CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022574Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

BIOPHARMACEUTICS REVIEW Office of New Drugs Quality Assessment			
Application No.:	NDAs 22-532 and 22-574	**Reviewer:** Sandra Suarez Sharp, Ph.D	
Division:	DRUP		
Sponsor:	Bayer HealthCare Pharmaceuticals	**Team Leader:** Angelica Dorantes, Ph.D .	
Trade Name:	YAZ and Yasmin fortified	**Supervisor:** Patrick J. Marroum, Ph.D	
Generic Name:	drospirenone+ ethinyl estradiol + levomefolate calcium	**Date Assigned:**	Aug 3, 2010
Indication:	Oral contraceptives	**Date of Review:**	Dec 8, 2010
Formulation	IR tablet		
Route of Administration	Oral		

SUBMISSIONS REVIEWED IN THIS DOCUMENT			
Submission date	**CDER Stamp Date**	**Date of informal/Formal Consult**	**PDUFA DATE (extended)**
Aug 24, 2020	Aug 24, 2010	NA	Dec 2010
Type of Submission:	Addendum to Original NDA reviews		
Type of Consult:	Dissolution method and specifications		

REVIEW SUMMARY:

YAZ® (3 mg of drospirenone and 0.02mg of ethinyl estradiol) and Yasmin (3 mg of drospirenone and 0.03mg of ethinyl estradiol) IR Tablets were approved on 2006 for the prevention of pregnancy in women who elect to use an oral contraceptive.

The sponsor (Bayer Health Pharmaceuticals) is seeking approval of NDA 22-532 and NDA 21-574, a folate fortified, oral contraceptive (OC) regimens which consist on the addition of Metafolin® (levomefolate calcium) to YAZ® and Yasmin®.

The following comments were sent to the sponsor via email regarding the dissolution specifications for both Yasmin + Metafolin and Yaz + Metafolin, respectively (refer to Biopharm reviews for NDAs 22-352 and 22-574 entered in DARRTS on 7/29/10 and 7/7/10, respectively):

1. The following dissolution method and specifications are recommended for Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet 3.0 mg + 0.02 mg + 0.451 mg based on the mean dissolution values from batches used in pivotal clinical trials and drug product stability:

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active ingredient (drospirenone, ethinylestradiol, and levomefolate calcium) is dissolved in 15 min.

2. The following dissolution method and specifications are recommended for Levomefolate calcium coated tablet 0.451 mg based on the mean dissolution values from batches used in pivotal clinical trials and drug product stability:

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active ingredient levomefolate calcium is dissolved in 15 min.

Please revise the dissolution specifications accordingly.

On Aug 10, 2010 the Agency received via email a response to the above comments as follows:

Bayer agrees to change the dissolution specification to Q (b) (4) at 15 minutes for the levomefolate calcium only tablets. However for the drospirenone+ethinylestradiol+levomefolate calcium tablets, we respectfully request that the specification remain Q= (b) (4) at 30 minutes, for the reasons presented below:

1. *Based on the data in section 2.7.1 "Summary of Biopharmaceutic Studies and Associated Analytical Methods", batch-to-batch differences in dissolution rate were observed at 15 minutes that had no impact on the overall bioavailability of the drug substances. These data demonstrate that the 15 minute draw time is overdiscriminatory and that the 30 minute draw time adequately demonstrates the release of the drug in-vivo and ensures its efficiency.*
2. *The dosage form was developed as an immediate release tablet, and not intended to fulfill the criteria for a rapidly dissolving tablet. Therefore we have minimal data at 15 minutes. Based on the data we do have, we believe that we may experience unnecessary stage 2 or 3 testing, or unnecessarily risk batch failures even though there is no impact on the in-vivo performance of the product. We are especially concerned that we only have minimal data regarding the 15 minute draw time during stability studies, and are concerned that setting the specification at 15 minutes without supporting stability data presents a risk of an unnecessary recall.*

Therefore Bayer respectfully requests that the specification for the drospirenone+ethinylestradiol+levomefolate calcium tablets remains Q= (b) (4) at 30 minutes.

Given that Yaz and Yaz + Metafolin were found bioequivalent and Yaz showed a slower dissolution profile with a mean value of (b) (4) dissolved in 30 min, this reviewer agrees with the sponsor's original

proposal for the dissolution specification of drospirenone+ethinylestradiol+levomefolate calcium tablets as Q= (b) (4) at 30 minutes.

On Aug 24, 2010 the sponsor submitted an updated dissolution specification sheet reflecting the above agreed dissolution specifications.

RECOMMENDATION:

The ONDQA/biopharmaceutics team has reviewed submission dated Aug 24, 2010 to NDAs 22-352 and 21-574. We found this NDA acceptable from biopharmaceutics perspective.

Sandra Suarez Sharp, Ph. D.
Biopharmaceutics Reviewer
Office of New Drugs Quality Assessment

Patrick J. Marroum, Ph. D.
Biopharmaceutics Supervisor
Office of New Drugs Quality Assessment

Cc: JDavid, ADorantes, Dchristner, HShroff

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

/s/

SANDRA SUAREZ
12/08/2010

PATRICK J MARROUM
12/08/2010

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 022574	Submission Dates under NDA 022574: 11/16/2009, 8/27/2010, 9/3/2010 Submission Dates under NDA 022532: 4/16/2010
Brand Name	Safyral
Generic Name	Drospirenone, ethinyl estradiol, and levomefolate calcium
Clinical Pharmacology Reviewer	Doanh Tran, Ph.D.
Clinical Pharmacology Team Leader	Myong-Jin Kim, Pharm.D.
OCP Division	Division of Clinical Pharmacology 3
OND Division	Division of Reproductive and Urologic Products
Sponsor	Bayer
Submission Type, Code	Original, 1
Formulation; Strength(s)	Immediate release oral tablet containing either 1) drospirenone 3 mg, ethinyl estradiol 0.03 mg, and levomefolate calcium 0.451 mg or 2) levomefolate calcium 0.451 mg only
Related IND/NDA	NDA 022532, IND 72,287
Indications	• Prevention of pregnancy • To raise folate levels for purpose of reducing risk of a neural tube defect

Table of Contents

1 Executive Summary

Safyral consists of 21 hormone-containing tablets (hereafter referred to as Safyral hormone) each containing 3 mg of drospirenone (DRSP), 0.03 mg of ethinyl estradiol (EE) and 0.451 mg of levomefolate calcium (Metafolin), and 7 tablets containing 0.451 mg of Metafolin only (Metafolin mono). Metafolin is a crystalline form of the calcium salt of L-5-methyltetrahydrofolate (L-5-MTHF). The dosage of Safyral is one hormone-containing tablet daily for 21 consecutive days followed by one Metafolin mono tablet daily for 7 days per treatment cycle.

There are 2 proposed indications for Safyral:

1. Prevention of pregnancy
2. To raise folate levels for purpose of reducing risk of a neural tube defect

The first indication is the same as in approved product Yasmin (NDA 021098), and is supported by a bioequivalence (BE) study (study 309662) comparing the pharmacokinetics (PK) of Safyral to Yasmin with respect to the DRSP and EE components. Yasmin is an approved product containing 21 tablets each containing 3 mg of DRSP and 0.03 mg of EE (hereafter referred to as Yasmin hormone), and 7 inert tablets. The difference between Safyral and Yasmin is the addition of 0.451 mg of Metafolin to each Safyral tablet.

The second indication, to raise folate levels for purpose of reducing risk of a neural tube defect, is the same indication as approved for Beyaz (NDA 022532). It is supported by 2 pharmacodynamic (PD) studies (studies 309763 and 310662). Study 309763 evaluated the increased red blood cell (RBC) and plasma folate concentrations following daily administration of Safyral or a combination of a Yasmin tablet and a folic acid 0.4 mg tablet for 24 weeks. Study 310662 also evaluated the folate concentrations but subjects were administered either a Beyaz tablet or a placebo tablet daily for 24 weeks. Beyaz consists of 24 hormone containing tablets (hereafter referred to as Beyaz hormone) each containing 3 mg of DRSP, 0.02 mg of EE and 0.451 mg of Metafolin, and 4 tablets containing 0.451 mg of Metafolin only.

In addition to these studies, the NDA includes a second BE study (study 309664), which provided additional PK information for the Metafolin mono tablet (this study was reviewed in NDA 022532). A summary of literature references related to absorption, distribution, metabolism, and excretion (ADME) properties and drug interactions was also provided in this NDA.

The Clinical Pharmacology review focused mainly on the BE studies and summary of ADME information. The 2 PD studies in support of the indication to raise folate levels for purpose of reducing risk of a neural tube defect are reviewed by the Clinical team.

1.1 Recommendation

The Division of Clinical Pharmacology 3/Office of Clinical Pharmacology finds NDA 022574 Acceptable from a Clinical Pharmacology perspective.

1.2 Phase IV Commitments and Requirements

None.

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Safyral differs from the approved Yasmin product by the addition of 0.451 mg Metafolin to each tablet (both hormone-containing and non-hormone-containing tablets). The acid form of metafolin is L-5-MTHF, the most common form of folate in circulation. The addition of metafolin is to provide folate

supplementation. The sponsor is seeking two indications, 1) prevention of pregnancy and 2) to raise folate levels for purpose of reducing risk of a neural tube defect.

The dose of 0.451 mg Metafolin is equimolar to 0.4 mg folic acid, a dose commonly formulated in vitamin supplements.

Pharmacokinetics:
Table 1 shows the mean EE and DRSP PK parameter values following single dose administration of a Safyral hormone tablet.

Table 1: Arithmetic mean (±SD) PK parameters following single dose of Safyral hormone

Drug substance	Parameter				
	C_{max} (pg/mL* or ng/mL**)	T_{max}*** (h)	$T_{1/2}$ (h)	AUC(0-inf) (pg*h/mL* or ng*h/mL**)	AUC(0-tlast) (pg*h/mL* or ng*h/mL**)
EE	63.8 ± 17.9	2 (1 – 4)	12.5 ± 3.3	777 ± 236	620 ± 179
DRSP	28.1 ± 7.0	1.5 (1 – 3)	32.2 ± 7.2	444 ± 105	413 ± 102
* = unit for EE, ** = unit for DRSP, *** = Median (range)					

Following single dose administration of a Safyral hormone tablet, serum L-5-MTHF concentration reached a baseline-corrected mean (SD) C_{max} of 53.9 (15.4) nmol/L. The median T_{max} was 0.5 hours (range 0.5 – 1.5 hours). Mean AUC(0-tlast) was 244 (63) nmol/L*hour. The apparent $t_{1/2}$ was 4.33 (0.55) hours. Similar PK parameter values were obtained following single dose administration of Metafolin mono tablet.

Distribution, metabolism, and excretion properties of Metafolin:
The sponsor did not conduct any studies with Safyral to address distribution, metabolism and excretion of metafolin. The sponsor surveyed the literature and the following pertinent information was gathered:

There is extensive first pass hepatic metabolism following oral folate absorption. Some of the folate up take by hepatocytes is excreted into the bile which can then be reabsorbed via enterohepatic recirculation. L-5-MTHF has low protein binding in serum (~35% protein bound). It is primarily bound to alpha2-macroglobulin and albumin. Folate kinetics is reported to be biphasic with a fast- and a slow-turnover pool. The fast-turnover pool has a half-life of hours (reported range from 9.9 – 31.5 hours) which probably reflects newly absorbed folate. The slow-turnover pool reflects turnover of folate polyglutamate and has a half life of days (reported range from 9.6 to more than 100 days).

The elimination of folates from the body occurs by urinary excretion of intact folates and catabolic products and fecal excretion.

Intrinsic factors:
Limited data from the literature suggests that renal impairment may increase the exposure of L-5-MTHF. No information is available on the effects of hepatic impairment or other intrinsic factors on the PK of L-5-MTHF.

No information was provided for DRSP and EE. The sponsor relied entirely on approved label for Yasmin.

Extrinsic factors:

Reports in the literature indicate that several drugs can reduce folate concentrations. The suggested mechanisms include: inhibition of the human dihydrofolate reductase (e.g. methotrexate and sulfasalazine), reduction of folate absorption (e.g. cholestyramine), and unknown mechanisms (e.g. antiepileptics such as carbamazepine, phenytoin, and valproic acid). The effects of these drugs on the bioavailability of Metafolin are not known.

Literature reports indicate that folates may alter the PK or PD of certain antifolate drugs such as phenytoin, methotrexate, and pyrimethamine.

No information was provided for DRSP and EE. The sponsor relied entirely on approved label for Yasmin.

Pharmacogenomics:
There does not appear to be significant relationship between the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677 C>T variant and folate responses to Metafolin administration.

Bioequivalence of DRSP and EE in Safyral and Yasmin:
The sponsor conducted a single dose BE study to compare the bioavailability of Safyral hormone tablets (test) to Yasmin hormone tablets (reference) under a fasting state. The results showed that the 90% CIs for test/reference ratio for DRSP and EE C_{max} and AUC were within the 80 – 125% BE limits indicating that the 2 formulations are bioequivalent with respect to DRSP and EE.

Formulation:
Both Safyral hormone and Metafolin mono tablets were formulated as immediate release tablets. The Safyral hormone and Metafolin mono formulations used in BE study 309662 and PD study 309763 were the same as the proposed to-be-marketed formulation. Throughout the clinical development program for Safyral, the tablet composition and manufacturing processes were not changed.

Bioanalytical methods:
Assays for BE studies:
Plasma DRSP, plasma EE, and serum L-5-MTHF concentrations from BE studies 309662 and 309664 were measured using validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methods. Inspection of the bioanalytical site for study 309662 revealed issues with robustness of the lower limit of quantitation (LLOQ) for DRSP and EE and inconsistency in deciding when a reassay was conducted. The Sponsor was requested to raise the LLOQ for DRSP and EE assays and to revise results for 3 samples based on agreed reassay procedure. The Sponsor complied and the revised dataset was considered acceptable.

Folate assay for PD studies:
Total folate in plasma and whole blood concentrations from PD studies 309763 (Safyral compared to Yasmin and 0.4 mg/day folic acid) and 310662 (Beyaz compared to Yaz) were measured using a microbiological assay with Lactobacillus casei. The assay had a calibration range of 25 – 500 ng/ml (56.64 – 1132.76 nmol/L) in blood and 1 – 20 ng/mL (2.27 – 45.31 nmol/L) in plasma. The validation report stated that the assay was not robust. Many plates were rejected due to calibration and/or quality control (QC) samples not meeting acceptance criteria. In some runs the calibration curve flattens at higher concentration and causing high bias for the High QC samples.

QC at LLOQ did not meet the ±20% criteria for accuracy and precisions. The bias in accuracy at LLOQ was -21.8% in whole blood and -23.3% in plasma. Intra run imprecision at LLOQ was 19.4% in whole blood and 22.7% in plasma. Inter run imprecision at LLOQ was 27.6% in whole blood and 36.3% in plasma. Accuracy and precision criteria were met at other QC levels including QC Low (i.e., 75 ng/mL

for whole blood and 3 ng/mL for plasma). Therefore, the assay had an "effective LLOQ" of 75 ng/mL in whole blood and 3 ng/mL in plasma. All QC samples used during bioanalysis of studies 309763 and 310662 were prepared in 5% BSA. Review of additional data from spiked plasma and blood samples by Division of Scientific Investigation (DSI) indicated that there was sufficient accuracy at the respective "effective LLOQ" levels.

Metabolite assay:
Concentrations of folic acid, tetrahydrofolate (THF), 5-methyl-THF, 5-formyl-THF, and 5,10-methyl-THF from study 309763 were measured using a semi-quantitative LC/MS assay. The assay was based on acceptance criteria of 50% for accuracy and precision at LLOQ (40% at other QC levels). The calibration range was 0.5 – 150 ng/mL. The QC levels (LLOQ, QC Low, QC Medium, and QC High) were at 0.5, 1.5, 20, and 100 ng/mL for 5-methyl-THF, and 0.5, 1.5, 5, and 10 ng/mL for the remaining analytes.

Available data supported the stability of folic acid and L-5-MTHF; bioanalytical results for these 2 moieties could be reviewed (taking into account the semi-quantitative nature of the assay). Long term and short term stability of 5-FTHF, 5,10-MTHF, and THF were not established. Available data suggests that 5,10-MTHF and THF are not stable following a freeze thaw cycle. Therefore, bioanalytical results for 5-FTHF, 5,10-MTHF, and THF are not considered reliable.

Briefing: An Optional Intra-Division Level Office of Clinical Pharmacology Briefing was held on October 25, 2010 with the following in attendance: Doanh Tran, Myong Jin Kim, E. Dennis Bashaw.

