U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION
Clinical Studies

NDA / Supporting Document Number:	22-574 / 001
Drug Name:	Safyral (0.03 mg ethinylestradiol + 3.0 mg drospirenone + 0.451 mg levomefolate calcium)
Indication(s):	Improvement in folate status in women who elect to use an oral contraceptive
Applicant:	Bayer HealthCare Pharmaceuticals Inc.
Date(s):	Letter Date: November 16, 2009 PDUFA Date: December 16, 2010
Review Priority:	1 Standard
Biometrics Division:	Division of Biometrics 3
Statistical Reviewer:	Sonia Castillo, Ph.D.
Biometrics Team Leader:	Mahboob Sobhan, Ph.D.
Medical Division:	Division of Reproductive and Urology Products
Clinical Team:	Daniel Davis, M.D., Clinical Reviewer Lisa Soule, M.D., Clinical Team Leader
Project Manager:	Pamela Lucarelli

Key Words: Clinical studies, NDA review

The Applicant has submitted this NDA for the addition of Metafolin (levomefolate calcium) to Yasmin in support of a folate-fortified, oral contraceptive (OC) regimen. The regimen consists of 21 tablets each containing 3 mg of drospirenone (DRSP), 0.03 mg of ethinyl estradiol (EE) and 0.451 mg of levomefolate calcium (cycle days 1-21) followed by 7 tablets containing 0.451 mg of levomefolate calcium only (cycle days 22-28). Based on an agreement with the Division of Reproductive and Urologic Products on June 25, 2009, this application incorporates by reference nonclinical and clinical information previously submitted (on August 21, 2009) in the YAZ plus Metafolin NDA 22-532.

A total of four studies, two bioequivalence studies and two pharmacodynamic studies, were conducted. These four studies are further described below. The Division agreed that the studies would support two separate NDAs for YAZ with Metafolin and Yasmin with Metafolin (refer to responses dated May 11, 2009 to the Pre NDA meeting briefing package for IND 72,287). The Division also agreed that the nonclinical and clinical information submitted in the YAZ plus Metafolin New Drug Application 22-532 could be cross referenced in this NDA submission (refer to email correspondence dated June 25, 2009).

According to the Applicant:

> A total of four studies, two bioequivalence studies and two pharmacodynamic studies, were conducted as discussed below. …
>
> The two bioequivalence studies were performed in order to demonstrate that the addition of levomefolate calcium has no influence on the rate and extent of absorption of both, estrogen and progestin, and the presence of estrogen and progestin has no influence on the rate and extent of absorption of Metafolin®. One study each was performed for Yasmin® (NDA 22-532, Module 5.3.1.2 Report A27410) and YAZ® (NDA 22-532, Module 5.3.1.2 Report A28575). Both studies were performed in Europe.
>
> The third study is referred to as the "long-term use of folate study" (NDA 22- 532, Module 5.3.4.1 Report A39814). This study was performed in order to investigate plasma and red blood cell (RBC) folate during a 24-week administration of a fortified oral contraceptive and to demonstrate that the selected dose of 0.451 mg levomefolate calcium and 0.4 mg folic acid result in equivalent levels of plasma and RBC folate. Furthermore, the depot effect after cessation of folate supplementation during a 20-week elimination phase was investigated. The oral contraceptive used in this study was Yasmin®. This study was also conducted in Europe.
>
> The forth study, referred to as the "folate benefit study", investigated plasma folate, red blood cell folate and homocysteine levels during a 24-week oral administration of an OC with or without levomefolate calcium (NDA 22-532, Module 5.3.5.1 Report A43598). This study was conducted to provide data showing that fortification of an oral contraceptive with levomefolate calcium is clinically beneficial even in women on a folate-fortified diet. The oral contraceptive used in this study was YAZ®. This study was conducted in the US. (Reference: pages 2 and 3 of the submission cover letter).

Per the clinical reviewer, it is assumed that the data from the U.S. pharmacodynamic trial using YAZ plus Metafolin can be extrapolated to the use of Yasmin plus Metafolin. They both use the same dose of DSRP and Metafolin but the EE dose is different. Based on this assumption, the review for this Yasmin plus Metafolin application cross references the statistical review for YAZ plus Metafolin (see attachments below) and no additional statistical input is necessary.

ATTACHMENTS

U.S. Department of Health and Human Services
Food and Drug Administration
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STATISTICAL REVIEW AND EVALUATION
Clinical Studies

NDA / Supporting Document Number:	22-532 / 001
Drug Name:	Beyaz (0.020 mg ethinylestradiol + 3.0 mg drospirenone + 0.451 mg levomefolate calcium)
Indication(s):	Improvement in folate status in women who elect to use an oral contraceptive
Applicant:	Bayer HealthCare Pharmaceuticals Inc.
Date(s):	Letter Date: August 21, 2009 PDUFA Date: September. 24, 2010
Review Priority:	1 Standard
Biometrics Division:	Division of Biometrics 3
Statistical Reviewer:	Sonia Castillo, Ph.D.
Biometrics Team Leader:	Mahboob Sobhan, Ph.D.
Medical Division:	Division of Reproductive and Urology Products
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Project Manager:	Pamela Lucarelli
Key Words:	Clinical studies, NDA review

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1. EXECUTIVE SUMMARY

1.1 Conclusions and Recommendations

A large amount of data submitted in support of this application was invalid due to poor blood sample preparation and was discarded from the efficacy evaluation. Blood sample preparation problems were discovered at two of the eight study sites during an interim analysis of blinded baseline plasma and red blood cell (RBC) folate data for all pre-treatment samples for all 385 randomized subjects in Study A43598. One of the sites was the largest study site that enrolled 31.2% of all subjects (120 of 385) and the other site enrolled 3.4% of all subjects (13 of 385).

