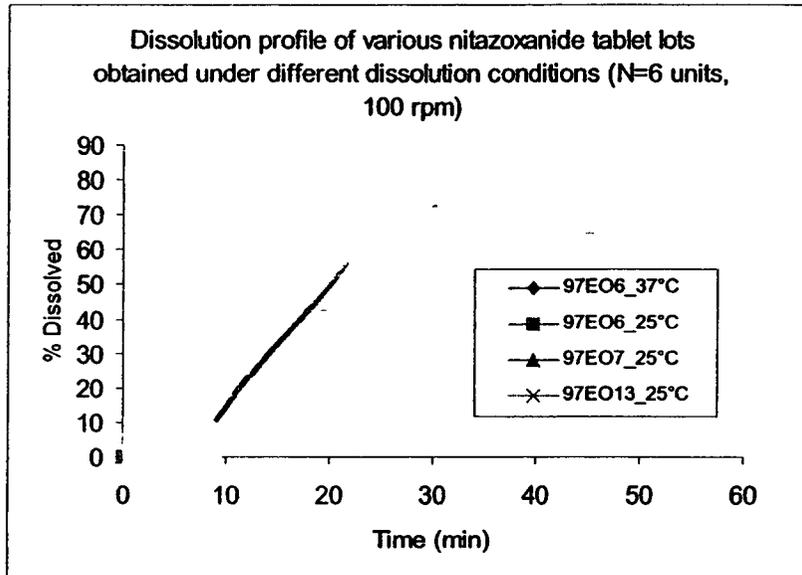
^b Lot no. 121201 is a pilot lot of nitazoxanide powder for oral suspension produced with the red dye to be used in the proposed formulation. The dissolution is similar to that of batches 26726, 26751 and 26752 which were produced with the red dye used in batches of powder for oral suspension that were used in the adequate and well-controlled clinical studies.

^c Lot 03117 is a batch of powder for suspension produced by [redacted] that used red dye instead of the proposed red dye #40, and it used a strawberry flavoring from a different supplier. This lot is identical to lot [redacted] used in uncontrolled studies no. CL-NTZ-05-001 and PRC-04-NTZ-03.

^d The dispersible tablet was used to treat children age 4-11 years enrolled in study no. PRC-04-NTZ-03 (an uncontrolled study).

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The dissolution data were plotted and are shown below in the following two figures.



If different-strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to-be-marketed product?

Table 2 and Figure 3 shows the comparison of plasma profiles for the suspension and tablet formulations. The suspension formulation was found to be not bioequivalent to the tablet formulation. The exposure of tizoxanide was found to be 41% less than the tablet formulation. When this finding was correlated with the results of the efficacy study, it is interesting to note that the efficacy in children, who were administered the suspension was found to be greater than in adults, who were administered tablets. This suggests that the better efficacy of tizoxanide in children may be the direct result of the higher drug concentrations in the gastrointestinal tract, which is the site of efficacy for this drug. In other words, the higher exposure of tizoxanide observed upon administration of tablets may actually result in lesser amount of the drug available at the site of action, that is, the stomach thus leading to less efficacy compared to the suspension dosage form.

Table 1. PK parameters of tizoxanide derived from the individual tizoxanide plasma profiles

PK parameter	Test 100 mg/5 mL suspension	Reference 500 mg tablet	p-value	Test/reference ratio	
				Point estimate	90% CI
C _{max} (µg/mL)	6.91	11.7	<0.001	59	51-68
AUC _t (µg-h/mL)	33.6	47.5	<0.001	71	63-80
AUC _{inf} (µg-h/mL)	33.9	47.8	<0.001	71	63-80
T _{max} (h)	1.50	1.50	0.067	-0.5h	-1.0 to 0.0 h
T _{1/2} (h)	1.46	1.38	0.365		