2 Question Based Review

2.1 General Attributes

2.1.1 What is Safyral? What are the proposed indications for Safyral?

Safyral consists of 21 tablets each containing 3 mg of DRSP, 0.03 mg of EE and 0.451 mg of Metafolin, and 7 tablets containing 0.451 mg of Metafolin only. Both Safyral hormone and Metafolin mono tablets are formulated as immediate release tablets. The dosage of Safyral is one hormone-containing tablet daily for 21 consecutive days followed by one Metafolin mono tablet daily for 7 days per treatment cycle.

The proposed indications for this product are as follows:

1. Prevention of pregnancy
2. To raise folate levels for purpose of reducing risk of a neural tube defect

The first indication is the same as in approved product Yasmin (NDA 021098), and is supported by a BE study comparing the PK of Safyral to Yasmin with respect to the DRSP and EE components.

The second indication is supported by 2 PD studies showing increased RBC and plasma folate due to administration of Metafolin. Metafolin is a crystalline form of the calcium salt of L-5-MTHF. L-5-MTHF is the predominant form of dietary folate and is the only species normally found in the circulation.

2.1.2 What Clinical Pharmacology data were provided in this NDA?

Review notes: *Most of the Clinical Pharmacology data for the current NDA are cross-referenced to data submitted to NDA 022532. When this reviewer refers to data submitted to the current NDA, it should be interpreted as data submitted either directly to this NDA or via cross reference to NDA 022532. For topics previously reviewed in NDA 022532, refer to Clinical Pharmacology review of NDA 022532 for details (DARRTS, date 7/29/2010).*

The NDA is supported mainly by BE study 309662 and 2 PD studies (studies 309763 and 310662). The endpoints for the PD studies were plasma folate, RBC folate, and plasma homocysteine concentrations. A second BE study (study 309664) provided additional PK information for the Metafolin mono tablet. Additional supporting data included a PK/PD population analysis using data from the above 4 studies, a relative bioavailability analysis of a literature report comparing L-5-MTHF and folic acid [Langenohl et al. 2003], and literature references related to ADME properties, drug interaction, and PD of levomefolate calcium.

2.2 General Clinical Pharmacology

Safyral contains Metafolin and the same dose of active ingredients in the approved product Yasmin, namely DRSP and EE. The safety and efficacy of DRSP and EE in Safyral is supported primarily by a BE study bridging to Yasmin. No additional clinical pharmacology information with respect to DRSP and EE was submitted in this NDA. Therefore, the Clinical Pharmacology review is mainly focused on the new active ingredient Metafolin, in addition to the BE assessment for DRSP and EE. For additional clinical pharmacology information on DRSP and EE, refer to product label for Yasmin and clinical pharmacology reviews of Yasmin NDA (NDA 021098).

2.2.1 What are the pharmacokinetic properties of the Safyral hormone tablet? Are Safyral hormone tablets BE to Yasmin hormone tablets with respect to DRSP and EE?

Bioavailability of Safyral hormone tablets was evaluated in single dose, crossover BE study 309662. This study evaluated plasma DRSP and EE concentrations and serum L-5-MTHF concentrations following a single oral dose of Safyral hormone or Metafolin mono tablet in 45 healthy young females. This study also provided BE bridging information for EE and DRSP between Yasmin hormone and Safyral hormone tablets. Each Yasmin hormone tablet contains 3 mg DRSP and 0.03 mg EE.

EE:

For Safyral hormone tablet, the mean (±SD) EE C_{max} was 63.8 ± 17.0 pg/mL and the median Tmax was reached 2 hours (range: 1 to 4 hours) after administration. Mean value for AUC(0–tlast) was 620 ± 179 pg*h/mL. The AUC(0-inf) and $t_{1/2}$ were 777 ± 236 pg*h/mL and 12.5 ± 3.3 hours respectively (Table 2).

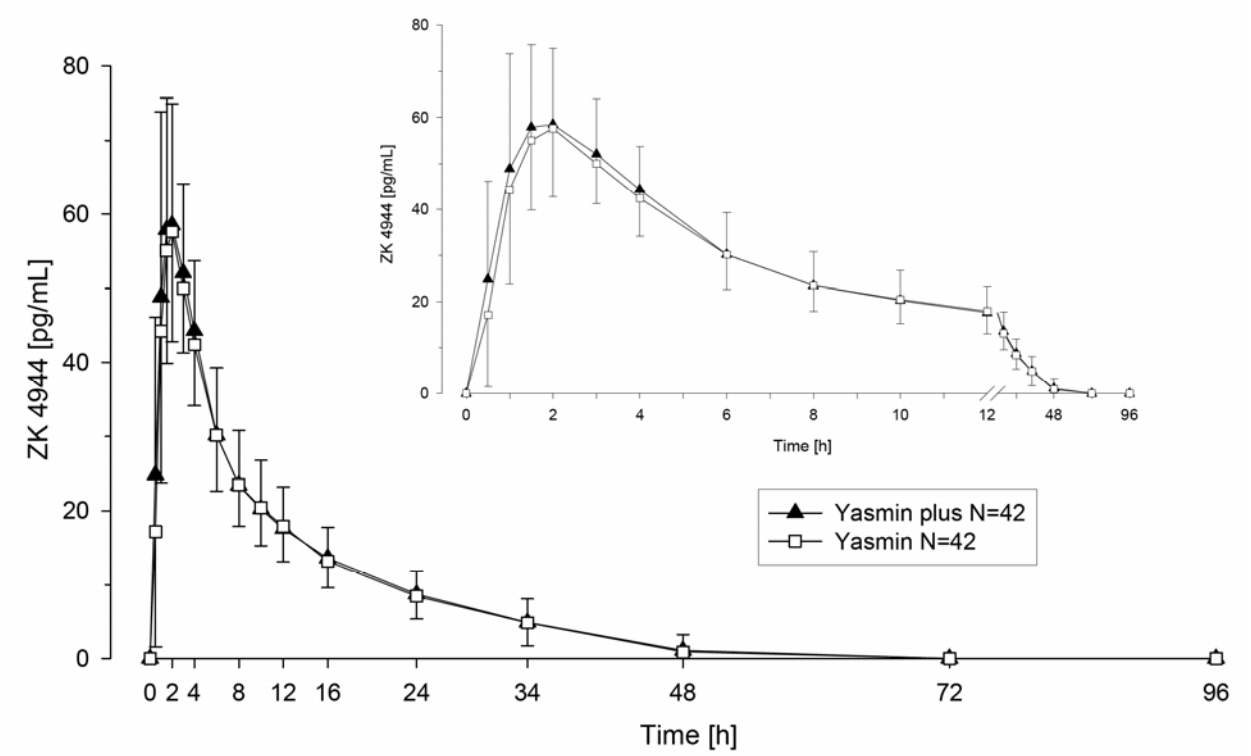
The mean PK profiles for Safyral hormone and Yasmin hormone tablets were similar (figure 1). BE analysis results (Table 3) showed that the 90% CI of Safyral/Yasmin ratio for EE C_{max} and AUC(0-tlast) were within the 80 – 125% limits, indicating that Safyral and Yasmin were BE with respect to EE.

Table 2: Arithmetic mean (± SD) pharmacokinetic parameters of EE

Treatment	Parameter				
	C_{max} (pg/mL)	T_{max} * (h)	$T_{1/2}$ (h)	AUC(0-inf) (pg*h/ml)	AUC(0-tlast) (pg*h/ml)
Safyral (N=42)	63.8 ± 17.9	2 (1 – 4)	12.5 ± 3.3 (N=17)**	777 ± 236 (N=17)**	620 ± 179
Yasmin (N=42)	61.2 ± 18.8	2 (0.5 – 4)	12.3 ± 3.0 (N=21)**	747 ± 212 (N=21)**	601 ± 184

* = Median (range), ** = There were insufficient data to calculate $T_{1/2}$ and hence AUC (0-inf) in some subjects; parameter values were based on evaluable subjects.

Figure 1: Mean (SD) plasma concentrations of EE (ZK4944)



Yasmin plus = Safyral, ZK 4944 = EE

Table 3: Bioequivalence assessment for EE

EE	N	Yasmin (reference)	Safyral (test)	Test/reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [pg*h/ml]	41	607.7	613.3	101.1	97.7, 104.8
Cmax [pg/mL]	41	62.1	63.2	102.3	96.7, 108.2

DRSP:

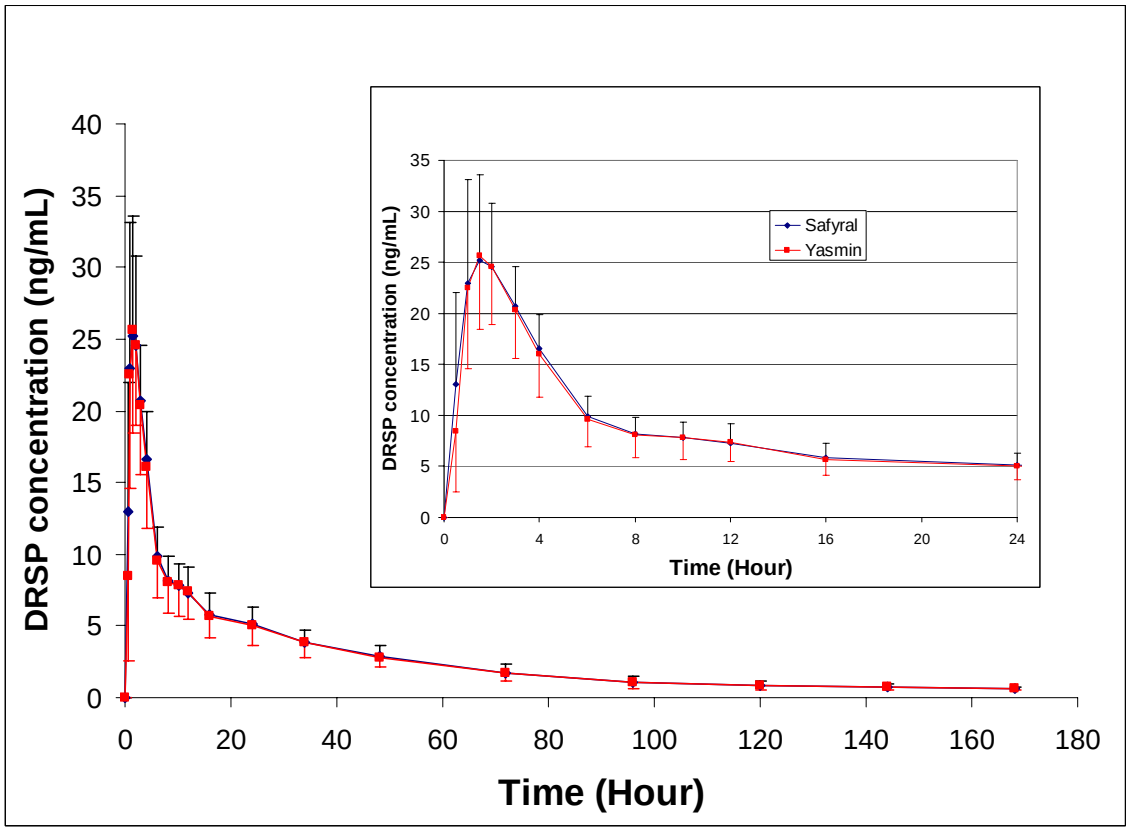
During bioanalysis of DRSP samples, the sponsor discovered that some samples had matrix interference in the chromatogram signal that equated to a maximum of 1 ng/mL DRSP. This was attributed to the type of tubes used for sample storage. There was matrix interference in the bioanalysis of DRSP for 19 subjects (affecting a total of 23 PK profiles). The sponsor remedied this by excluding samples from subjects with matrix interference observed in the chromatogram and the concentration of DRSP was calculated to be <5 ng/mL. However, this matrix interference is expected to be present in samples with calculated concentration ≥5 ng/mL, even though it may not be detectable. In contrast to the sponsor's analysis, this reviewer considered data in subjects where no matrix interference was identified in any sample in the same treatment period. Table 4 shows the PK parameters in this population. These results are similar to those using the sponsor's method (See Appendix 4.1 for further discussion of matrix interference and for calculated PK parameters based on the sponsor's method).

For Safyral hormone tablet, the mean (±SD) DRSP C_{max} was 28.1 ± 7.0 ng/mL and the median Tmax was reached 1.5 hours (range: 0.5 to 3 hours) after administration. Mean values for AUC(0–tlast) was 420 ± 96 ng*h/mL. The AUC(0-inf) and $t_{1/2}$ were 452 ± 100 ng*h/mL and 32.1 ± 8.1 hours, respectively (table 4). The mean DRSP PK profiles for Safyral hormone and Yasmin hormone tablets were similar (figure 2).

Table 4: Arithmetic mean (± SD) pharmacokinetic parameters of DRSP (Only subjects with no matrix interference)

Treatment	Parameter				
	C_{max} (ng/mL)	T_{max}* (h)	$T_{1/2}$ (h)	AUC(0-inf) (ng*h/ml)	AUC(0-tlast) (ng*h/ml)
Safyral (N=30)	28.1 ± 7.0	1.5 (0.5 – 3)	32.1 ± 8.1	452 ± 100	420 ± 96
Yasmin (N=28)	27.3 ± 6.8	1.5 (1 – 3)	32.2 ± 7.2	444 ± 105	413 ± 102
* = Median (range)					

Figure 2: Mean (SD) plasma concentrations of DRSP (Only subjects with no matrix interference)



BE analysis for DRSP:

Because matrix interference was identified in some samples, the sponsor performed analysis of bioequivalence using 4 different sets of subjects in order to assess the impact of analytical interferences. The following sets of subjects were analyzed:

1. All subjects, regardless whether interferences were present or not
2. Subjects without interference in any treatment
3. Subjects without interference or interference in only 1 treatment
4. Subjects without interference or interference in both treatments

This reviewer considered set #2 (only subjects without interference in any treatment) to be the primary data set for BE consideration. This set does not include data from samples with interference and include only those subjects with data from both test and reference treatments, which is consistent with the original

statistical analysis plan. The BE results are shown below for each data set (Tables 5 – 8). In all cases the 90% CI were within the 80 – 125% limits. Safyral is bioequivalent to Yasmin with respect to DRSP.

Table 5: BE results – set #1, all subjects

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	39	455.24	453.85	99.45	96.70, 102.28
Cmax [ng/mL]	39	27.78	27.48	98.66	93.37, 104.24

Table 6: BE results – set #2, only subjects without interference in any treatment.

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	21	458.09	459.19	100.20	96.02, 104.57
Cmax [ng/mL]	21	27.79	27.39	97.82	89.94, 106.39

Table 7: BE results – set #3, subjects without interference or interference in only 1 treatment

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	35	455.09	455.46	100.04	96.61, 103.59
Cmax [ng/mL]	35	28.12	27.60	98.01	91.96, 104.46

Table 8: BE results – set #4, subjects without interference or interference in both treatments

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	25	457.84	456.08	99.53	95.56, 103.66
Cmax [ng/mL]	25	27.32	27.24	98.80	90.24, 108.16

L-5-MTHF:

Table 9 shows the PK parameters for serum L-5-MTHF following single dose administration of Safyral hormone and Metafolin mono tablets. Safyral hormone showed a median T_{max} of 0.5 hours for L-5-MTHF. The apparent (baseline corrected) $t_{1/2}$ was 4.33 hours. Figure 3 shows the baseline-uncorrected mean serum L-5-MTHF PK profiles. The figure includes mean profile following treatment with Yasmin, which represents the profile without administration of metafolin.

Baseline correction was performed by subtracting the value of predose measurement (at -30 minutes) from each observed concentration. Individual L-5-MTHF concentration time profiles in Yasmin treated period (i.e., no Metafolin) generally showed a flat pattern, indicating that a single predose sample was adequate to capture baseline L-5-MTHF concentration.