Samples from both sites were not processed correctly due to incorrect dilution during sample preparation and/or a failure to protect blood samples from excessive light exposure. This resulted in higher than expected levels of folate which were invalid and/or biologically implausible. Valid plasma folate, whole blood folate, and hematocrit values are needed to calculate RBC folate. Both the clinical reviewer and the Division of Scientific Investigations report recommended that these RBC folate samples be removed from the RBC folate primary efficacy analysis. There were no sample preparation issues with plasma folate levels.

Due to these blood sample preparation errors, the analysis for RBC folate dropped 41% of the Beyaz data and 34% of the comparator data. So instead of declaring statistical significance, a descriptive presentation using the 95% confidence interval for the treatment difference is used.

Despite dropping this substantial amount of data, the two submitted studies provide supportive evidence demonstrating the efficacy of the oral contraceptive Beyaz (0.020 mg ethinylestradiol + 3.0 mg drospirenone + 0.451 mg levomefolate calcium) to improve the folate status in women who elect to use an oral contraceptive. There was an increase in RBC folate and plasma folate levels with Beyaz use.

1.2 Background

This two study submission is a new drug application for the oral contraceptive Beyaz. Study A43598 is a randomized, multicenter, double-blind, parallel-group, placebo-controlled Phase 3 trial conducted in the U.S. comparing the efficacy and safety of Beyaz and YAZ for the improvement of folate blood levels in women who choose to use an oral contraceptive. Study A39814 is a single center, open-label, parallel-group, controlled Phase 1 trial conducted in Germany to assess the pharmacodynamic effect on plasma folate and red blood cell folate and to compare the profile of circulating folate metabolites during 24 weeks of treatment with the oral contraceptive Yasmin containing levomefolate calcium or Yasmin co-administered with folic acid followed by 20 weeks of open-label treatment with Yasmin only in women seeking contraception.

The information from these two studies is used to demonstrate an increase in red blood cell and plasma folate levels and to describe the duration of time red blood cell folate levels remain above 906 nmol/L after discontinuation of treatment with levomefolate calcium added to the oral contraceptive. Folate dietary supplementation is clinically recommended for women who plan to become pregnant to aid in the reduction of neural tube defects during fetal development.

The Applicant's proposed indication is:

(b) (4).

1.3 Statistical Issues and Findings

Two statistical issues were identified in this submission. One was how to address the efficacy analyses when presented with a large amount of invalid and/or biologically implausible red blood cell (RBC) and plasma folate data due to poor blood sample preparation at two of the eight clinical sites in Study A43598. The other was changing the primary efficacy endpoints for RBC folate and plasma folate levels from the folate level value at Week 24 to the folate level's change from baseline at Week 24. This issue was raised by the clinical reviewer because the change from baseline at Week 24 adjusts for the variable baseline folate levels among women of childbearing age in the general population. Otherwise, the Applicant adhered to statistical methods for the primary endpoints as specified in the protocol and Statistical Analysis Plan.

From a statistical perspective, the two submitted studies (24-week Study A43598 and 44-week Study A39814) provide supportive evidence demonstrating the efficacy of Beyaz to improve the folate status in women who elect to use an oral contraceptive based on the endpoints of RBC folate level and plasma folate level. There was an increase in RBC folate and plasma folate levels with Beyaz use compared to YAZ.

2. INTRODUCTION

2.1 Overview

The Applicant has submitted two clinical studies (A43598 and A39814) designed to demonstrate the safety and efficacy of Beyaz (0.020 mg ethinylestradiol + 3.0 mg drospirenone + 0.451 mg levomefolate calcium) to improve the folate status in women who elect to use an oral contraceptive. For the remainder of this review, I will refer to **levomefolate calcium** as metafolin. Table 2.1 presents a brief summary of these studies.

Table 2.1
Brief Summary of Clinical Studies for YAZ + Metafolin and Yasmin + Metafolin

Study Number (Study Type) (No. of Sites / Country) Dates of Study Conduct	Subject Population	Treatment	Number Randomized (ITT[1])	Design[2]
A43598 (8 / United States) April 2007 to Sept. 2008	Healthy female subjects of reproductive age, 18 to 40 years old	Beyaz (YAZ + Metafolin) YAZ **Total**	291 94 **385**	DB, R, PG, AC, MC, 24 weeks
A39814 (1 / Germany) Dec. 2006 to Jan. 2008		Yasmin/Metafolin + Placebo Folate (Period 1) followed by Yasmin (Period 2) Yasmin + Folate (Period 1) followed by Yasmin (Period 2) **Total**	86 86 **172**	OL, SC, PG, 44 weeks

Source: Statistical Reviewer's listing.
[1] ITT = Intent to Treat
[2] DB = Double-blind, OL = Open-label, R = Randomized, AC = Active Control, PG = Parallel Group, MC = Multicenter, SC = Single Center

2.2 Data Sources

The study report and additional information for these studies were submitted electronically. The submitted SAS data sets for each study were complete and well documented. These items are located in the Electronic Document Room at \\Cdsesub1\evsprod\NDA022532\0000 under submission dates 8-21-2009, 11-17-2009, and 2-4-2010.