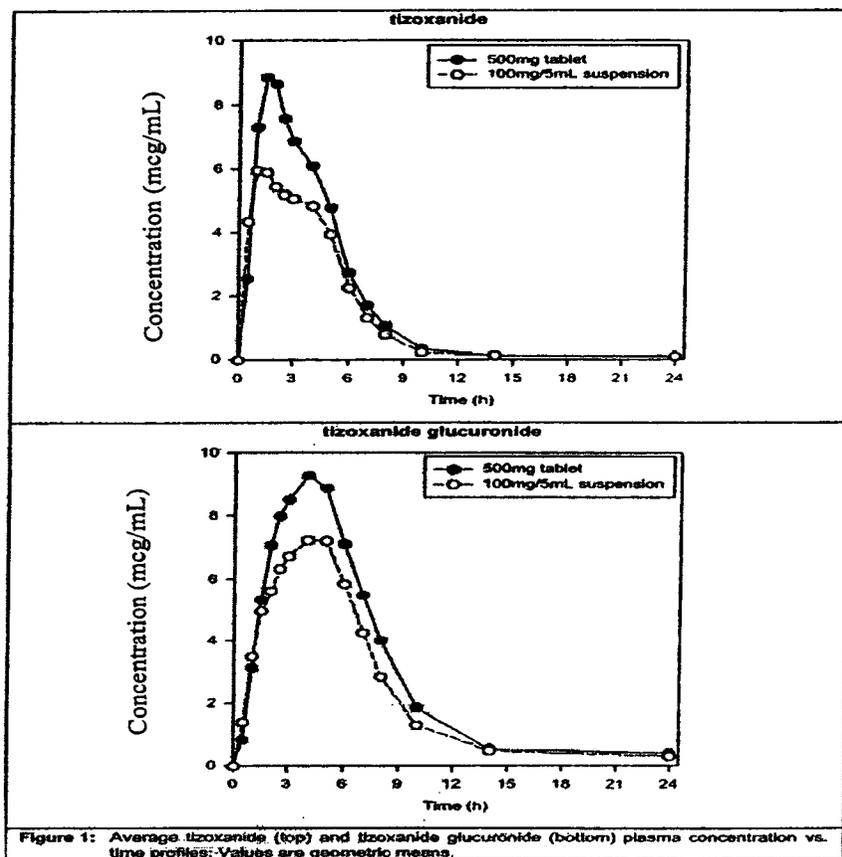
Values are medians for t_{max} and t_{1/2}

1: probability associated with the hypothesis of no difference between formulations (Koch's test for t_{max}. ANOVA for the other parameters)

2: expected geometric means test/reference ratio (%) and standard 90% CI, derived from ANOVA, except for t_{max}: non-parametric 90% CI of the treatment difference.

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Figure 3. Comparison of the plasma profiles of tizoxanide and tizoxanide glucuronide for the tablet and suspension formulations.



4.6. Analytical Methodology

What analytical methodology was used to determine nitazoxanide?

Clinical Pharmacokinetic studies Study B099597, RM01/02-1015 and 198.637:

The following assays were validated and used to determine nitazoxanide, tizoxanide and tizoxanide glucuronide in plasma from the above-mentioned studies. A review of the analytical methodology is presented below:

HPLC Conditions:

Mobile phase: /

Column:

Internal Standard: nifuroxanide

Detection: Mass Spectrometer

Linearity: — $\mu\text{g/mL}$ for tizoxanide an' — $\mu\text{g/mL}$ for tizoxanide glucuronide.

QC samples: — $\mu\text{g/mL}$ for tizoxanide and — $\mu\text{g/mL}$ for tizoxanide glucuronide, respectively.

Recovery: Average recovery was found to be close to 100% at every level.

Limit of Quantitation: — $\mu\text{g/mL}$ for tizoxanide an' — $\mu\text{g/mL}$ for tizoxanide glucuronide.

Specificity: The extraction and chromatographic procedures allowed a good separation of the components of interest from endogenous compounds.

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