Table 9: Arithmetic mean (±SD) L-5-MTHF PK parameters (Study 309662)

Baseline method	Treatment	C_{max} (nmol/L)	AUC(0-tlast) (nmol/L*h)	T_{max}^{*} (h)	$T_{1/2}$ (h)
Baseline - uncorrected	Safyral (n=41)	68.0 ± 19.6	413 ± 129	0.5 (0.5 – 1.5)	NC

	Metafolin mono (n-43)	64.3 ± 17.8	411 ± 140	0.5 (0.5 – 1.5)	NC
Baseline - corrected	Safyral (n=41)	53.9 ± 15.4	244 ± 62.9	0.5 (0.5 – 1.5)	4.33 ± 0.554 (n=11)**
	Metafolin mono (n=43)	50.7 ± 13.8	247 ± 63.7	0.5 (0.5 – 1.5)	4.92 ± 0.352 (n=9)**
* T_{max} is shown as median and range. ** = There were insufficient data to calculate $T_{1/2}$ in some subjects; parameter values were based on evaluable subjects. NC Not calculated					

Figure 3: Mean serum concentration (±SD) of baseline-uncorrected L-5-MTHF (ZK270898)

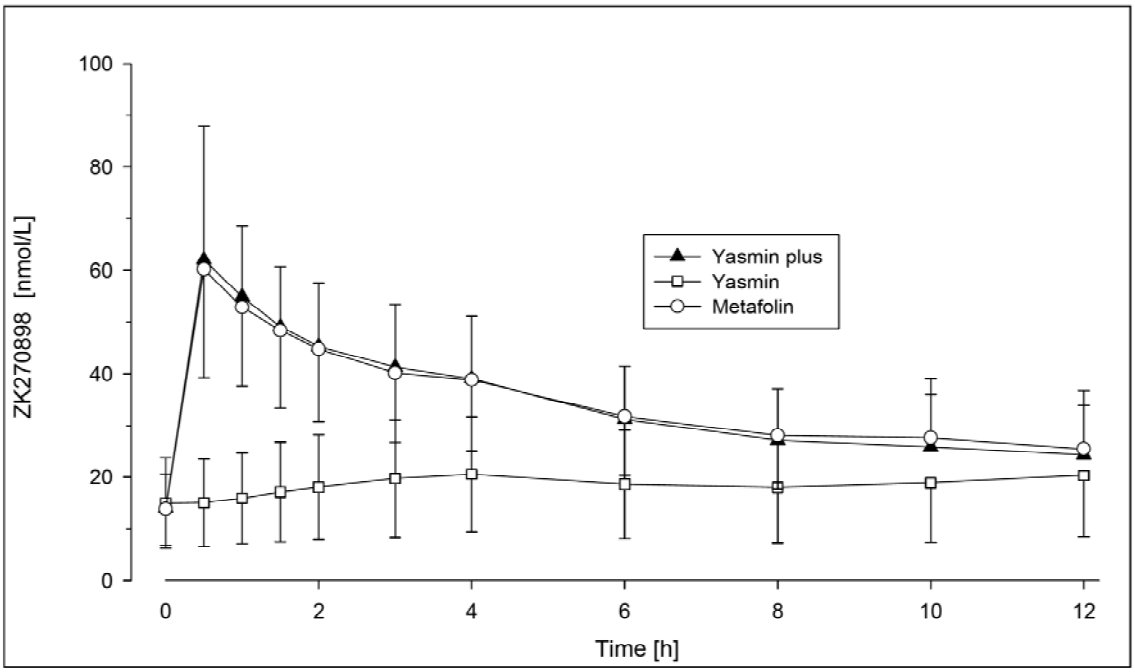


Figure note: Yasmin plus = Safyral; Yasmin does not contain Metafolin and L-5-MTHF profile for Yasmin represents endogenous concentrations.

2.2.2 What are the pharmacokinetic properties of Metafolin mono tablet?

As discussed in section 2.2.1, BE study 309662 included administration of Metafolin mono tablet in one treatment period. The PK parameters and profile of L-5-MTHF following single dose administration of Metafolin mono tablet are shown in Table 9 and Figure 3, respectively. The results showed similar PK properties for Metafolin mono tablet and Safyral hormone tablet. The Sponsor performed BE analyses comparing baseline-corrected and baseline-uncorrected L-5-MTHF between Safyral hormone and Metafolin mono tablets (Tables 10 and 11). These BE comparisons were not required from a regulatory perspective. Nevertheless, the results showed that the 90% CIs for the ratios of geometric means for C_{max} and AUC were within the 80 – 125% range, indicating the both the Safyral hormone and Metafolin mono tablets provided the same L-5-MTHF bioavailability.

The sponsor asserted that these results also indicate that there is no metabolic interaction between DRSP/EE and L-5-MTHF. Since the formulation for Safyral hormone and Metafolin only tablets are similar (see section 2.5.2), this reviewer agrees that there is likely no metabolic interaction present following single dose administration. However, potential confounding effects of the different formulations could not be completely ruled out. It should also be noted that this was a single dose study

and may not capture the maximum interaction potential between DRSP/EE and L-5-MTHF. The product label for Yasmin indicates that upon multiple dose administration, serum C_{max} and AUC accumulated by about 2 – 3 fold for DRSP and about 1.5 – 2 fold for EE.

Table 10: Bioequivalence assessment for L-5-MTHF (baseline-corrected, PPS)

L-5-methyl-THF	N	Metafolin (reference)	Safyral (test)	Relative bioavailability (test / reference) in %	Two-sided 90% confidence interval in %
AUC(0–tlast) [nmol*h/l]	41	241.5	236.7	98.0	(94.2, 101.9)
Cmax [nmol/l]	41	48.9	51.9	106.2	(99.2, 113.7)

Table 11: Bioequivalence assessment for L-5-MTHF (baseline-uncorrected, PPS)

L-5-methyl-THF	N	Metafolin (reference)	Safyral (test)	Relative bioavailability (test / reference) in %	Two-sided 90% confidence interval in %
AUC(0–tlast) [nmol*h/l]	41	396.0	394.5	99.6	(95.7, 103.7)
Cmax [nmol/l]	41	62.3	65.4	104.9	(98.8, 111.3)

2.2.3 What is the relative bioavailability of Metafolin and folic acid?

It is difficult to provide a conclusive answer to this question because there are many formulations of folic acid available as over-the-counter and they may potentially have different absolute bioavailability due to differences in formulation performance. Nevertheless, available data suggest that administration of an equimolar dose of Metafolin would yield similar or higher folate exposure compared to administration of folic acid. PD study (study 309763) administered either Safyral combination tablet or Yasmin and a 0.4 mg folic acid tablet provided a relative comparison of the degree of folate supplementation from the 2 specific formulations evaluated. The results indicated that the steady state plasma and RBC folate concentrations were similar in women taking Safyral or Yasmin + 0.4 mg folic acid. Additionally, a study in the literature [Prinz-Hangenohl et al., 2003] evaluated the relative bioavailability of single doses of 0.416 mg L-5-MTHF (906 nmol, equivalent to 0.451 mg Metafolin) or 0.400 mg folic acid, with or without folic acid preload (1 mg/day for 10 days), in 21 healthy female volunteers. The authors reported that the bioavailability of L-5-MTHF (baseline corrected AUC) was equivalent (with folic acid preload) or higher (without folic acid preload [AUC ratio 156%]) than an equimolar dose of folic acid (i.e., 0.400 mg). It should be noted that the folic acid formulations used in these studies may have different bioavailability compared to those used in studies relating to prevention of neural tube defects.

2.2.4 Does administration of Safyral lead to increase in plasma and RBC folate concentration?

The effect of daily administration of 0.451 mg levomefolate calcium for 24 weeks on plasma folate, RBC folate, and plasma homocysteine concentrations were compared to no folate administration in study 310662 (Beyaz vs. Yaz) and to 0.4 mg/day folic acid in study 309763 (Safyral vs. Yasmin and 0.4 mg/day folic acid). The details of these studies are reviewed and discussed in the Clinical review by Dr. Daniel Davis.

The results (as reported by sponsor) indicated that daily administration of 0.451 mg Metafolin increased plasma and RBC folate concentrations. The geometric mean plasma and RBC folate concentrations after once daily administration of Safyral for 24 weeks were 47.8 nmol/L and 1324 nmol/L, respectively. The geometric mean plasma and RBC folate concentrations after once daily administration of Beyaz for 24 weeks were 57.5 nmol/L and 1346 nmol/L, respectively.

The increases in plasma and RBC folate concentrations were observed regardless of whether women were from area with or without a food folate fortification program. However, women from areas with a food folate fortification program had higher baseline folate and a smaller net increase due to Metafolin administration. For example, larger increase was observed in study 309763 (mean increase of 30.5 nmol/L [95% CI 27.4, 33.9 nmol/L] at 24 weeks from a baseline of 15.0 [95% CI 13.6, 16.4]) which was conducted in Germany, where there is no food fortification program. Study 310662, conducted in the US where there is a food fortification program, showed a mean baseline plasma folate concentration of 41.3 nmol/L [95% CI 38.8, 43.9 nmol/L] and a smaller net increase of 16.0 nmol/L [95% CI 13.8, 18.6 nmol/L] at 24 weeks. Similar observations were made for RBC folate concentrations.

The sponsor also provided a population PK/PD report evaluating the kinetics of folate in plasma and RBCs. The PK/PD modeling did not provide additional insights beyond what was already apparent. Due to long runtime, the sponsor did not evaluate effects of covariates. Differences in assays used for PK and PD studies also presented additional challenge in interpreting the modeling results. Therefore, PK/PD results were not reviewed in details.

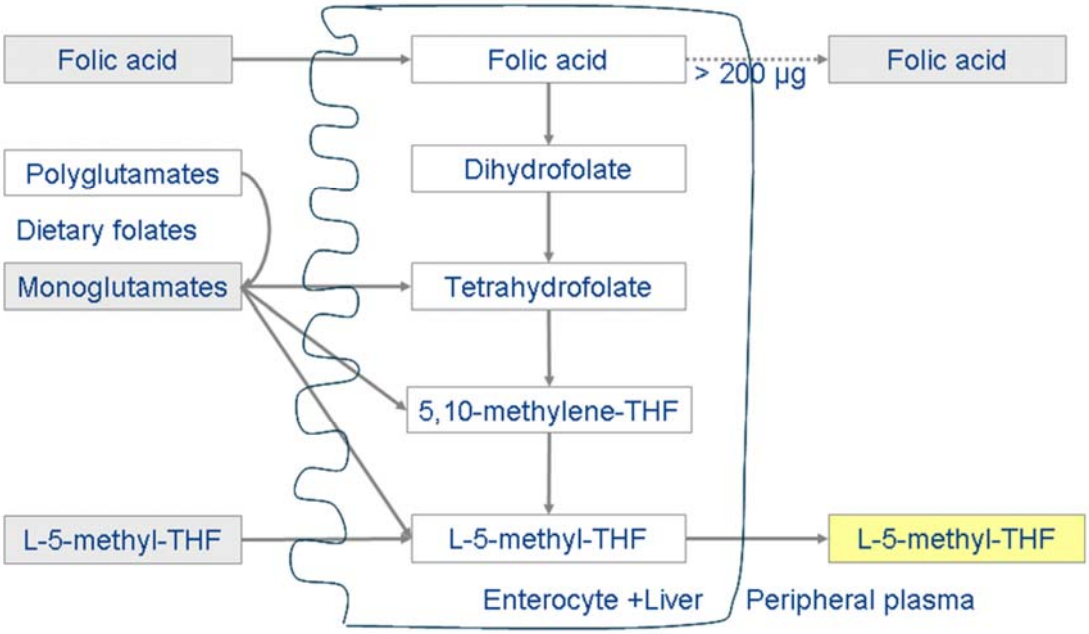
2.2.5 What are the ADME properties of Metafolin?

Other than BE study 309662, which provided information on the absorption of L-5-MTHF from the Safyral formulation, the sponsor did not conduct any other studies relating to ADME properties of L-5-MTHF from Safyral. The sponsor surveyed the literature and provided a summary of ADME information on folate in general, which in many cases are applicable to L-5-MTHF. This reviewer reviewed the sponsor's summary and associated references when needed. Several review articles/book chapters were relied upon for general information on folate. The following is a summary of relevant ADME properties.

Absorption:

Absorption of folates is a saturable, pH-dependent process that occurs throughout the length of the small intestine, but absorption is most efficient in the proximal small intestine [Steinberg 1984]. Polyglutamates from food are deconjugated by glutamate carboxypeptidase II into monoglutamates prior to absorption in the small intestine. However, metabolism is not required for transport across the intestine. Following administration of folic acid, particularly at high doses, unmetabolized folic acid could be detected in the systemic circulation. L-5-MTHF is absorbed unchanged. 5-MTHF is the most common form of folates circulated in humans.

Figure 4: schematic of folates absorption



After oral administration of Metafolin (from Safyral tablets), absorption was rapid with the median T_{max} for L-5-MTHF of 0.5 hour (range 0.5 to 1.5 hour). Mean serum L-5-MTHF C_{max} of about 50 nmol/L above baseline was observed following single administration of 0.451 mg Metafolin (based on mean serum L-5-MTHF C_{max} data from studies 309662 and 309664 as follows: Safyral: 53.9 ± 15.4 nmol/L, Metafolin mono from study 309662: 50.7 ± 13.8 nmol/L, Beyaz: 46.5 ± 14.3 nmol/L, Metafolin mono from study 309664: 47.5 ± 18.9 nmol/L).

Distribution:
There is extensive first pass hepatic metabolism following oral folate absorption [Rogers et al. 1997]. Some of the folate up take by hepatocytes is excreted into the bile which can then be reabsorbed via enterohepatic recirculation.

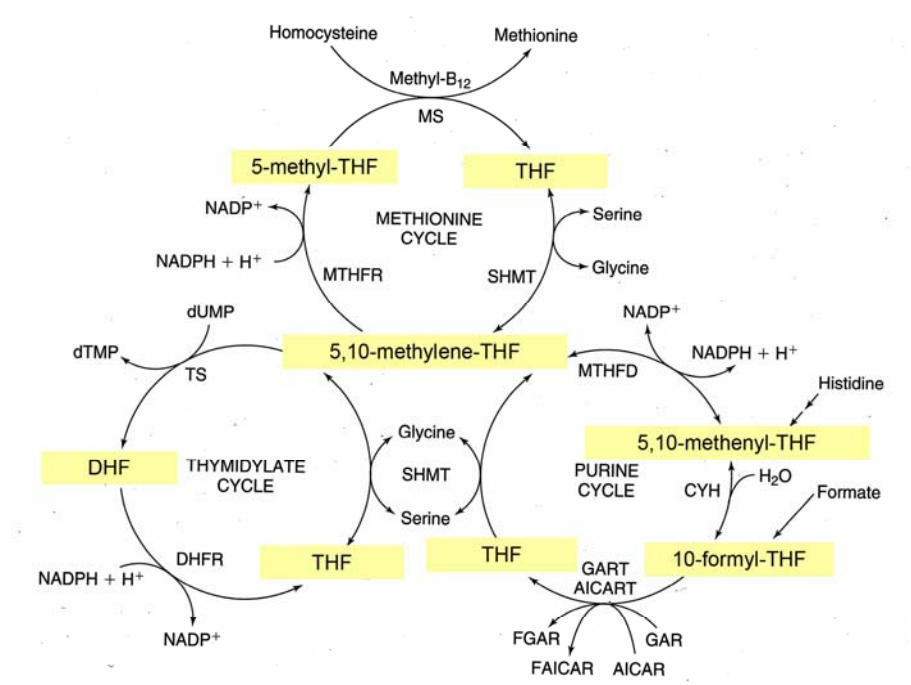
L-5-MTHF has low protein binding in serum (~35% protein bound based on measurement of folic acid activity in donor serum [Markkanen et al. 1972]). It is primarily bound to alpha2-macroglobulin and albumin.

Folate kinetics is reported to be biphasic with a fast- and a slow-turnover pool. The fast-turnover pool has a half-life of hours (reported range from 9.9 – 31.5 hours [von der Porten et al. 1992]) which probably reflects newly absorbed folate. This is in line with the apparent terminal half-life of approximately 4 - 5 hours after single oral administration of 0.451 mg Metafolin. The slow-turnover pool reflects turnover of folate polyglutamate and has a half life of days (reported range from 9.6 to 19 days [von der Porten et al. 1992]) to more than 100 days [Krumdieck et al. 1978]).

L-5-MTHF is the predominant folate in the circulation and therefore likely the folate form normally transported into peripheral tissues to be used for cellular folate metabolism. There are three physiological mechanisms for the transport and the uptake of folates by various cell types: two carrier-mediated, active transport mechanisms (the reduced folate carrier and the folate receptor), and passive diffusion [Sirotnak and Tolner 1999, Henderson et al. 1995].

Metabolism:
Accumulation of intracellular folates requires the conversion of monoglutamates into polyglutamate forms by the enzyme folylpolyglutamate synthetase (FPGS). L-5-MTHF is a poor substrate for FPGS [Cichowicz and Shane 1987] suggesting that the incorporation of L-5-MTHF into the cellular folate metabolism is preceded by the conversion to THF via the methionine synthase reaction before effective polyglutamylation and tissue retention is achieved. Folate coenzymes are involved in 3 major interrelated metabolic cycles in the cytosol of cells.