3. STATISTICAL DESIGN

3.1 Study Design

3.1.1 Study A43598

This was a multicenter (8 U.S. sites), randomized, double-blind, active-controlled, parallel-group Phase 3 study to investigate plasma folate and red blood cell folate levels in healthy women of reproductive age, between 18 to 40 years of age, requesting contraception during a 24-week oral administration of YAZ + Metafolin compared to YAZ alone. Eligible subjects were randomized in a 3:1 ratio to either YAZ + Metafolin or YAZ. According to the Applicant, the 3:1 ratio was chosen to acquire more data for YAZ + Metafolin.

The 24-week blinded oral treatment consisted of:

> **Test Treatment:** YAZ +Metafolin (0.020 mg ethinylestradiol (EE) + 3.0 mg drospirenone (DRSP) + 0.451 mg Metafolin). Each 28-day treatment cycle consisted of once daily hormone and Metafolin treatment for 24 days followed by hormone-free Metafolin only tablet for 4 days.

Reference Treatment: YAZ (0.020 mg EE + 3.0 mg DRSP). Each 28-day treatment cycle consisted of once daily hormone treatment for 24 days followed by once daily hormone-free tablet for 4 days.

Before starting treatment, three blood samples used for calculation of RBC and plasma folate levels were taken and their medians were used as the baseline values. Blood samples were then drawn every 4 weeks during the treatment period (Weeks 4 through 24) for determination of RBC and plasma folate levels. These collected blood samples were prepared for laboratory analysis at the study site and then sent to centralized laboratories for assessment.

The primary study objective was to demonstrate an increase in RBC and plasma folate levels as a result of metafolin supplementation in healthy, reproductive-aged female subjects.

The co-primary efficacy variables were RBC folate and plasma folate levels at Week 24. RBC folate levels were calculated by:

*RBC folate = ([whole blood folate * 100] – [plasma folate * (100 - hematocrit)])/hematocrit*

Because RBC folate levels rely on valid whole blood folate, plasma folate, and hematocrit levels, if any of these values are either invalid and/or biologically implausible, RBC cannot be calculated. Due to blood sample preparation errors, that is what happened in this study. The details are given after description of the remainder of this protocol.

The following were the secondary efficacy variables and were not considered for labeling claims:

- The mean Neural Tube Defect (NTD) risk reduction evaluated as change from Baseline to Week 24 in NTD risk
- The mean changes from Baseline to Weeks 4, 8, 12, 16, and 20 in RBC folate levels
- The mean changes from Baseline to Weeks 4, 8, 12, 16, and 20 in plasma folate levels
- The mean changes from Baseline to Weeks 4, 8, 12, 16, 20, and 24 in plasma homocysteine levels

Two efficacy analysis populations were described in the protocol. The full analysis set (FAS) was defined as all randomized subjects who took at least 1 dose of study medication. The per protocol set (PPS) was defined as all FAS subjects who:

- did not violate any of the inclusion and exclusion criteria, with the potential of influencing primary endpoints,
- did not take any medications influencing folate uptake, distribution, metabolism or excretion,
- had at least 75% study medication compliance per cycle [treatment compliance calculated as 100 times the number of tablets taken per cycle divided by the length of the cycle in days (i.e., 28 days)],
- had no other major protocol violation affecting primary endpoints,
- completed 24 weeks of the treatment course, and
- had valid plasma folate for Baseline and Week 24 or valid RBC folate values for Baseline and Week 24.

This definition of the PPS was the result of Amendment 4, dated Nov. 6, 2008. As pre-specified in the protocol, the eligibility of each FAS subject for PPS inclusion was determined prior to unblinding the data for analysis.

The primary efficacy analyses of RBC folate and plasma folate levels at Week 24 used an analysis of covariance (ANCOVA) with treatment as factor and their respective baseline folate level as covariate. The primary efficacy population was the PPS, which used only subjects who had both baseline and Week 24 folate levels. As documented in Amendment 4 (Nov. 6, 2008), the Applicant decided that based on the smaller number of evaluable subjects and valid samples for RBC folate analysis because of blood sample preparation errors, the PPS became the primary efficacy population instead of the FAS using last observation carried forward (LOCF).

Analyses were also conducted for the FAS with and without LOCF. LOCF was used for prematurely discontinued subjects, or subjects with no plasma or RBC folate level at Week 24.

All hypotheses (superiority) were tested at the 2-sided 5% significance level and the 95% confidence intervals for the treatment differences are provided.