Figure 5: Folate mediated metabolism (adapted from Shane 2006 by Sponsor)



Abbreviations: AICAR, aminomidazole carboxamide ribonucleotide; AICART, AICAR formyltransferase; B12, vitamin B12 (cobalamin); CYH, methenyltetrahydrofolate cyclohydrolase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; FAICAR, formyl-aminoimidazole carboxyamide ribonucleotide; FGAR, formyl-glycinamide ribonucleotide; GAR, glycinamide ribonucleotide; GART, GAR formyltransferase; MS, methionine synthase; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylenetetrahydrofolate reductase; SHMT, serine hydroxymethyltransferase; THF, L-tetrahydrofolate; TS, thymidylate synthase

Excretion:
The elimination of folates from the body occurs by urinary excretion of intact folates and catabolic products and fecal excretion. The body folates pools exhibit biphasic kinetics with a rapid and a slow turnover pool. A rapid decline in urinary and fecal radioactivity with a half-life of hours (reported range 9.9 – 31.5 hours) is followed by a long decline with a reported half-life ranging from 9.6 to more than 100 days [von der Porten et al. 1992, Krumdieck et al. 1978]. Buchholz et al. (1999) reported that urinary and fecal excretion data from a male adult suggested that it would takes 360 days to completely eliminate an oral dose of 35 µg [14C]-labeled folic acid by linear extrapolation. If exponential extrapolation was done, the elimination $t_{1/2}$ was estimated to be about 300 days.

2.3 Intrinsic Factors

2.3.1 Were the effects of renal impairment or hepatic impairment on the pharmacokinetic of L-5-MTHF evaluated?

No specific studies were conducted to evaluate the effect of hepatic or renal impairments on L-5-MTHF. However, studies for L-5-MTHF in patients with renal or hepatic impairment are not necessary since the use of Safyral is not recommended in these patients due to existing concerns. The current Yasmin label states that Yasmin is contraindicated in patients with renal impairment. Patient with moderate hepatic impairment had mean DRSP exposure that is approximately 3 times higher than the exposure in women with normal liver function.

Limited data from the literature suggested that patients with renal impairment may have higher exposure following exogenous administration of L-5-MTHF. One literature reported a high dose of L-5-MTHF (17 mg once daily for 12 weeks) was administered to subjects with end stage renal disease [Bostom et al. 2000]. The authors reported that renal impairment resulted in significant elevation in folate concentration. After 12 weeks of daily folate intake, plasma folate concentrations increased by a mean of 838 nmol/L. There was no increase in adverse events reported due to the elevated folate concentration.

2.3.2 Are genetic 5,10-methylenetetrahydrofolate reductase (MTHFR) variants associated with differential effects of Safyral on folate biomarkers?

The pharmacogenomics reviewer, Dr. Li Zhang, evaluated the data regarding MTHFR polymorphism as part of the Clinical Pharmacology review of NDA 022532 (DARRTS, date 7/29/2010). She concluded that "[b]ased on Sponsor descriptive results, there does not appear to be significant relationship between the MTHFR 677 C>T variant and folate responses to Yaz or Beyaz. Mean RBC and plasma folate levels at Baseline were comparable for the wild type (CC) and heterozygote (CT) genotype groups and lowest for the variant (TT) genotype group. For the homozygote (variant) TT genotype group of MTHFR polymorphism 677C>T, an increase in plasma and RBC folate was observed in the Beyaz group that was similar to the CC and the CT genotype groups. For homocysteine, Baseline level was slightly higher in the TT (variant) genotype group compared with the other genotype groups. During treatment, homocysteine levels were similar for all genotypes." A similar conclusion of lack of "significant relationship between the MTHFR 677 C>T variant and folate responses" may be drawn for Safyral since both Safyral and Beyaz contain the same 0.451 mg Metafolin per tablet.

2.4 Extrinsic Factors

2.4.1 What is the effect of food on the bioavailability of Safyral tablets?

The effect of food on bioavailability of Safyral tablets was not evaluated. The label for Yasmin states that DRSP and EE Cmax decreased by about 40% due to a high fat meal while DRSP AUC was unchanged. EE AUC decreased by about 20% due to food. The Yasmin label does not restrict dosing with respect to meals.

There were no data submitted on the effect of food on bioavailability of Metafolin for either Safyral or Metafolin mono tablets. The sponsor proposed that Safyral can be taken without regards to meals. Since the Yasmin approved product is an immediate release formulation and its label does not restrict dosing with respect to meals, there is no need to conduct a fed BE study or a food effect study of the new formulation with respect to assessing the bioavailability of DRSP and EE. The PD study evaluating the total folate exposure administered Safyral without regards to meals. Therefore, this reviewer concurs that

Safyral could be administered without regards to meals. However, since the effect of food intake on Metafolin bioavailability was not evaluated, the label should reflect this fact.

2.4.2 What are the effects of L-5-MTHF on the pharmacokinetics of other drugs?

The Sponsor did not conduct any drug interaction studies with metafolin. The sponsor conducted a review of the literature. The referenced literature is consistent with the sponsor's conclusion that folates may modify the PK or PD of certain antifolate drugs (e.g. antiepileptics (such as phenytoin), methotrexate, and pyrimethamine) and may result in decreased pharmacological effect of the antifolate drug. Specific recommendations on dosage adjustments could not be made.

2.4.3 What are the effects of other drugs on the concentration of plasma and RBC folate concentrations?

The Sponsor did not conduct any drug interaction studies with metafolin. The sponsor submitted a survey of the literature and indicated that "several drugs have been reported to reduce folate levels and decrease the efficacy of folates by inhibition of the human dihydrofolate reductase (e.g. methotrexate, trimethoprim, sulfasalazine, and triamterene) or by reducing folate absorption (e.g. cholestyramine), or via unknown mechanisms (e.g. antiepileptics such as carbamazepine, phenytoin, and valproic acid)."

Dihydrofolate reductase: The referenced literature supports the effects of methotrexate and sulfasalazine on reducing folate concentration. However, it suggests that trimethoprim and triamterene do not alter serum or RBC folate concentrations.

Cholestyramine: Cholestyramine is a quaternary ammonium salt of a high molecular weight copolymer. The substance is hydrophobic and insoluble in water. In the small intestine, the chloride ions of cholestyramine exchange with bile acids, thereby interrupting bile acid enterohepatic circulation. In addition to binding bile acids, cholestyramine may also bind acidic and neutral drugs and reduce or delay their absorption. Reduced folate concentrations have been reported in patient chronically treated with cholestyramine [Cayen 1985].

Antiepileptic drugs: The sponsor referenced literature reports indicating that antiepileptic drugs like phenytoin, carbamazepine, barbiturates, primidone and valproic acid may induce folate deficiency. The are various suggested mechanisms including induction of liver enzymes [Maxwell et al. 1972], impairment of folate absorption [Ibbotson et al. 1967], competitive interaction between folate co-enzymes and drugs [Girdwood and Lenham 1956], and increased demand for folate as a cofactor in the hydroxylation of the anticonvulsant [Jensen and Olesen 1970].

It should be noted that the above observations are for total folate concentrations in the body. Since metafolin administration is expected to contribute to the body's folate pool, it is likely that these observations would hold in the presence of metafolin administration. However, the magnitude of effects on bioavailability of metafolin is not known.

2.5 General Biopharmaceutics

2.5.1 Were the formulations used in the 2 Safyral clinical studies the same as the proposed to-be-marketed formulation?

Yes. Both clinical studies that administered Safyral (BE study 309662 and PD study 309763) were performed with the proposed to-be-marketed product. Throughout the whole clinical development the tablet composition and manufacturing processes were not changed.

Yasmin product used in the BE study 309662 was the approved product. Yasmin tablets used in PD study 309763 were in the color of Safyral tablets. Yaz tablets used in PD study 310662 were in the color of Beyaz tablets. Release dissolution testing results for Yasmin and Yaz formulations used in the PD studies were provided, which showed that they met the release dissolution specification.

Please note that BE of Safyral and Yasmin with respect to EE and DRSP was discussed under section 2.2.1.

2.5.2 What is the formulation composition of Safyral hormone tablet and Metafolin only tablet?

Tables 12 and 13 show the formulation composition of Safyral hormone tablets and Metafolin mono tablets, respectively.

Table 12: Composition of Safyral hormone tablet

Composition	Reference to standard	Function	Amount [mg]
Drug substance			
Drospirenone micronized	specification	drug substance	3.000
Ethinylestradiol betadex clathrate micronized [a]	specification	drug substance	0.030
Levomefolate calcium (b) (4)	specification	drug substance	0.451
Excipients			(b) (4)
Lactose monohydrate	Ph. Eur., USP/NF, Ph. Jap		
Cellulose microcrystalline	Ph. Eur., USP/NF		
Croscarmellose sodium	Ph. Eur., USP/NF, Ph. Jap		
Hydroxypropylcellulose (b) (4)	Ph. Eur., NF		
Magnesium stearate	Ph. Eur., USP/NF, Ph. Jap		
(b) (4)	specification		
or alternatively			
Hypromellose (b) (4) (	Ph. Eur., USP/NF, Ph. Jap		
(b) (4)	Ph. Eur., USP/NF		
Talc	Ph. Eur., USP/NF, Ph. Jap		
Titanium dioxide	Ph. Eur., USP/NF,, Ph. Jap		
Ferric oxide yellow	Directive 95/45/EC USP/NF, JPE		
Ferric oxide red	Directive 95/45/EC USP/NF, JPE		
(b) (4)	Directive 95/45/EC		

a calculated as ethinylestradiol

(b) (4)

Table 13: Composition of Metafolin only tablet

Composition	Reference to standard	Function	Amount [mg]
Drug substance			
Levomefolate calcium (b) (4)	specification	drug substance	0.451
Excipients			
Lactose monohydrate	Ph. Eur., USP/NF, Ph. Jap	(b) (4)	
Cellulose microcrystalline	Ph. Eur., USP/NF		
Croscarmellose sodium	Ph. Eur., USP/NF, Ph. Jap		
Hydroxypropylcellulose (b) (4)	Ph. Eur., NF		
Magnesium stearate	Ph. Eur., USP/NF, Ph. Jap		
(b) (4)	specification		
or alternatively			
Hypromellose (b) (4) (b) (4)	Ph. Eur., USP/NF, Ph. Jap Ph. Eur., USP/NF		
Talc	Ph. Eur., USP/NF, Ph. Jap		
Titanium dioxide	Ph. Eur., USP/NF, Ph. Jap		
Ferric oxide red	Directive 95/45/EC USP/NF, JPE		
Ferric oxide yellow	Directive 95/45/EC USP/NF, JPE Directive 95/45/EC		
(b) (4)			
(b) (4)			

2.6 Analytical Section

2.6.1 What bioanalytical methods were used to assess concentrations of DRSP, EE, and MTHF in bioequivalence studies 309664 (Beyaz vs. Yaz) and 309662 (Safyral vs. Yasmin)? Were these methods adequately validated?

Plasma DRSP and EE, and serum L-5-MTHF concentrations from BE studies 309664 and 309662 were measured using LC/MS/MS methods. Review of the method validation reports indicated that the methods met the FDA recommended validation criteria. However, there were storage stability (samples stored for longer than proven stability period prior to analysis) and assay performance issues identified. Following corrective action by the Sponsor, the assay was considered acceptable for analysis of BE study 309662.

A summary of the issues and the corrective actions related to study 309662 are presented below. Please see Clinical Pharmacology review for NDA 022532 (DARRTS, date 7/29/2010) for discussion of issues related to study 309664.

Storage stability issues:
Review of the bioanalytical reports indicated a potential issue regarding sample stability for both studies 309664 and 309662. Requests for clarification were sent to sponsor on 1/25/2010 and 3/2/2010 for studies 309664 and 309662, respectively. The following section summarizes the issues and the outcomes for issues related to study 309662. Sponsor's response related to storage stability of EE and DRSP samples for study 309664 is addressed in Clinical Pharmacology review of NDA 022532.

1. The DRSP bioanalytical report for study 309662 stated that storage stability for DRSP in human plasma was demonstrated for 200 days. The report indicated that samples were stored for ≤ 111 days

at [redacted]. However, it is not clear if samples were analyzed within 200 days from the day of sample collection (i.e., the total time stored at [redacted] and time stored elsewhere before being shipped to [redacted]). It appears that the first sample collected from study 309662 was on 16 August 2006 and the last analytical run for DRSP analysis was performed on 9 October 2007. Therefore the maximum storage time could be up to 419 days. Provide data supporting long term storage stability of DRSP in human plasma for at least 419 days. Alternatively, provide evidence that each sample from this study was analyzed for DRSP within 200 days of sample collection.

- Sponsor replied on 3/11/2010 and stated that for study 309662, all DRSP plasma sample except 1 were analyzed within 200 days. One sample was analyzed after 228 days. This was a baseline sample from period 1 that was recorded as being below LLOQ. This sample should not affect the interpretation of the DRSP results from study 309662.

2. The EE bioanalytical report for study 309662 states that storage stability for EE in human plasma was demonstrated for 268 days. The report indicated that samples were stored for ≤ 245 days at [redacted]. However, it is not clear if samples were analyzed within 268 days from the day of sample collection (i.e., the total time stored at [redacted] and time stored elsewhere before being shipped to [redacted]). It appears that the first sample collected from study 309662 was on 16 August 2006 and the last analytical run for EE analysis was performed on 11 October 2007. Therefore the maximum storage time could be up to 421 days. Provide data supporting long term storage stability of EE in human plasma for at least 421 days. Alternatively, provide evidence that each sample from this study was analyzed for EE within 268 days of sample collection.
   - Sponsor replied on 3/11/2010 and stated that for study 309662, all EE plasma samples were analyzed within 234 days.

Assay performance issues:

<u>EE and DRSP assays:</u>
A DSI consult was sent (DARRTS, date 1/13/2010) for inspection of the clinical study site and bioanalysis sites for DRSP, EE, and MTHF for BE study 309662. DSI inspection report for study 309662 was received on 8/10/2010 (see DSI memorandum in DARRTS, date 8/10/2010). The DSI inspection found several deficiencies and recommended the following actions:

1. The firm should recalculate subject concentrations with new calibration curves for ethinyl estradiol from 4 pg/mL to 1000 pg/mL and use 4 pg/mL as LLOQ (483 Item 1). The reviewer should evaluate re-calculated ethinyl estradiol concentrations using the amended report (amendment 02) that the firm planned on submitting by August 18, 2010.
2. The firm should recalculate subject concentrations with new calibration curves for drospirenone from 0.5 ng/mL to 100 ng/mL and use 0.5 ng/mL as LLOQ (483 Item 1). The reviewer should evaluate re-calculated drospirenone concentrations in the amended report (amendment 02) that the firm planned on submitting by August 18, 2010.
3. The reviewer should replace the data for the following samples with the values in the table included in the firm's written response to Form FDA 483 (483 Item 3) in bioequivalence evaluation.

ID	Sample ID	Original result	Reported result	Comment
0819	PID 3, Period 3, 8 hr	36.5 pg/mL	32.4 pg/mL	Result from run AQ17-002 is reported, because this run is accepted upon rejection of STD A. Low IS was observed in run AQ17-008 (reanalysis run, now unnecessary analysis)
1059	PID 12, Period 2, -0.50 hr	<LLQ	<4.00 pg/mL	Was reanalysed at the time
1735	PID 26, Period 2, 4 hr	30.2 pg/mL	NR	Set to NR as a result of applying the new 30-170% rule.

This reviewer concurred with DSI's recommendations. The following recommendations were forwarded to the Sponsor on 8/20/2010:

Based on the findings of the FDA DSI inspection of (b) (4), we have the following recommendations regarding the bioanalysis of samples from Study 309662:

1. Establish new calibration curves for ethinyl estradiol (EE) from 4 pg/mL to 1,000 pg/mL and re-calculate all sample concentrations using the new calibration curves with 4 pg/mL as the lower limit of quantitation (LLOQ). Bioequivalence (BE) assessment should not include any EE concentration below 4 pg/mL.
2. Establish new calibration curves for drospirenone (DRSP) from 0.5 ng/mL to 100 ng/mL and re-calculate all sample concentrations using the new calibration curve with 0.5 ng/mL as the LLOQ. BE assessment should not include any DRSP concentration below 0.5 ng/mL.
3. There were inconsistencies in deciding when a reassay was conducted due to internal standard variability that affected 3 samples. Replace the "original result" values with the new "reported result" values for the 3 affected samples as shown in the table below. BE assessment should use the values as indicated under the "reported result" column for these samples.

ID	Sample ID	Original result	Reported result	Comment
0819	PID 3, Period 3, 8 hr	36.5 pg/mL	32.4 pg/mL	Result from run AQ17-002 is reported, because this run is accepted upon rejection of STD A. Low IS was observed in run AQ17-008 (reanalysis run, now unnecessary analysis)
1059	PID 12, Period 2, -0.50 hr	<LLQ	<4.00 pg/mL	Was reanalysed at the time
1735	PID 26, Period 2, 4 hr	30.2 pg/mL	NR	Set to NR as a result of applying the new 30-170% rule.

Upon completing the recommended corrective actions, the new data set should be assessed to determine if the data are adequate to permit calculation of bioequivalence (e.g., Are there samples missing that would prevent adequate calculation of individual pharmacokinetic parameters? Is there a sufficient number of subjects remaining?). If the data set is deemed acceptable for bioequivalence assessment, BE analysis should be performed. The results of the BE analysis, PK profiles and calculated PK parameter values should be included as an amendment to Study Report A27410 and submitted to the NDA. The raw data set should also be submitted to the NDA in SAS Transport (.xpt) format. Submit the revised results and data files as soon as possible.

The Sponsor agreed to all recommendations. Revised datasets and BE analysis results were submitted on 8/27/2010. A new study report for study 309664 incorporating these changes was submitted on 9/7/2010.

L-5-MTHF assay:

DSI recommended that L-5-MTHF data from study 309662 be accepted for review (DSI memorandum to NDA 22532, DARRTS, date 5/24/2010).

Clinical site conduct for study 309662:
DSI indicated that there was no significant finding at the clinical site (DSI memorandum, DARRTS, date 8/10/2010).

2.6.2 What bioanalytical methods were used to assess concentrations of RBC and plasma folate in PD studies 310662 (Yaz vs. Beyaz) and 309763 (Yasmin + folic acid vs. Safyral)? Were these methods adequately validated?

Total folate in plasma and whole blood concentration from PD studies 309763 (Safyral) and 310662 (Beyaz) were measured using a microbiological assay with Lactobacillus casei. The assay had an effective lower limit of quantitation of 75 ng/mL in whole blood and 3 ng/mL in plasma.

The assay was validated by (b) (4) (validation report A32903, (b) (4) report V7002). The assay had a calibration range of 25 – 500 ng/ml (56.64 – 1132.76 nmol/L) in blood and 1 – 20 ng/mL (2.27 – 45.31 nmol/L) in plasma. The validation report stated that the assay is not robust. Many plates were rejected due to calibration and/or QC samples not meeting acceptance criteria. In some runs the calibration curve flattens at higher concentration and causing high bias for the High QC samples.

QC at LLOQ did not meet ±20% for accuracy and precisions. The bias in accuracy at LLOQ was -21.8% in whole blood and -23.3% in plasma. Intra run imprecision at LLOQ was 19.4% in whole blood and 22.7% in plasma. Inter run imprecision at LLOQ was 27.6% in whole blood and 36.3% in plasma. Accuracy and precision criteria were met at other QC levels including QC Low (i.e., 75 ng/mL for whole blood and 3 ng/mL for plasma). The sponsor accepted the LLOQ with the expectation that study samples would not be below the QC Low level. Review of the datasets from studies 309763 and 310662 by statistics reviewer, Dr. Sonia Castillo, indicated that there were a limited number of samples that were below the effective LLOQ. These samples were excluded from analysis.

Selectivity against DRSP and EE was evaluated. The assay was selective against DRSP and EE. Sensitivity for PGA (folic acid), 5-MTHF (racemic mixture), and effect of depletion of total folate by folate binding protein (FBP) were also evaluated. The results indicated that the assay could accurately detect spiked PGA and 5-MTHF. Depletion by FBP was inconclusive but was suggestive that FBP could deplete folate.

Based on the validation results alone, absolute accuracy of observed in vivo study sample could not be assured since the QC low and QC medium were prepared in 5% bovine serum albumin (BSA) and not the true matrix (i.e., blood or plasma). QC high was based on spiked matrix and was acceptable. All QC samples used during bioanalysis of studies 309763 and 310662 were prepared in 5% BSA. At the request of DSI, the bioanalysis firm (b) (4) conducted additional accuracy test for spiked plasma and blood samples. DSI stated that "(b) ( ) has demonstrated sufficient accuracy for assays of folate in plasma and whole blood, except for folate determination of 3 ng/mL spiked into plasma." DSI recommended that plasma folate results below 3 ng/mL be omitted from analysis (DSI memorandum to NDA 22532 in DARRTS, date 6/30/2010). Therefore, it could be concluded that there was sufficient accuracy at the "effective LLOQ" of 3 ng/mL in plasma and 75 ng/mL in blood.

Freeze/thaw stability in blood and plasma was established for 3 freeze/thaw cycles. Storage stability at < minus 70ºC was acceptable for up to 3 years 2 months (based on additional data provided to DSI as documented in DSI memorandum to NDA 22532 on 5/24/2010). Blood samples could be diluted up to 5-fold and plasma samples could be diluted up to 100-fold.

DSI inspection recommendations (NDA 22532 DARRTS, date 5/24/2010):

The DSI inspected 2 clinical sites and the bioanalytical sites related to PD studies 309763 and 310662. A team meeting was held on 6/28/2010 with representative from Clinical Pharmacology, DSI, Clinical, and Biostatistics disciplines to discuss the issues raised by DSI. The following section lists the DSI recommendations that required a response and this reviewer's response, with concurrence from the review team.

Clinical Site Observations

A. Subjects identified using concomitant medication listed in the exclusion criteria should be excluded from analysis (Subjects #026 and 003) (See 483 Item 1 (b) (4)).

Response: Defer to Clinical reviewer.

B. Failure to protect samples from light exposure may have compromised folate stability. The identified samples should be excluded from further analysis. Note, only 60 of the 121 subjects at the investigated site were reviewed so the list of samples exceeding recommended light exposure may be incomplete (See 483 Item 1 (b) (4)).

Response: This observation was made at (b) (4). The time from blood draw to RBC folate processing ranged up to 4 hours 3 minutes in the subjects investigated. In response to DSI's observation (attachment YEG2 in DSI's memorandum to NDA 22532 on 5/24/2010), (b) (4) (b) (4) agreed that they did not follows the instruction to place the blood samples for RBC analysis in the dark within 5 minutes after blood draws. A table provided by (b) (4) showed that about 64% of all samples at his site were processed after 5 minutes. This reviewer agrees that the identified samples should be excluded. The sponsor should identify and exclude all blood samples that were processed after 5 minutes. Alternatively, the sponsor can provide data to show that extended exposure to light do not alter the stability of folates in blood.

Review note: The Clinical Team decided to exclude all data from this site due to other reasons. Therefore, this issue will not be pursued further. Please see Clinical review for details.

Analytical Site Observations

A. Long-term frozen stability evaluations for whole blood are not adequate for the period of study sample storage. The High- and Medium-concentration QCs reported at 2 years, 11 months are outside the study sample acceptance criterion of ±15% from nominal. Additionally, the potential changes in folate recovery and concentrations during the first freeze/thaw cycle have not been evaluated. The Review Division should request whole blood folate stability data for approximately 2 years, and during the first freeze/thaw cycle.

Response:
Long term stability: Table 14 shows the results of long-term stability data as provided by (b) (4) (i.e., the firm that conducted the bioanalysis of PD studies 310662 and 909763) to DSI (attachment SYK2 in DSI memorandum to NDA 022532 on 5/24/2010). The failed stability samples were at nominal concentrations of 200 and 400 ng/mL in phosphate buffered saline with bovine serum albumin (PBS-BSA). Both 400 ng/mL samples showed a recovery of 118%, slightly outside of the typical acceptance range of ±15%. The 200 ng/mL samples showed a recovery of 129% and 113%, respectively. One was within the acceptable range while the second sample failed on the high side. Other tested stability samples in PBS-BSA, blood, and plasma were within the ±15% criterion. Also, other stability data showed that total folate

was stable for up to 10 months. A discussion with the Clinical team indicated that a precision of ± 30% would not be of a concern. Therefore, considering that the failed stability samples failed on the high side, other stability samples all passed, total folate samples were considered stable at up to 10 months, and the lack of need for a highly precise estimate of plasma and blood folate concentrations in the PD studies, this reviewer recommends that the samples be considered acceptable from a long term stability perspective. This recommendation was discussed at the review team meeting on 6/28/2010.

Table 14: summary of long term stability data submitted by (b) (4) to DSI (from attachment SYK2 in DSI memorandum on 5/24/2010)

QC	Matrix	result 1 ng/ml	result 2 ng/ml	avg	%sd	nominal ng/ml	recovery of nominal recovery1	recovery of nominal recovery2	Time interval
7002 QC endo	Blood	92.6	100.5	96.6	5.8%	endo	n.a.	n.a.	3 year 2 months
7002 QC high	Blood	316.0	317.0	316.5	0.2%	endo+ 200	110%	110%	3 year 2 months
7227 QC High	PBS-BSA	470.9	471.6	471.2	0.1%	400	118%	118%	2 year 11 months
7227 QC medium	PBS-BSA	257.9	226.7	242.3	9.1%	200	129%*	113%	2 year 11 months
7227 QC low	PBS-BSA	79.6	81.9	80.7	2.1%	75	106%	109%	2 year 11 months
7002 QC endo	Plasma	12.8	11.0	11.9	11%	endo	n.a.	n.a.	3 year 2 months
7002 QC high	Plasma	21.1	20.3	20.7	2.7%	endo+ 8	115%	105%	3 year 2 months
7002 QC endo	Plasma	11.9	11.5	11.7	2.4%	endo	n.a.	n.a.	3 year 6 months
7002 QC High	Plasma	20.7	20.2	20.4	1.7%	endo+ 8	112%	106%	3 year 5 months
7227 QC High	PBS-BSA	16.0	15.9	16.0	0.3%	16	100%	100%	2 year 11 months
7227 QC medium	PBS-BSA	8.1	7.8	7.9	3.2%	8	101%	97%	2 year 11 months
7227 QC low	PBS-BSA	3.1	3.0	3.0	2.5%	3	103%	99%	2 year 11 months

All QCs have been prepared twice, and each prepared sample was incubated in duplicate.
Result 1 = averaged result from a duplicate incubation in ng/ml. Result 2 = averaged result from a duplicate incubation in ng/ml.
Recovery = recovery of nominal value or nominal spike in %.
Recovery in Blood or Plasma is calculated as (measurement QC High - average measurements corresponding QC Endo)/nominal spike in %.
Recovery in PBS-BSA is calculated as measurement/nominal value in %. n.a.: not available, since QC Endo reflects the endogenous (unknown) concentration.
*Slightly above the acceptance criterion (recovery between 80 and 120%).

Stability following the first freeze/thaw cycle: Assay validation was conducted such that all QC samples were prepared and stored at -70 ºC prior to use. For freeze/thaw stability testing the sponsor compared the expected value based on mean of QC endo and QC high in the same assay run. Since the QC endo and QC high have been frozen before, the question remains whether there is an effect of the first freeze/thaw cycle (the effect of second and third freeze thaw cycles can be delineated from available data and do not show a difference between the first, second, and third cycles).

(b) (4) provided additional data to DSI and DSI concluded that there are sufficient data to demonstrate stability of folate during the first freeze/thaw cycle (see DSI memorandum to NDA 022532 in DARRTS, date 6/30/2010).

B. (b) (4) should evaluate the recovery of folate in plasma and whole blood at three concentrations to ensure the accuracy of study sample determinations. As mentioned, (b) (4) expected to provide these data by 5/15/10 but, due to technical issues, will provide the data by 5/31/10. In the absence of these data, the majority of the reported concentrations in the audited studies cannot be assured.

Response: (b) (4) provided additional data to DSI. (b) (4) evaluated folate concentrations in whole blood samples spiked at 100, 200, and 300 ng/mL and in plasma samples spiked at 3, 8, and 12 ng/mL. DSI stated that (b) (4) has demonstrated sufficient accuracy for assays of folate in plasma and whole blood, except for folate determinations of 3 ng/mL spiked into plasma. If the l15% acceptance criterion is used, DSI recommends that plasma folate results below 3 ng/mL should be omitted from analysis" (see DSI memorandum to NDA 022532, date 6/30/2010). Since the review team has already used an "effective LLOQ" threshold of 3 ng/mL for plasma, no further action is needed.

C. In the absence of demonstration of dilution linearity for 8-fold diluted samples, the six diluted samples should be omitted from analysis.

Response: DSI memorandum to NDA 022532 on 6/30/2010 stated that additional data at 8-fold diluted did not meet acceptance criteria and the prior recommendation stands. This reviewer agrees that the six 8-fold diluted samples should be omitted from analysis.

D. The subject samples accepted with only 4 QCs (two concentrations each in duplicate) should be omitted from analysis. Samples identified by firm as approved outside the study plan should also be excluded.

Response:
Samples from runs with only 2 QC: the Sponsor accepted 2 series (Blood series 22, 3 out of 18 plates and Plasma series 8, 24 out of 24 plates) where the QC high was accidentally not pipetted into the plates and only the low and medium QCs were available for applying the acceptance criteria. The sponsor provided the following justifications:

- All the measured concentrations were within the calibrated range.
- For both series (series 22 for whole blood, series 8 for plasma, which represent all data mentioned in the observation), all 4 out of 4 remaining QCs were within specification (< 15% deviation).
- In addition, in each series of the study for whole blood and for plasma, the WHO International Standard Folate, (NIBSC code: 95/528) was included on each plate. The folate concentration in the reconstituted material is 13 ng/ml (if reconstituted to represent plasma) or 130 ng/ml (if reconstituted to represent whole blood). For all individual plasma plates, the accuracy for both duplicates of this material was within 85-115% (actual values 97-107%). For all individual whole blood plates, the accuracy for both duplicates of this material was within 85-115% (actual values 96-101%).
- For QC High in this study ((b) (4) study 7227), zero out of all 206 plasma plates, and only one out of a total of 270 whole blood plates failed the acceptance criteria.

The standard acceptance criteria are 1) at least 4 of 6 QC samples should be between ±15% and 2) at least one of 2 QC should be valid at each level. Due to the absence of the high QC, the second acceptance criteria could not be met. However, based on the above observations that all remaining QC samples were within the ±15% criteria and further supported by the WHO International Standard Folate duplicate

samples being within ±15% of their expected values, these runs could be considered acceptable. This recommendation was discussed at the review team meeting on 6/28/2010.

Samples approved outside the study plan: These samples should be excluded unless the sponsor can provide adequate justification.

2.6.3 What bioanalytical methods were used to assess concentration of folate metabolites?

Concentrations of PGA (folic acid), THF (tetrahydrofolate), 5-MTHF (5-methyl-THF), 5-FTHF (5-formyl-THF), and 5,10-MTHF (5,10-methyl-THF) from study 309763 were measured using a semi-quantitative LC/MS assay. The assay was based on acceptance criteria of 50% for accuracy and precision at LLOQ (40% at other QC levels). The calibration range was 0.5 – 150 ng/mL. The QC levels (LLOQ, QC Low, QC Medium, and QC High) were at 0.5, 1.5, 20, and 100 ng/mL for 5-MTHF, and 0.5, 1.5, 5, and 10 ng/mL for the remaining analytes.

The accuracy for THF failed to meet the acceptance criteria at initial validation runs. The sponsor noted that runs 2 and 3 conducted after a freeze/thaw cycle had lowed calculated concentration suggesting that THF was not stable. Subsequently, runs 2 and 3 were repeated (noted as runs 1a and 1b) with freshly prepared stocks and did meet the acceptance criteria for accuracy, except for the LLOQ sample at 0.5 ng/mL.

No data on storage stability was provided in the validation report. There was evidence that a freeze/thaw cycle may cause degradation of THF as observed concentrations in validation runs 2 and 3 (previously frozen samples) were lower as compared to those from run 1 (fresh samples).

Based on the original validation report the following conclusion was drawn: the data generated from this assay is not considered quantitative due to the wide acceptance criteria and the lack of storage and freeze/thaw stability data. The Clinical review team indicated that for their purpose, they would accept qualitative data on relative metabolite profiles. Therefore, the sponsor was requested to submit the information on storage and freeze/thaw stability to ensure that the samples were stable prior to analysis. The following request was sent to sponsor on 4/15/2010 (under NDA 022532).

Information request for sponsor:

Clinical Study Report A34010 included a validation report ((b) (4) Report V 7430/01) and a bioanalysis report ((b) (4) Report V 7430/02) for determination of folate metabolites in human plasma from clinical study 309763. The validation report did not include the following stability assessments: freeze/thaw, short-term storage (e.g., at room temperature) and long-term storage (e.g., at minus 20ºC or minus 70ºC). In addition, it appears that at least 1 or 2 of the tested moieties (i.e., tetrahydrofolate [THF] and 5,10-methyl-THF [5,10-MTHF]) were not stable following a freeze/thaw cycle, as indicated by the results from validation Runs 2 and 3 compared to Run 1. Provide the following data as soon as possible:

1. Evidence of long-term storage stability for each tested moiety. In addition, provide a table showing the storage time (i.e., time from sample collection to time of analysis) for each study sample and whether or not it was analyzed within the demonstrated storage stability period. Samples analyzed outside of the demonstrated stability period should not be used.
2. Evidence of stability upon 3 freeze and thaw cycles for each tested moiety. In addition, provide a table showing how many freeze and thaw cycles each sample incurred prior to analysis. Given that all samples were stored at below minus 70ºC upon receipt by (b) (4) (b) (4), all would have incurred at least 1 freeze and thaw cycle.