Analyses that used all valid baseline and valid Week 24 data from all eight sites were referred to as Scenario A in the study report. The analyses mentioned above belong to Scenario A. Due to the blood sample preparation problems at two of the eight sites, the Applicant decided, prior to unblinding the data, to analyze plasma folate and RBC folate levels in two other ways:

- Scenario B analyzed RBC folate and plasma folate as in Scenario A, but excluded data from the two sites
- Scenario C evaluated all whole blood folate values after normalization to a dilution factor of 0.1 using all 8 sites

The secondary efficacy variables were analyzed for the PPS and FAS with and without LOCF. Descriptive statistics were provided by treatment group for each of the variables.

The sample size was estimated based on a two-sided, two-group t-test of equal means, 5% significance level, 90% power, a 3:1 randomization, and the following published data: a change in mean RBC folate levels from baseline to Week 24 (for YAZ + Metafolin group) of at least 100 ng/ml, mean baseline RBC folate level of 263.6 ng/mL and standard deviation of 200 ng/mL. Based on these assumptions, the total evaluable sample size is 228 subjects (57 YAZ subjects and 171 YAZ + Metafolin subjects). Furthermore, assuming a 33% drop-out rate, a total of 344 subjects were enrolled in the study.

Plasma folate was added as a co-primary endpoint after start of the study (Amendment 2, Oct. 16, 2007) and the sample size was based on RBC folate because, according to the Applicant, both primary endpoints were expected to be highly correlated.

The Applicant recalculated the sample size after availability of results from the blinded interim analysis of baseline samples in this study and results from the Applicant's European Long-term Folate study (Amendment 4; Nov. 6, 2008). According to the Applicant, the sample size recalculation was done because "these results differed considerably from initial expectations described in the original protocol." Their intent was to show that the study was still powered to detect a difference, using the estimated number of completers, even though the number of evaluable subjects was less than the 228 subjects in the original sample size calculation. The revised total sample size of 148 subjects (37 subjects in the YAZ group, and 111 subjects in YAZ Plus group) was based on the following criteria: the initial assumption of an increase in RBC folate of at least 100 ng/mL was adjusted to 125 ng/mL, the SD remained at 200 ng/mL, and 90% power.

Blood Sample Preparation Issues

The study report stated that there were blood sample preparation problems at two of the eight study sites. Because of these problems, the Applicant decided to remove these sites from the analysis (Amendment 4, Nov. 6, 2008) in addition to any invalid and/or biologically implausible values at any site. Recall that valid plasma folate, whole blood folate, and hematocrit values are needed to calculate RBC folate. On January 25, 2010, the Division of Reproductive and Urologic Products (DRUP) sent an Information Request to the Applicant to describe the problems in detail. The Applicant's February 4, 2010 response stated that during the interim analysis of blinded baseline plasma and RBC folate data for all pre-treatment samples for all 385 randomized subjects, "it appeared that all samples from study site 104 and most samples from study site 108 were not processed correctly." The response also stated that: "During the bioanalytical assessment of whole blood samples, unexpectedly high folate levels were revealed which were mostly associated with a darker red color that could have been caused by incorrect dilution during the sample preparation."

This blinded interim analysis of baseline plasma and RBC folate data was the result of Amendment 3, dated May 28, 2008. The Applicant's rationale for conducting this blinded interim analysis was for "the purpose of meeting with the FDA to discuss baseline folate data. (Based on the results of the interim analysis, a meeting with the FDA was no longer deemed necessary.)" Of note is that no inferential statistics were performed.

A consult was sent by DRUP to the Division of Scientific Investigations (DSI) to investigate the sample preparation problems at both sites 104 and 108. Of note is that site 108 was the largest study site, enrolling 31.2% of all subjects (120 of 385), and site 104 enrolled 3.4% of all subjects (13 of 385). The DSI report, dated May 24, 2010, documented that the clinical central laboratory identified the samples with dilution problems and noted an additional sample preparation error at site 108 that compromised folate stability due to failure to protect blood

samples from excessive light exposure. The report recommended that the samples from site 108 with excessive light exposure be removed from the RBC folate calculation and analysis.

The DSI report did not recommend removal of sites 104 and 108 from the analyses due to the substantial blood sample preparation errors that resulted in invalid and/or biologically implausible blood folate values but the clinical reviewer wanted these sites removed from the analysis of RBC folate. Also, the clinical reviewer does not agree with the protocol specified primary efficacy analyses based on RBC and plasma folate levels at Week 24 because the baseline folate levels vary for each subject and depend on the amount of dietary intake of folate. Instead the clinical reviewer would rather see analyses based on the change from baseline at Week 24 in RBC and plasma folate levels.

Although the Applicant has presented results based on their protocol specified analyses for three different scenarios, in this review I analyzed the RBC folate levels using the change from baseline at Week 24, excluding sites 104 and 108, and using those subjects who took at least one dose of study medication (FAS) and using LOCF. Since there were no sample preparation issues with the plasma folate levels, my analysis used the change from baseline at Week 24 and the FAS subjects with LOCF. In addition, my analyses did not use the invalid RBC or plasma folate values that arise in routine laboratory blood work analysis. Therefore, baseline or Week 24 folate levels were not available for some subjects and the number of subjects included in the individual analyses presented in section 3.2.1 varies.