3. Evidence of short-term storage stability for each tested moiety. The duration of demonstrated short-term storage stability should be sufficient to cover the time allotted to process the study samples.

Sponsor's response:
The Sponsor provided response to the above request on 7/16/2010. A summary of the response is outlined below for each request.

1. The sponsor stated that long term storage stability at -70 ºC was demonstrated for folic acid for 38 months in whole blood and 41 months in plasma (b) (4) report V8888). Long term stability at -80 ºC for L-5-MTHF in serum was demonstrated for 552 days based on validation study performed in support of the BE study (Anapharm report 55161MIH). However, no stability data is available for the other metabolites (5,10-MTHF, 5-FTHF/10-FTHF, THF).

   The sponsor stated that all bioanalyses for folic acid and L-5-MTHF were completed within the proven long-term storage stability range (as stated above). However, a similar conclusion can't be drawn for the other metabolites. Therefore, the results for metabolites 5,10-MTHF, 5-FTHF/10-FTHF, THF are not considered reliable.

2. The sponsor stated that freeze thaw stability over 3 freeze/thaw cycles for total folate in plasma and whole blood was demonstrated in (b) (4) bioanalytical report V7002 (spiked folic acid samples analyzed with microbiological assay with Lactobacillus casei that is sensitive to total folate). Freeze thaw stability of L-5-MTHF over 3 freeze/thaw cycles was demonstrated in (b) (4) bioanalytical report 55161MIH.

   The Sponsor also provided an analysis showing results of effect of 1 freeze thaw cycle showing that in addition to folic acid and L-5-MTHF, 5-FTHF was stable after 1 freeze thaw cycle (The majority of samples [481 out of 501] were thawed only once). It should be noted that this analysis by sponsor was based on 3 different sets of stability samples. All 3 sets were analyzed fresh but only one set were analyzed following a freeze thaw cycle. Additionally, this data was based on a semi-quantitative assay and do not provide clear evidence of freeze thaw stability. However, assuming the samples were prepared correctly, the data may be considered reliable for use in conjunction with the current semi-quantitative assay. The results of this analysis also suggested that 5,10-MTHF and THF were not stable after the freeze thaw cycle with mean recovery of 61% and 21%, respectively.

   Overall for the current purpose of use as it relates to the current semi-quantitative assay for folate metabolites, the issue with freeze thaw stability for folic acid, L-5-MTHF, and 5-FTHF is considered resolved. The data suggests that 5,10-MTHF and THF may be unstable following a freeze thaw cycle and therefore the observed values may lower than the actual values.

3. Short term storage stability at room temperature up to 24 hours is available for total folate in whole blood and plasma and L-5-MTHF in serum. Short term storage stability for other metabolites was not established.

Overall assessment of the metabolite LC/MS assay:
This assay is a semi-quantitative assay. Available data supports the stability of folic acid and L-5-MTHF and bioanalytical results for these 2 moieties could be reviewed (taking into account the semi-quantitative nature of the assay). Stability of 5-FTHF, 5,10-MTHF, and THF were not established. Available data suggests that 5,10-MTHF and THF are not stable following a freeze thaw cycle. Therefore, bioanalytical results for 5-FTHF, 5,10-MTHF, and THF are not considered reliable.

DSI inspection recommendations (NDA 022532 DARRTS, 5/24/2010):

The DSI inspected the bioanalytical sites relating to the folate metabolite assay for clinical study 309763. The following section lists the DSI recommendations and this reviewer's response.

A. The lack of audit trail during manual chromatogram reintegrations prevents reconstruction of the events, and therefore prevents assurance of the data. However, for semi-quantitative purposes of this assay (risk of neural tube defects; pharmacogenomics), these data may be usable.

Response: Noted.

B. The procedure for Sponsor-requested re-assays was not defined prior to study initiation. The original values should be used for pharmacokinetic assessments.

Response: Twenty-one (21) out of 485 samples were reanalyzed for "implausibility" at the request of sponsor. The median of the duplicate reassays and the original value was reported in Annex 5 of (b) (4) report V7430/02. Even though (b) (4) did not pre-specify the calculation method, the method used was a standard practice that is used at (b) (4). A review of the data indicated that the median values were generally consistent with the original values. The use of one set of values versus the other would unlikely to alter the conclusion from this study.

C. Documentation (especially dates of events) was incomplete for the preparation of calibrator and QC samples. However, the available records and data are consistent with preparation according to the documented procedure.

Response: Noted.

3 Detailed Labeling Recommendations

The sponsor has revised the label to reflect agreements made with Beyaz label. There are no major labeling recommendations from Clinical Pharmacology perspective. Other minor labeling changes will be communicated directly with the review team.

4 Appendix

4.1 Individual Study Reviews

4.1.1 Review of bioequivalence study report A27410 (Protocol 309662)

Title: Open-label, randomized, three-fold crossover study to investigate the bioequivalence of two different tablet formulations containing 0.03 mg EE and 3 mg DRSP without [SH T470FA, Yasmin] and with [SH T04532A, Safyral] 0.451 mg Metafolin, and to investigate the bioequivalence of two different tablet formulations containing 0.451 mg Metafolin without [SH T04532C, Metafolin mono] and with 0.03 mg EE/ 3 mg DRSP [SH T04532A] in healthy young women.

Objective: To investigate 1) the BE of EE and DRSP in Yasmin and Safyral tablets, and 2) the BE of L-5-MTHF in Safyral and Metafolin mono tablets.

Study design: Single-center, open-label, randomized, cross-over study with three treatments (one dose each), three study periods separated by at least one menstrual cycle, and six different treatment sequences. Forty five (45) healthy women aged 18 – 37 years of age (mean 29.7 years)with body mass index (BMI) of 19 – 28 kg/m^2 (mean 22.6 kg/m^2) were enrolled (Full analysis set or FAS). Forty one (41) subjects completed the study (Per protocol set or PPS). The study enrolled mostly Caucasians.

The study comprised six periods: Screening (visits 1 and 2), pre-dose, treatment period 1, treatment period 2, treatment period 3 and follow-up. A washout of at least one menstrual cycle occurred between treatment periods.

Each treatment was administered between the third and sixth menstrual cycle day. The study drug was administered after an over night fast (at least 10 hours) in the morning between 7:00 am and 10:00 am. The study drug was taken with 240 ml of water. Fasting is continued for 4 hours post drug administration.

A folate-free (<5 µg folate/100 g powder) shake (85 g of diet powder containing 1831 kJ, 4 g protein, 58 g carbohydrate, 21 g fat mixed with 240 ml water) was ingested between 3 to 2 hours before drug administration. To minimize folate intake, the same shake was also administered at 4, 7, and 10 hours post drug administration in lieu of regular food intake (i.e., lunch). The shake administration at 3 to 2 hours predose should not interfere with the interpretation of the BE results. Since the shake is administered as a liquid suspension, after 2 hours it should be emptied from the stomach. Additionally, this condition is applied to all treatment period.

Study period:
Date of first volunteer, first visit: 8/16/2006
Date of last volunteer, last visit: 7/4/2007

Test products:
Treatment A: Single oral administration of 1 coated tablet SH T470FA (Yasmin) containing 0.030 mg EE and 3 mg DRSP
Treatment B: Single oral administration of 1 coated tablet SH T04532A (Safyral) containing 0.030 mg EE, 3 mg DRSP, and 0.451 mg Metafolin
Treatment C: Single oral administration of 1 coated tablet SH T04532C (Metafolin) containing 0.451 mg Metafolin

PK samplings:

Samples for assessment of serum L-5-MTHF, and plasma EE and DRSP were obtained at the time points indicated in the table below. EE and DRSP were not measured in Treatment C.

Table 15: PK sampling times

Time (h)	-.5	.5	1	1.5	2	3	4	6	8	10	12	16	24	34	48	72	96	12	144	168
L-5-MTHF	x	x	x	x	x	x	x	x	x	x	x									
EE	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
DRSP	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			

Bioanalytical analysis:
The concentration of EE and DRSP in plasma samples and L-5-MTHF in serum samples was determined by a validated LC/MS/MS with a LLOQ of 2 pg/ml (EE), 100 pg/ml (DRSP) and 1.09 nmol/L (L-5-methyl-THF). Results were reported in Bioanalytical Report number A32464.

The DSI made several recommendations, including raising the LLOQ for EE and DRSP assays (see discussion within the Question Based Review for further details). The Sponsor has performed the recommended corrective actions and the study report for study 309662 was amended.

There was matrix interference with the analysis for DRSP identified by sponsor for 19 subjects and affected 23 PK profiles for DRSP in the study (10 profiles for Safyral and 13 profiles for Yasmin). The bioanalysis report stated that this was due to the use of different tubes in the clinical study and an evaluation at the end of the study by sponsor indicated that the interference has a maximum potential signal of 1 ng/ml. No further details were provided. The Sponsor was requested to clarify what the interference was and how it was determined (via email 3/30/2010).

The sponsor provided a response on 4/16/2010 to NDA 022532. The sponsor stated that there was an interfering peak in the DRSP chromatograms of some samples. Although the retention times of the interfering peak (3.38 – 3.49 minutes) and of the DRSP peak (3.26 minutes) were well distinguished, they were not baseline separated. The different retention time suggests that the nature of the interference was not related to contamination or carryover of DRSP.

The sponsor identified the relationship of the cryo tube used and the observed interference through a retrospective investigation. The sponsor observed that the interfering peak did not occur in any of the samples of the entire treatment period for a specific subject if the peak was not present in the pre-dose samples. The sponsor provided a list of subjects that had samples with matrix interference as well as several subjects that had no samples with matrix interference. In some cases, a subject may have interference in one period but not in another period. In all cases, subjects with samples stored in small 1 mL cryo tubes (b) (4) (b) (4)) had matrix interference while those stored in larger 2 mL cryo tubes ((b) (4)) did not have interference. For certain subjects where the tube type was changed in the middle of PK sampling period from 1 ml to 2 ml tubes (Subject 20 period 1, subject 21 period 1, subject 23 period 1), the same pattern holds in that there was interference in pre-dose samples where 1 ml tubes were used and no interference at end of sampling period where the 2 mL tubes were used. These results are consistent with the sponsor's conclusion that the interference was related to the type of tube used for sample storage. However, the actual cause of the interfering peak was not identified. The sponsor stated that they have attempted to reproduce the interference but they were not able to obtain the (b) (4) 1 mL tubes used at the clinical site since they were used up.

The Sponsor clarified that the interference was evaluated in all affected pre-dose samples against a calibration standard curve of DRSP and it was concluded that the interference had at maximum a concentration of 1 ng/mL. The sponsor accepted all samples with concentration ≥5.00 ng/mL (i.e., ≥5x the interfering peak) regardless of whether or not there were interference in the pre-dose sample using the same tubes. Only samples with interference observed in the chromatogram AND the concentration of DRSP determined to be <5.00 ng/mL were excluded. The sponsor acknowledged that for "DRSP concentrations >5 ng/mL an [interference] of up to 1 ng/mL most likely still may have been present in 'the remaining samples in the same treatment period' but was not visible any more in the chromatograms as interfering peaks due to the low concentration of the interference."

The sponsor calculated PK parameters based on all data remaining after exclusion of selected samples with interference as described above. However, the presence of interference in at least one sample for a given subject during a study period suggested that there would likely be interference in other samples from the same subject in that period since it is likely that the same tube type would be used. Therefore, this reviewer calculated descriptive PK parameters only in subjects in which no matrix interference was identified at all.

The sponsor also decided to perform additional BE analyses excluding subjects with any samples that showed interference.

Statistical analysis:
The primary BE variables are C_{max} and AUC(0-inf). In cases where AUC(0-inf) could not be reliably calculated, the AUC(0-tlast) would be considered the primary variable instead of AUC(0-inf). This was the case for EE and L-5-MTHF.

90% confidence intervals were calculated for Treatment A vs. Treatment B (bioequivalence of EE and DRSP) and Treatment B vs. Treatment C (bioequivalence of L-5-MTHF).

The sequence effect was tested significant for the AUC(0-inf) of DRSP and the AUC(0-tlast) of EE. A study inherent carryover effect can be excluded since it was a single dose study, the drug is not an endogenous moiety, and adequate washout period was included (washout period of 1 menstrual cycle with dosing between day 3 and day 6 of menses). Therefore, the significant sequence effect should not affect the interpretation of the BE results.

Protocol Deviations:
There were 4 major protocol deviations, withdrawal of consent (RNRs 8 and 24), inability to obtain blood samples as required in the protocol due to vasovagal reaction (RNR 35), and pregnancy (RNR 109). These subjects did not complete the study and were excluded from PK analysis.

PK results:

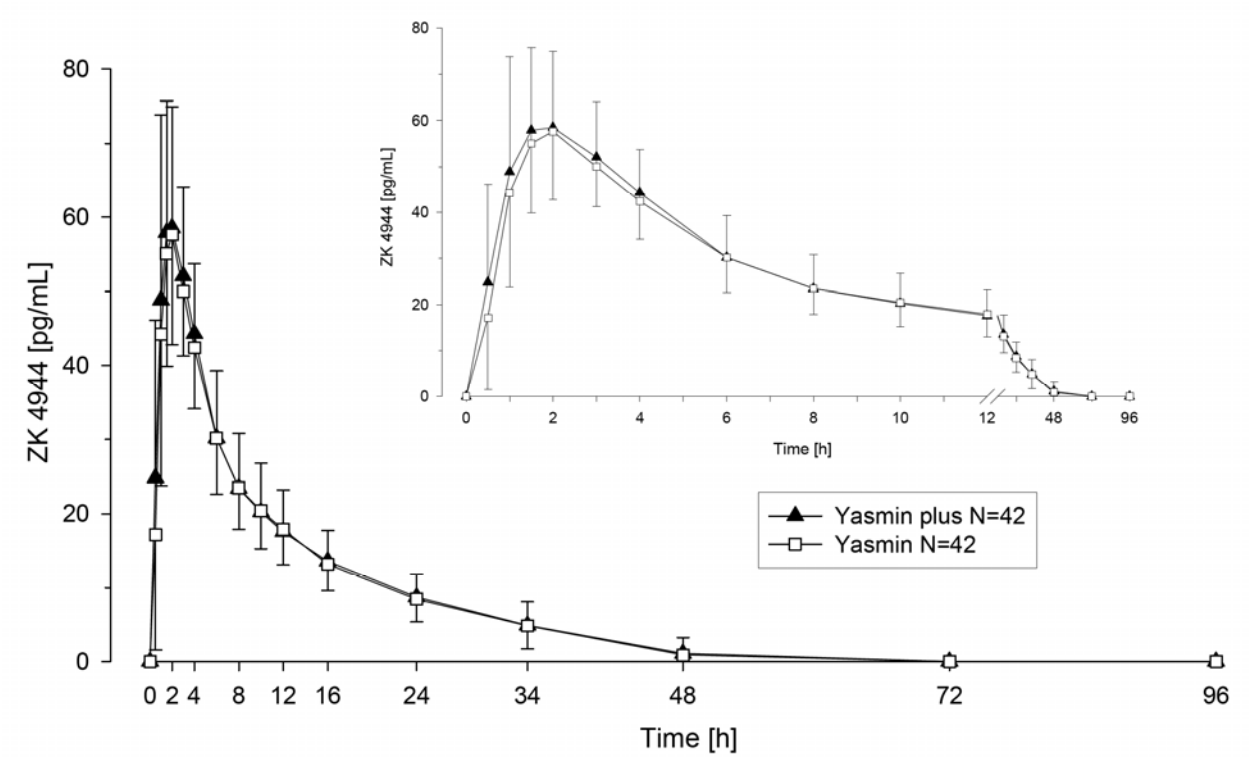
EE:
For Safyral hormone tablet, the mean (±SD) EE C_{max} was 63.8 ± 17.0 pg/mL and the median Tmax was reached 2 hours (range: 1 to 4 hours) after administration. Mean values for AUC(0–tlast) was 620 ± 179 pg*h/mL. There were insufficient data to calculate $T_{1/2}$ and hence AUC(0-inf) in some subjects. Based on the evaluable subjects (n=17), the AUC(0-inf) and $t_{1/2}$ were 777 ± 236 pg*h/mL and 12.5 ± 3.3 hr respectively (Table 16).

PK profiles were similar between Safyral and Yasmin tablets (Figure 6). Results of BE analysis (Table 17) showed that the 90% CI of the test/reference ratios for AUC(0-tlast) and C_{max} were within the 80 – 125% limits.

Table 16: Arithmetic mean (± SD) pharmacokinetic parameters of EE

Treatment	Parameter				
	C_{max} (pg/mL)	T_{max} * (h)	$T_{1/2}$ (h)	AUC(0-inf) (pg*h/ml)	AUC(0-tlast) (pg*h/ml)
Safyral (N=42)	63.8 ± 17.9	2 (1 – 4)	12.5 ± 3.3 (N=17) **	777 ± 236 (N=17) **	620 ± 179
Yasmin (N=42)	61.2 ± 18.8	2 (0.5 – 4)	12.3 ± 3.0 (N=21) **	747 ± 212 (N=21) **	601 ± 184
* = Median (range), ** = There were insufficient data to calculate $T_{1/2}$ and hence AUC (0-inf) in some subjects; parameter values were based on evaluable subjects.					

Figure 6: Mean plasma concentrations of EE (ZK4944)



Yasmin plus = Safyral, ZK 4944 = EE

Table 17: Bioequivalence assessment for EE

EE	N	Yasmin (reference)	Safyral (test)	Test/reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [pg*h/ml]	41	607.7	613.3	101.1	97.7, 104.8
Cmax [pg/mL]	41	62.1	63.2	102.3	96.7, 108.2

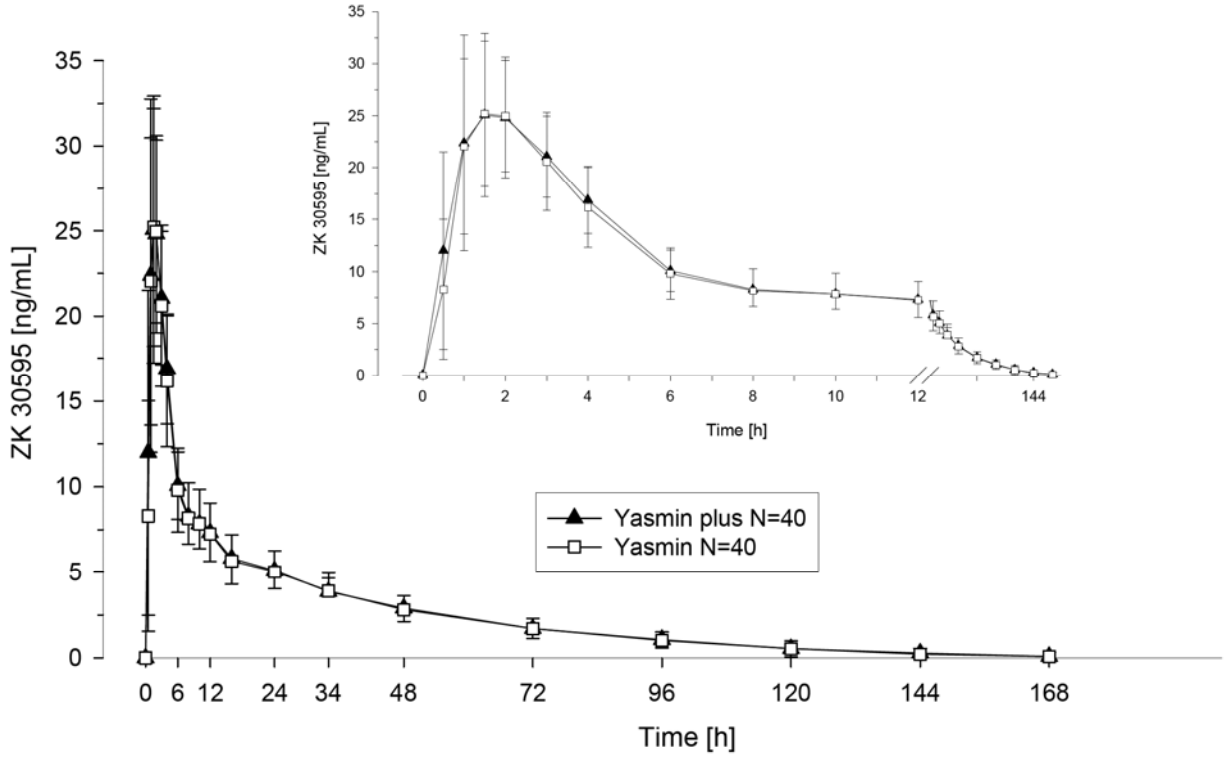
DRSP:

Results based on PPS population:
For Safyral hormone tablet, the mean (±SD) DRSP C_{max} was 27.9 ± 6.6 ng/mL and the median Tmax was reached 1.5 hours (range: 0.5 to 3 hours) after administration. Mean values for AUC(0–tlast) was 426 ± 88 ng*h/mL. The AUC(0-inf) and $t_{1/2}$ were 456 ± 92 ng*h/mL and 33.7 ± 8.5 hr, respectively (Table 18). The mean DRSP PK profiles for Safyral hormone and Yasmin hormone tablets were similar (figure 7).

Table 18: Arithmetic mean (± SD) pharmacokinetic parameters of DRSP (PPS population)

Treatment	Parameter				
	C_{max} (ng/mL)	T_{max}* (h)	$T_{1/2}$ (h)	AUC (ng*h/ml)	AUC(0-tlast) (ng*h/ml)
Safyral (N=40)	27.9 ± 6.6	1.5 (0.5 – 3)	33.7 ± 8.5	457 ± 92	426 ± 88
Yasmin (N=40)	27.3 ± 6.4	1.5 (1 – 3)	33.2 ± 7.8	447 ± 100	416 ± 97
* = Median (range)					

Figure 7: Mean (SD) plasma concentrations of DRSP (ZK 30595)



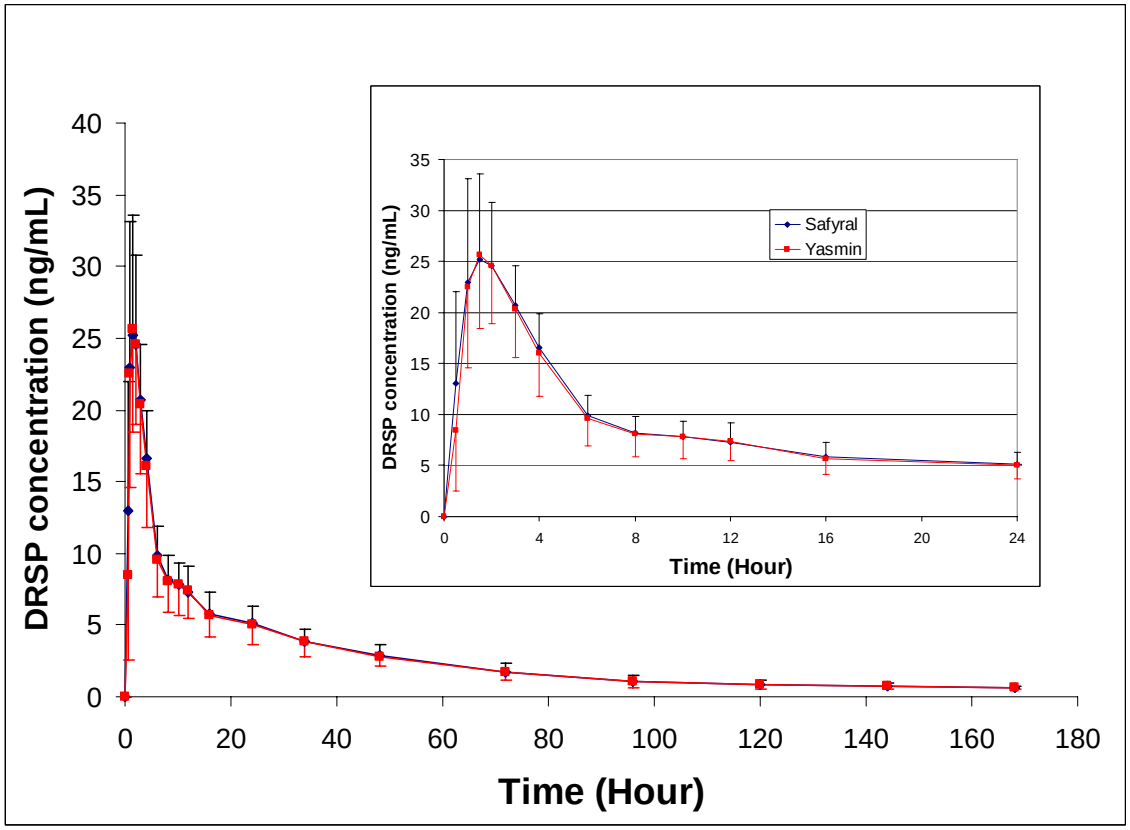
Yasmin plus = Safyral, ZK 30595 = DRSP

Results based on subjects with no samples identified as having matrix interference:
For Safyral hormone tablet, the mean (±SD) DRSP C_{max} was 28.1 ± 7.0 ng/mL and the median Tmax was reached 1.5 hours (range: 0.5 to 3 hours) after administration. Mean values for AUC(0–tlast) was 420 ± 96 ng*h/mL. The AUC(0-inf) and $t_{1/2}$ were 452 ± 100 ng*h/mL and 32.1 ± 8.1 hr, respectively (Table 19). The mean DRSP PK profiles for Safyral hormone and Yasmin hormone tablets were similar (figure 8)

Table 19: Arithmetic mean (± SD) pharmacokinetic parameters of DRSP (Only subjects with no matrix interference)

Treatment	Parameter				
	C_{max} (ng/mL)	T_{max}* (h)	$T_{1/2}$ (h)	AUC(0-inf) (ng*h/ml)	AUC(0-tlast) (ng*h/ml)
Safyral (N=30)	28.1 ± 7.0	1.5 (0.5 – 3)	32.1 ± 8.1	452 ± 100	420 ± 96
Yasmin (N=28)	27.3 ± 6.8	1.5 (1 – 3)	32.2 ± 7.2	444 ± 105	413 ± 102
* = Median (range)					

Figure 8: DRSP mean (SD) plasma PK profile



BE analysis:

The sponsor performed analysis of bioequivalence using 4 different sets of subjects in order to estimate the impact of analytical interferences. The following sets of subjects were analyzed:

1. All subjects, regardless whether interferences were present or not
2. Subjects without interference in any treatment
3. Subjects without interference or interference in only 1 treatment
4. Subjects without interference or interference in both treatments

The BE results are shown below for each data set. In all cases the 90% CI were within the 80 – 125% limits. This reviewer considers set #2 (only subjects without interference in any treatment) to be the primary data set for BE consideration. This set does not include data from samples with interference and

include only those subjects with data from both test and reference treatment, which is consistent with the original statistical analysis plan.

Table 20: BE results – set #1, all subjects

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	39	455.24	453.85	99.45	96.70, 102.28
Cmax [ng/mL]	39	27.78	27.48	98.66	93.37, 104.24

Table 21: BE results – set #2, only subjects without interference in any treatment.

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	21	458.09	459.19	100.20	96.02, 104.57
Cmax [ng/mL]	21	27.79	27.39	97.82	89.94, 106.39

Table 22: BE results – set #3, subjects without interference or interference in only 1 treatment

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	35	455.09	455.46	100.04	96.61, 103.59
Cmax [ng/mL]	35	28.12	27.60	98.01	91.96, 104.46

Table 23: BE results – set #4, subjects without interference or interference in both treatments

DRSP	N	Yasmin (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-inf) [ng*h/mL]	25	457.84	456.08	99.53	95.56, 103.66
Cmax [ng/mL]	25	27.32	27.24	98.80	90.24, 108.16

L-5-MTHF:

PK profiles were similar between Safyral and Metafolin mono tablets. The sponsor conducted BE analysis on both baseline-corrected and baseline-uncorrected PK parameter values. Results of BE analysis showed that the 90% CI of the test/reference ratios for AUC(0-tlast) and C_{max} were within the 80 – 125% limits with or without baseline correction.

Table 24: Arithmetic mean (±SD) for L-5-MTHF PK parameters

Baseline method	Treatment	C_{max} (nmol/L)	AUC(0-tlast) (nmol/L*h)	T_{max}* (h)	$T_{1/2}$ (h)
Baseline - uncorrected	Safyral (n=41)	68.0 ± 19.6	413 ± 129	0.5 (0.5 – 1.5)	NC
	Metafolin mono (n-43)	64.3 ± 17.8	411 ± 140	0.5 (0.5 – 1.5)	NC
Baseline - corrected	Safyral (n=41)	53.9 ± 15.4	244 ± 62.9	0.5 (0.5 – 1.5)	4.33 ± 0.554 (n=11)**
	Metafolin mono (n=43)	50.7 ± 13.8	247 ± 63.7	0.5 (0.5 – 1.5)	4.92 ± 0.352 (n=9)**

* T_{max} is shown as median and range. ** = There were insufficient data to calculate $T_{1/2}$ in some subjects; parameter values were based on evaluable subjects. NC Not calculated

Figure 9: Mean (±SD) baseline-uncorrected serum concentration of L-5-MTHF

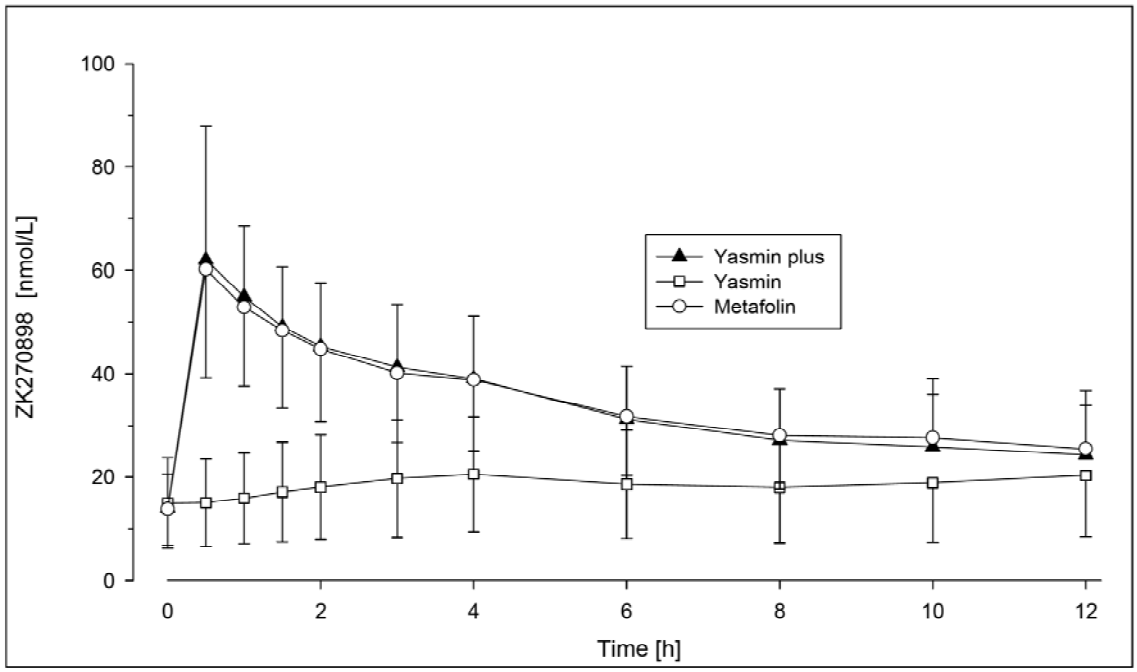


Figure note: Yasmin plus = Safyral

Table 25: Bioequivalence assessment for L-5-MTHF (baseline corrected, PPS)

Metafolin	N	Metafolin mono (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [nmol*h/mL]	41	241.51	236.65	97.98	94.19, 101.93
Cmax [nmol/mL]	41	48.86	51.88	106.19	99.18, 113.68

Table 26: Bioequivalence assessment for L-5-MTHF (baseline uncorrected, PPS)

Metafolin	N	Metafolin mono (Reference)	Safyral (Test)	Test/Reference ratio in %	Two-sided 90% CI in %
AUC(0-tlast) [nmol*h/mL]	41	395.95	394.49	99.63	95.73, 103.69
Cmax [nmol/mL]	41	62.32	65.37	104.90	98.83, 111.34

Conclusions:

The results indicate that Safyral tablets are bioequivalent to Yasmin tablets with respect to EE and DRSP. Similarly, the results indicate that Safyral tablets are bioequivalent to Metafolin mono tablets with respect to L-5-MTHF.