3.1.2 Study A39814

This is a single center (Germany), randomized, double-blind, double-dummy, parallel group Phase 1 clinical trial to assess the pharmacodynamic effect on plasma folate and red blood cell folate and to compare the profile of circulating folate metabolites during 24 weeks of treatment with the oral contraceptive Yasmin containing Metafolin or Yasmin co-administered with folic acid followed by 20 weeks of open-label treatment with Yasmin alone in women seeking contraception.

Subjects were healthy women, 18 to 40 years of age, who were seeking contraception, had an RBC folate > 317 nmol/L and < 906 nmol/L, and no concomitant intake of vitamin supplements or medication containing folate or interacting with folate. A total of 172 healthy female subjects were enrolled and equally randomized to either Yasmin + Metafolin or Yasmin alone (86 subjects per treatment).

The study consisted of 24 weeks of blinded treatment followed by 20 weeks of open-label treatment (folate elimination phase) and is described as follows:

Period 1 (Weeks 1 to 24):

> **Test Treatment:** Yasmin + Metafolin (0.030 mg ethinylestradiol (EE) + 3 mg drospirenone (DRSP) + 0.451 mg Metafolin), in combination with folic acid placebo tablets (encapsulated). Each 28-day treatment cycle consisted of once daily hormone and Metafolin treatment for 21-days followed by once daily hormone free, 0.451 mg Metafolin only tablet for 7 days. A folic acid placebo tablet (encapsulated) was taken each day.
>
> **Reference Treatment:** Yasmin (0.030 mg EE + 3 mg DRSP), in combination with 0.4 mg folic acid tablets (encapsulated). Each 28-day treatment cycle consisted of once daily hormone and folic acid treatment for 21 days followed by once daily hormone free, folic acid only tablet (encapsulated) for 7 days.

Period 2 (Weeks 25 to 44):

> **Both Treatment Groups:** Yasmin alone. Each 28-day treatment cycle consisted of once daily hormone treatment for 21 days followed by once daily hormone free tablet for 7 days.

Before starting treatment, three blood samples for RBC and plasma folate were drawn and used to calculate the baseline values. Blood samples were then drawn every 14 days during the treatment period (Weeks 2 through 44) for determination of plasma folate and RBC folate.

The primary pharmacodynamic study objectives were:

- To demonstrate whether 0.451 mg Metafolin from test treatment and 0.4 mg folic acid from reference treatment results in similar plasma folate and RBC folate exposures as measured by plasma folate $AUC_{(0\text{-}24\ weeks)}$ and RBC folate $AUC_{(0\text{-}24\ weeks)}$
- To determine the duration of time following the end of blinded treatment (24-weeks) during which a red blood cell folate concentration equal to or greater than 906 nmol/L was maintained in the Yasmin + Metafolin group, i.e. time to falling of RBC folate < 906 nmol/L in the Yasmin + Metafolin group

The primary pharmacodynamic variables were:

- AUC(0-24weeks) for plasma folate
- AUC(0-24 weeks) for RBC folate
- Time to falling of RBC folate < 906 nmol/L in the Yasmin + Metafolin group

RBC folate concentrations were calculated by:

*RBC folate = ([whole blood folate * 100] – [plasma folate * (100 - hematocrit)])/hematocrit*

The total sample size of 172 subjects (86 per group) was based on the following assumptions:

- Proportion of subjects not belonging to the Per Protocol Set: 37%
- True ratio (Test AUC plasma folate /Reference AUC plasma folate) of 110%
- Bioequivalence limits of (80%; 125%)
- Power of 90%
- Standard deviation of 0.24 on the log-scale

The full analysis set (FAS) included all subjects who were treated with at least one dose of the study drug and had at least one clinical observation after start of treatment. The per protocol set (PPS) included all treated subjects without any major protocol deviations (e.g. missing data preventing a reliable calculation of RBC folate, or insufficient drug exposure during treatment Period 1). According to the protocol, the analysis on the PPS was the relevant analysis set for the co-primary variables.

Since this is a Phase 1 trial, no formal statistical tests were conducted. This review describes the time to falling of RBC folate to less than 906 nmol/L in the Yasmin + Metafolin group because the clinical reviewer requested that this endpoint be verified. The time to falling of RBC folate level below 906 nmol/L was calculated using the Kaplan Meier estimate and the two-sided 95% confidence interval for the median is provided.

3.2 Evaluation of Efficacy

3.2.1 Study A43598 Subject Disposition and Baseline Characteristics

Table 3.1 presents the number of randomized subjects and their disposition for Study A43598. A total of 385 subjects were randomized, 291 subjects to the YAZ + Metafolin group and 94 to the YAZ group. For the primary efficacy endpoint, 379 of the 385 randomized subjects were included in the Full Analysis Set (FAS) (285 for YAZ + Metafolin and 94 for YAZ). Six subjects were not included because they did not ingest study medication and all six were in the YAZ + Metafolin group.

Discontinuation rates were similar in both treatment groups (28.2% for YAZ + Metafolin and 25.5% for YAZ). The primary reasons for study discontinuation were subject lost to follow-up (8.6% for YAZ + Metafolin and 8.5% for YAZ), protocol deviation (6.2% for YAZ + Metafolin and 8.5% for YAZ), adverse events (4.5% for YAZ + Metafolin and 3.2% for YAZ), and withdrawal of consent (4.1% for YAZ + Metafolin and 3.2% for YAZ).

Table 3.1
Study A43598: Randomization and Disposition of All Subjects

	YAZ + Metafolin	YAZ
Number Randomized	291	94
Received but did Not Ingest Study Medication*	6 (2.1)	0 (0.0)
Treated (FAS)*	285 (97.9)	94 (100)
Completed n (%)*	203 (69.8)	70 (74.5)
Discontinued n (%)*	82 (28.2)	24 (25.5)
Primary Reason for Discontinuation n (%)*:		
Subject Lost to Follow-up	25 (8.6)	8 (8.5)
Protocol Deviation	18 (6.2)	8 (8.5)
Adverse Event	13 (4.5)	3 (3.2)
Withdrawal of Consent	12 (4.1)	3 (3.2)
Pregnancy	2 (0.6)	0 (0.0)
Other	12 (4.1)	2 (2.1)

Source: Text Table 10, page 75, Study A43598 report.
* With respect to number of randomized subjects.

Both groups were similar with respect to baseline and demographic characteristics based on the FAS. For the YAZ + Metafolin group, the majority of subjects were Caucasian. (63.5%) and 35 years of age or younger (94.0%) and had a mean age of 24.8 years and mean BMI of 24.1 kg/m^2. For the YAZ group, the majority of the subjects were Caucasian (63.8%) and 35 years of age or younger (97.9%) and had a mean age of 24.6 years and a mean BMI of 23.9 kg/m^2.

3.2.2 Study A43598 Efficacy Results

Table 3.2 presents the results for the change from baseline at Week 24 for RBC folate. Because of the sample preparation problems with the RBC folate data, results for the FAS using LOCF and without sites 104 and 108 are presented. Dropping these sites removes 41% (118/285) of the YAZ + Metafolin and 34% (32/94) of the YAZ RBC folate data from the analysis. Although the p-value for this analysis is small, the large amount of missing data makes it difficult to conclude in this situation that the probability of a false positive is less than 0.0001. Instead, presenting the 95% confidence interval to describe the treatment effect on RBC folate is preferred.

RBC folate level when taking YAZ + Metafolin compared to YAZ increased by 368.9 nmol/L (95% C.I.: 285.7 nmol/L to 452.2 nmol/L). For completeness, additional sensitivity analyses using: 1) the PPS with and without sites 104 and 108, 2) the FAS with and without LOCF, and 3) the FAS without sites 104 and 108 and without LOCF are presented in Tables A.1 to A.3 in the Appendix.

Table 3.2
Study A43598: Red Blood Cell (RBC) Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24 (Full Analysis Population with LOCF Excluding Sites 104 and 108)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value*
YAZ + Metafolin	167	949.4	402.3	368.9 (285.7, 452.2)	< 0.0001
YAZ	62	995.7	33.4		

Source: Statistical Reviewer's analysis and Table 112, page 728 of 870, and Table 124, page 744 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.
* p-value should be used with caution since 41% (118/285) of the YAZ + Metafolin folate data and 34% (32/94) of the YAZ RBC folate data were dropped from the analysis.

Table 3.3 presents the results for the change from baseline at Week 24 for plasma folate. Because the plasma folate data did not have the sample preparation problems found with the RBC folate data, results using the FAS with LOCF are presented. Although not all subjects had complete data, 7.4% (21/285) of the YAZ + Metafolin and 5.3% (5/94) of the YAZ plasma folate data were dropped from the analysis, which is considerably less than the percentages for the RBC folate analysis. In this case, the p-value can be used to describe the treatment effect.

The plasma folate level when taking YAZ + Metafolin compared to YAZ increased by 18.9 nmol/L (95% C.I.: 14.6 nmol/L to 23.1 nmol/L; p-value < 0.0001). For completeness, additional sensitivity analyses using: 1) the Per Protocol Set (PPS) with and without sites 104 and 108, 2) the FAS with and without LOCF, and 3) the FAS without sites 104 and 108 and without LOCF are presented in Tables A.4 to A.6 in the Appendix.

Table 3.3
Study A43598: Plasma Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Full Analysis Population with LOCF Using All Sites)

	n*	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value
YAZ + Metafolin	264	44.6	16.8	18.9 (14.6, 23.1)	< 0.0001
YAZ	89	41.6	-2.0		

Source: Statistical Reviewer's analysis and Table 148, page 776 of 870, and Table 160, page 792 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.
* 7.4% (21/285) of the YAZ + Metafolin and 5.3% (5/94) of the YAZ plasma folate data were dropped from the analysis

3.2.3 Study A39814 Subject Disposition and Baseline Characteristics

The results for the Per Protocol Set (PPS) analysis of the Yasmin + Metafolin group are presented. The PPS included the 75 subjects who did not have major protocol deviations and were assessed as being appropriate for inclusion in the pharmacodynamic analysis by the Applicant. The FAS had 86 subjects but 11 were removed due to major protocol violations. All subjects were Caucasian and had a mean age of 28.2 years, body mass index of 23.0 kg/m2, and a mean baseline RBC folate value of 578.3 nmol/L.

3.2.4 Study A39814 Efficacy Results

Table 3.4 presents the time to RBC folate falling below 906 nmol/L in the folate elimination phase. The total number of subjects at Week 24 was 75, with four subjects having an RBC folate level < 906 nmol/L. At the end of treatment Period 1 (Week 24), 95% of subjects had RBC folate levels ≥ 906 nmol/L. The proportion of subjects with RBC folate levels ≥ 906 nmol/L decreased over time during the elimination phase: 4 weeks after stopping folate intake 85% of the subjects had RBC folate levels ≥ 906 nmol/L, 8 weeks after stop 60%, 10 weeks after stop 47%, 12 weeks after stop 29% and 20 weeks after stop (at the last sampling point) 9%. The median time to RBC folate falling below 906 nmol/L after 24 weeks of treatment occurred at study week 34 (95% C.I. of 32 to 36 weeks). That is, the median time to RBC folate falling below 906 nmol/L after 24 weeks of treatment occurred after 10 weeks. Of note is that the results for the FAS were similar to those for the PPS and are presented in Table A.7 in the Appendix.

Table 3.4
Study A39814: Kaplan Meier (KM) Estimates and the Proportion of Subjects for the Time to RBC Folate Falling Below 906 nmol/L for the Yasmin + Metafolin Treatment Group in the Folate Elimination Phase (Week 26 to Week 44) – Per Protocol Set

Week	Number of subjects with RBC folate ≥ 906 nmol/L	KM estimate (Proportion of subjects with RBC folate ≥ 906 nmol/L)
24	71	0.947
26	70	0.933
28	64	0.853
30	59	0.787
32	45	0.600
34	35	0.467
36	22	0.293
38	18	0.240
40	13	0.173
42	10	0.133
44	7	0.093

Source: Table 117, page 762 of 942, Study A39814 report.

3.3 Evaluation of Safety

For information about the evaluation of safety, refer to the safety section of the clinical review.

4. FINDINGS IN SUBGROUP POPULATIONS

There were no subgroup populations of interest in this review.

5. CONCLUSIONS

A large amount of data submitted in support of this application was invalid due to poor blood sample preparation and was discarded from the efficacy evaluation. Blood sample preparation problems were discovered at two of the eight study sites during an interim analysis of blinded baseline plasma and red blood cell (RBC) folate data for all pre-treatment samples for all 385 randomized subjects in Study A43598. One of the sites was the largest study site that enrolled 31.2% of all subjects (120 of 385) and the other site enrolled 3.4% of all subjects (13 of 385).

Samples from both sites were not processed correctly due to incorrect dilution during sample preparation and/or a failure to protect blood samples from excessive light exposure. This resulted in higher than expected levels of folate which were invalid and/or biologically implausible. Valid plasma folate, whole blood folate, and hematocrit values are needed to calculate RBC folate. Both the clinical reviewer and the Division of Scientific Investigations report recommended that these RBC folate samples be removed from the RBC folate primary efficacy analysis. There were no sample preparation issues with plasma folate levels.

Due to these blood sample preparation errors, the analysis for RBC folate dropped 41% of the Beyaz data and 34% of the comparator data. So instead of declaring statistical significance, a descriptive presentation using the 95% confidence interval for the treatment difference is used.

Despite dropping this substantial amount of data, the two submitted studies provide supportive evidence demonstrating the efficacy of the oral contraceptive Beyaz (0.020 mg ethinylestradiol + 3.0 mg drospirenone + 0.451 mg levomefolate calcium) to improve the folate status in women who elect to use an oral contraceptive. There was an increase in RBC folate and plasma folate levels with Beyaz use compared to YAZ.

6. APPENDIX

Table A.1
Study A43598: Red Blood Cell (RBC) Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Per Protocol Population, Using All Sites and Excluding Sites 104 and 108)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value*
Using All Sites					
YAZ + Metafolin	124	986.2	419.7	384.7 (282.4, 487.0)	< 0.0001
YAZ	45	990.0	35.0		
Excluding Sites 104 and 108					
YAZ + Metafolin	122	961.4	436.1	403.4 (311.4, 495.4)	< 0.0001
YAZ	44	987.6	32.7		

Source: Statistical Reviewer's analysis and Table 104, page 718 of 870, and Table 116, page 734 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.
* p-value should be used with caution since more than 56% (161/285) of the YAZ + Metafolin folate data and more than 52% (49/94) of the YAZ RBC folate data were dropped from the analysis.

Table A.2
Study A43598: Red Blood Cell (RBC) Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Full Analysis Population, Using All Sites and Excluding Sites 104 and 108)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value*
Using All Sites					
YAZ + Metafolin	128	985.5	415.2	380.11 (278.8, 481.4)	< 0.0001
YAZ	45	990.0	35.1		
Excluding Sites 104 and 108					
YAZ + Metafolin	126	961.6	431.0	398.3 (307.0, 489.5)	< 0.0001
YAZ	44	987.6	32.8		

Source: Statistical Reviewer's analysis and Table 108, page 723 of 870, and Table 120, page 739 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.
* p-value should be used with caution since more than 55% (157/285) of the YAZ + Metafolin folate data and more than 52% (49/94) of the YAZ RBC folate data were dropped from the analysis.

Table A.3
Study A43598: Red Blood Cell (RBC) Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Full Analysis Population with LOCF, Using All Sites)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value*
Using All Sites					
YAZ + Metafolin	170	968.6	388.7	346.0 (259.0, 433.0)	< 0.0001
YAZ	65	1001.81	42.7		

Source: Statistical Reviewer's analysis and Table 112, page 728 of 870, and Table 124, page 744 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.
* p-value should be used with caution since 40% (115/285) of the YAZ + Metafolin folate data and 31% (29/94) of the YAZ RBC folate data were dropped from the analysis.

Table A.4
Study A43598: Plasma Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Per Protocol Population, Using All Sites and Excluding Sites 104 and 108)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value
Using All Sites					
YAZ + Metafolin	196	60.6	16.0	18.9 (14.0, 23.7)	< 0.0001
YAZ	66	41.7	-2.9		
Excluding Sites 104 and 108					
YAZ + Metafolin	129	41.9	16.2	17.4 (11.5, 23.3)	< 0.0001
YAZ	47	41.2	-1.2		

Source: Statistical Reviewer's analysis and Table 140, page 766 of 870, and Table 152, page 782 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.

Table A.5
Study A43598: Plasma Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Full Analysis Population, Using All Sites and Excluding Sites 104 and 108)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value
Using All Sites					
YAZ + Metafolin	201	45.2	16.2	19.2 (14.4, 24.0)	< 0.0001
YAZ	66	43.2	-2.9		
Excluding Sites 104 and 108					
YAZ + Metafolin	133	42.3	16.2	17.6 (11.7, 23.4)	< 0.0001
YAZ	47	41.2	-1.3		

Source: Statistical Reviewer's analysis and Table 144, page 771 of 870, and Table 156, page 787 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.

Table A.6
Study A43598: Plasma Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Full Analysis Population with LOCF, Excluding Sites 104 and 108)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value
Excluding Sites 104 and 108					
YAZ + Metafolin	168	41.7	17.4	18.3 (13.1, 23.4)	< 0.0001
YAZ	62	38.5	-0.8		

Source: Statistical Reviewer's analysis and Table 148, page 776 of 870, and Table 160, page 792 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.

Table A.7
Study A39814: Kaplan Meier (KM) Estimates and the Proportion of Subjects for the Time to RBC Folate Falling Below 906 nmol/L for the Yasmin + Metafolin Treatment Group in the Folate Elimination Phase (Week 26 to Week 44) - FAS

Week	Number of subjects with RBC folate ≥ 906 nmol/L	KM estimate (Proportion of subjects with RBC folate ≥ 906 nmol/L)
24	77	0.951
26	75	0.926
28	67	0.826
30	62	0.763
32	48	0.588
34	38	0.463
36	25	0.296
38	20	0.231
40	15	0.167
42	12	0.129
44	9	0.090

Source: Table 118, page 763 of 942, Study A39814 report

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22532	ORIG-1	BAYER HEALTHCARE PHARMACEUTICALS INC	YAZ Folate

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SONIA CASTILLO
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MAHBOOB SOBHAN
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Addendum to Statistical Review

NDA Number:	22-532 / Supporting Document 001
Drug Name:	Beyaz (0.020 mg ethinylestradiol + 3.0 mg drospirenone + 0.451 mg levomefolate calcium)
Indication(s):	Improvement in folate status in women who elect to use an oral contraceptive
Applicant:	Bayer HealthCare Pharmaceuticals Inc.
Date(s):	Letter Date: August 21, 2009 PDUFA Date: September 24, 2010
Review Priority:	1 Standard
Biometrics Division:	Division of Biometrics 3
Biometrics Reviewer:	Sonia Castillo, Ph.D.
Biometrics Team Leader:	Mahboob Sobhan, Ph.D.
Medical Division:	Division of Reproductive and Urologic Drug Products
Clinical Team:	Daniel Davis, M.D., Clinical Reviewer Lisa Soule, M.D., Clinical Team Leader
Project Manager:	Pamela Lucarelli
Key Words:	Clinical studies, NDA review

This addendum to the statistical review for this application corrects typographical errors in Table A.4 in the Appendix. The revised table is presented below with the affected values underlined.

Table A.4
Study A43598: Plasma Folate Levels (nmol/L) - Treatment Difference for Change from Baseline at Week 24
(Per Protocol Population, Using All Sites and Excluding Sites 104 and 108)

	n	Baseline	LS Mean[1]	LS Mean Difference[1] (95% C.I.)	p-value
Using All Sites					
YAZ + Metafolin	196	<u>45.0</u>	16.0	18.9 (14.0, 23.7)	< 0.0001
YAZ	66	<u>43.1</u>	-2.9		
Excluding Sites 104 and 108					
YAZ + Metafolin	129	41.9	16.2	17.4 (11.5, 23.3)	< 0.0001
YAZ	47	41.2	-1.2		

Source: Statistical Reviewer's analysis and Table 140, page 766 of 870, and Table 152, page 782 of 870 of Study A43598 report.
[1] Least Squares mean estimates, confidence intervals, and p-values based on an ANCOVA model with treatment as factor and baseline value as covariate.

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