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

/s/

DOANH C TRAN
11/04/2010

MYONG JIN KIM
11/05/2010

BIOPHARMACEUTICS REVIEW Office of New Drugs Quality Assessment			
Application No.:	NDAs 22-532 and 22-574	**Reviewer:** Sandra Suarez Sharp, Ph.D	
Division:	DRUP		
Sponsor:	Bayer HealthCare Pharmaceuticals	**Team Leader:** Angelica Dorantes, Ph.D .	
Trade Name:	YAZ and Yasmin fortified	**Supervisor:** Patrick J. Marroum, Ph.D	
Generic Name:	drospirenone+ ethinyl estradiol + levomefolate calcium	**Date Assigned:**	Aug 3, 2010
Indication:	Oral contraceptives	**Date of Review:**	Aug 9, 2010
Formulation	IR tablet		
Route of Administration	Oral		

SUBMISSIONS REVIEWED IN THIS DOCUMENT

Submission date	CDER Stamp Date	Date of informal/Formal Consult	PDUFA DATE (extended)
Aug 9, 2010	Aug 9, 2010	NA	Sep 2010
Type of Submission:	Addendum to Original NDA reviews		
Type of Consult:	Dissolution method and specifications		

REVIEW SUMMARY:

YAZ® (3 mg of drospirenone and 0.02mg of ethinyl estradiol) and Yasmin (3 mg of drospirenone and 0.03mg of ethinyl estradiol) IR Tablets were approved on 2006 for the prevention of pregnancy in women who elect to use an oral contraceptive.

The sponsor (Bayer Health Pharmaceuticals) is seeking approval of NDA 22-532 and NDA 21-574, a folate fortified, oral contraceptive (OC) regimens which consist on the addition of Metafolin® (levomefolate calcium) to YAZ® and Yasmin®.

The following comments were sent to the sponsor via email regarding the dissolution specifications for both Yasmin + Metafolin and Yaz + Metafolin, respectively (refer to Biopharm reviews for NDAs 22-352 and 22-574 entered in DARRTS on 7/29/10 and 7/7/10, respectively):

1. The following dissolution method and specifications are recommended for Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet 3.0 mg + 0.02 mg + 0.451 mg based on the mean dissolution values from batches used in pivotal clinical trials and drug product stability:

Dosage	USP	Speed	Medium	Volume	Specification

IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active ingredient (drospirenone, ethinylestradiol, and levomefolate calcium) is dissolved in 15 min.

2. The following dissolution method and specifications are recommended for Levomefolate calcium coated tablet 0.451 mg based on the mean dissolution values from batches used in pivotal clinical trials and drug product stability:

Dosage Form	**USP Apparatus**	**Speed (rpm)**	**Medium**	**Volume (mL)**	**Specification**
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active ingredient levomefolate calcium is dissolved in 15 min.

Please revise the dissolution specifications accordingly.

On Aug 10, 2010 the Agency received via email a response to the above comments:

Bayer agrees to change the dissolution specification to Q (b) (4) at 15 minutes for the levomefolate calcium only tablets. However for the drospirenone+ethinylestradiol+levomefolate calcium tablets, we respectfully request that the specification remain Q= (b) (4) at 30 minutes, for the reasons presented below:

1. *Based on the data in section 2.7.1 "Summary of Biopharmaceutic Studies and Associated Analytical Methods", batch-to-batch differences in dissolution rate were observed at 15 minutes that had no impact on the overall bioavailability of the drug substances. These data demonstrate that the 15 minute draw time is overdiscriminatory and that the 30 minute draw time adequately demonstrates the release of the drug in-vivo and ensures its efficiency.*
2. *The dosage form was developed as an immediate release tablet, and not intended to fulfill the criteria for a rapidly dissolving tablet. Therefore we have minimal data at 15 minutes. Based on the data we do have, we believe that we may experience unnecessary stage 2 or 3 testing, or unnecessarily risk batch failures even though there is no impact on the in-vivo performance of the product. We are especially concerned that we only have minimal data regarding the 15 minute draw time during stability studies, and are concerned that setting the specification at 15 minutes without supporting stability data presents a risk of an unnecessary recall.*

Therefore Bayer respectfully requests that the specification for the drospirenone+ethinylestradiol+levomefolate calcium tablets remains Q= (b) (4) at 30 minutes.

Given that Yaz and Yaz + Metafolin were found bioequivalent and Yaz showed a slower dissolution profile with a mean value of (b) (4) dissolved in 30 min, this reviewer agrees with the sponsor's original proposal for the dissolution specification of drospirenone+ethinylestradiol+levomefolate calcium tablets

RECOMMENDATION:

The ONDQA/biopharmaceutics team has reviewed submission dated Aug 3, 2010 to NDAs 22-352 and 21-574. We found this NDA acceptable from biopharmaceutics perspective. The following comments should be conveyed to the sponsor:

1. The following dissolution method and specifications are recommended for Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet 3.0 mg + 0.02 mg + 0.451 mg and Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet 3.0 mg + 0.03 mg + 0.451 mg based on the mean dissolution values from batches used in pivotal clinical trials, bioequivalence study, and drug product stability:

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active ingredient (drospirenone, ethinylestradiol, and levomefolate calcium) is dissolved in 30 min.

2. The following dissolution method and specifications are recommended for Levomefolate calcium coated tablet 0.451 mg based on the mean dissolution values from batches used in pivotal clinical trials and drug product stability:

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active ingredient levomefolate calcium is dissolved in 15 min.

3. Please revise the dissolution specifications accordingly and submit updated specifications sheets for levomefolate tablets for both NDA 22-532 and NDA 22-574.

Sandra Suarez Sharp, Ph. D.
Biopharmaceutics Reviewer
Office of New Drugs Quality Assessment

Patrick J. Marroum, Ph. D.
Biopharmaceutics Supervisor
Office of New Drugs Quality Assessment

Cc: JDavid, ADorantes, Dchristner, HShroff

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22574	ORIG-1	BAYER CORP PHARMACEUTICAL DIV	YASMIN PLUS (DEOSPIRENONE ETHINYL ESTRAD

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

/s/

SANDRA SUAREZ
08/12/2010

PATRICK J MARROUM
08/13/2010

BIOPHARMACEUTICS REVIEW Office of New Drugs Quality Assessment			
Application No.:	NDA 22-574	**Reviewer:** Sandra Suarez Sharp, Ph.D	
Division:	DRUP		
Sponsor:	Bayer HealthCare Pharmaceuticals	**Team Leader:** Angelica Dorantes, Ph.D .	
Trade Name:	Yasmin	**Supervisor:** Patrick J. Marroum, Ph.D	
Generic Name:	drospirenone 3 mg + ethinyl estradiol 0.03 mg + levomefolate calcium 0.451 mg	**Date Assigned:**	Dec 7, 2009
Indication:	Oral contraceptive	**Date of Review:**	July 5, 2010
Formulation	IR tablet		
Route of Administration	Oral		

SUBMISSIONS REVIEWED IN THIS DOCUMENT

Submission date	CDER Stamp Date	Date of informal/Formal Consult	PDUFA DATE
Nov 17, 2009, Feb 9, 2010	Nov 17, 2009	Dec 7, 2009	Sep 16, 2010
Type of Submission:	Original NDA		
Type of Consult:	Dissolution method and specifications---**NDA REVIEW**		

REVIEW SUMMARY:

Yasmin® (3 mg of drospirenone and 0.03 mg of ethinyl estradiol) has been approved for the prevention of pregnancy in women who elect to use an oral contraceptive (NDA 21-098).

The sponsor (Bayer Health Pharmaceuticals) is seeking approval of NDA 22-574, a folate fortified, oral contraceptive (OC) regimen which consists on the addition of Metafolin® (levomefolate calcium) to Yasmin®. 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 (LMCA) (cycle days 1-21) followed by 7 tablets containing 0.451 mg of LMCA only (cycle days 22-28).

In support of this NDA the sponsor included the results of total of four studies, two bioequivalence studies and two pharmacodynamic studies. The bioequivalence studies were performed in order to demonstrate that the addition of LMCA 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®. The PK studies are being reviewed by OCP.

The proposed dissolution method and specifications for this IR tablets are as follows:

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification (Q)
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active is dissolved in 30 min.

It is noted that the approved dissolution method (water) for Yasmin is different from that for the product

under investigation. As part of the dissolution method development, different media covering a pH-range from 1 to 6.8 were tested. Drug release of drospirenone, EE and LMCA was very fast throughout the physiological pH. Stability and sufficient solubility of the drug substances were obtained with phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid.

The three active ingredients dissolved very fast (i.e. > (b) (4) in 15 min). Therefore, this reviewer proposes to tighten the sponsor's specifications for the three active components of Yasmin.

RECOMMENDATION:

The ONDQA/biopharmaceutics team has reviewed NDA 22-574(000) submitted on Nov 17, 2009. We found this NDA acceptable from biopharmaceutics perspective. The following comment should be conveyed to the sponsor:

1. The following dissolution method and specifications are recommended for Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet 3.0 mg + 0.03 mg + 0.451 mg based on the mean dissolution values from batches used in pivotal clinical trials and drug product stability:

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active ingredient (drospirenone, ethinylestradiol, and levomefolate calcium) is dissolved in 15 min.

Please revise the dissolution specifications accordingly.

Sandra Suarez Sharp, Ph. D.
Biopharmaceutics Reviewer
Office of New Drugs Quality Assessment

Patrick J. Marroum, Ph. D.
Biopharmaceutics Supervisor
Office of New Drugs Quality Assessment

Cc: JDavid, ADorantes, Dchristner, HShroff

Background

Chemistry

Yasmin tablet formulation contains the drug substances DRSP, EE as ethinylestradiol betadex clathrate and LMCA. The drug substances drospirenone and EE are well known and were approved by the Agency for use in Yaz® film-coated tablets (0.020 mg EE as ethinylestradiol betadex clathrate and 3 mg drospirenone, NDA 21-676) in 2006.

LMCA is a white to slightly yellowish, almost odorless, crystalline powder. LMCA is utilized as (b) (4) drug substance to ensure the uniformity of distribution, no change of crystalline structure occurs during (b) (4). LMCA is sparingly soluble either in water (1.07 g/100 g at 20 °C) or 0.9 % sodium chloride solution at 20 °C, but it is very

slightly soluble or insoluble in most organic solvents. It is fairly stable as a solid; during long-term storage it shows sensitivity to oxygen, heat and humidity. LMCA is slightly hygroscopic when exposed to the air. For this reason, the product has to be stored protected from air and moisture.

The drug substances drospirenone, EE and LMCA are soluble in aqueous media over a range from pH 1 to 6.8. The solubilities in the tested media at 37 °C are given in Table 1.

Table 1. Solubilities of drospirenone, EE and LMCA in different media

Medium	Drospirenone mg dissolved at 37 °C in 1000 mL	Ethinylestradiol mg dissolved at 37 °C in 1000 mL	Levomefolate calcium mg dissolved at 37 °C in 1000 mL
0.1 M Hydrochloric acid pH 1	12.0	13.7	2770
Acetate buffer pH 4.5	16.6	14.4	8320
Phosphate buffer pH 6.8	13.4	13.2	> 8320

Reviewer's Comments
The three active ingredients can be considered highly soluble since it requires less than 240 mL of media for the solubilization of the amount of each drug in the formulation throughout the physiological pH.

Drug Product
The proposed product is a folate fortified, OC regimen which consists on the addition of Metafolin® (levomefolate calcium) to Yasmin®. The regimen consists of 21 tablets each containing 3 mg of DRSP, 0.03 mg of EE and 0.451 mg of LMCA (cycle days 1-21) followed by 7 tablets containing 0.451 mg of LMCA only (cycle days 22-28). The components and composition for this product are summarized in Table 2.

Table 2. Tablet Formulation for Drospirenone + Ethinylestradiol + Levomefolate calcium film coated tablets

Composition	Reference to standard	Function	Amount [mg]
Drug substance			
Drospirenone micronized	specification	drug substance	3.000
Ethinylestradiol betadex clathrate micronized [a]	specification	drug substance	0.030
Levomefolate calcium (b) (4)	specification	drug substance	0.451
Excipients			
Lactose monohydrate	Ph. Eur., USP/NF, Ph. Jap.	(b) (4)	
Cellulose microcrystalline	Ph. Eur., USP/NF		
Croscarmellose sodium	Ph. Eur., USP/NF, Ph. Jap.		
Hydroxypropylcellulose (b) (4)	Ph. Eur., NF		
Magnesium stearate	Ph. Eur., USP/NF, Ph. Jap.		
(b) (4)	specification		
or alternatively			
Hypromellose (b) (4)	Ph. Eur., USP/NF, Ph. Jap.		
(b) (4)	Ph. Eur., USP/NF		
Talc	Ph. Eur., USP/NF, Ph. Jap.		
Titanium dioxide	Ph. Eur., USP/NF,, Ph. Jap.		
Ferric oxide yellow	Directive 95/45/EC USP/NF, JPE		
Ferric oxide red	Directive 95/45/EC USP/NF, JPE		
(b) (4)	Directive 95/45/EC		

a calculated as ethinylestradiol

(b) (4)

Dissolution Method and Specifications

The following method was used to perform the dissolution testing (release and stability) of all batches of the proposed IR product manufactured in support of this application:

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification (Q)
IR tablet	II (Paddle)	50	phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid	900	No less than (b) (4) (Q) of the labeled amount of each active is dissolved in 30 min.

It is noted that the approved USP dissolution method (see table below)[1] for Yasmin is different from the one proposed for the product under investigation.

Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Specification
IR tablet	II (Paddle)	50	water	900	NLT (b) (4) at 30 min for Drospirenone; NLT (b) (4) at 30 minutes for Ethinyl Estradiol

Dissolution Method Development

In the course of method development the following parameters were evaluated:

- Detection/quantification: The analytical procedure (HPLC with UV and fluorescence detection) for the quantification of the drug substances was evaluated and validated.
- Dissolution medium: Different media covering a pH-range from 1 to 6.8 were tested. Stability and sufficient solubility of the drug substance were obtained with phosphate buffered saline pH 6.8 (Ph. Eur.) containing 0.03 % ascorbic acid.
- Dissolution apparatus: The USP-paddle apparatus 2 was selected.
- Time point: During development dissolution profiles with sampling time points after 5, 10, 15, 30, 45 and 60 minutes were recorded. The specification was finally set after 30 minutes.
- Rotation speed: Methods using 50 and 75 rpm were compared. 50 rpm was found to be most suitable to ensure both discriminatory power and robustness of the dissolution method.
- Discriminatory power: For drospirenone the discriminatory power of the method has been demonstrated. The dissolution behavior of ethinylestradiol and LMCA fulfils the criteria for a "very rapidly dissolving" formulation.
- Robustness: The robustness of the dissolution test method has been investigated to assess the impact of deaeration on the dissolution conditions. There is no difference in the dissolution profiles when deaerated and not deaerated media are used. For the routine testing no deaeration is performed.

Analytical procedure for quantification and its Validation

An HPLC method for quantification of the dissolved drug substances was developed. The detection wavelengths were as follows:

- Detection wavelength (detector 1) UV detector (for the drospirenone and isodrospirenone evaluation peaks) 270 nm
- Detection wavelength (detector 2) Fluorescence detector (for evaluation peak LMCA) excitation 292 nm, measurement 356 nm
- Fluorescence detector (for evaluation peak EE) excitation 281 nm, measurement 305 nm

[1] Clinical Pharmacology review for NDA 21-098 dated 2/24/00.

The following summarizes the results of the validation procedure for the three active ingredients:

Specificity	demonstrated in the presence of dissolution medium and placebo	
Linearity of standard solution	5 to 125 %	
Accuracy	Drospirenone	101.7 %
	Ethinylestradiol	101.2 %
	Levomefolate calcium	100.0 %
Precision		
Repeatability of the method	Drospirenone	RSD ≤ 0.3 % (for 100 % level)
	Ethinylestradiol	RSD ≤ 0.9 % (for 100 % level)
	Levomefolate calcium	RSD ≤ 0.4 % (for 100 % level)
Repeatability of the chromatographic system	Drospirenone	RSD ≤ 0.2 % (for 100 % level)
	Ethinylestradiol	RSD ≤ 0.5 % (for 100 % level)
	Levomefolate calcium	RSD ≤ 0.4 % (for 100 % level)
Intermediate precision	no difference under comparable conditions	
Stability of test solution	stable for at least 24 hours at 12 °C (auto sampler)	
Range	Linearity	5 to 125 %
	Accuracy	50 to 125 %
Robustness of the HPLC-method	demonstrated for several analytical parameters	

Reviewer's Comments

The validation data demonstrated that the analytical procedure is adequate to measure dissolution of Drospirenone + EE + LMCA coated tablet 3.0 mg + 0.03 mg + 0.451 mg

in 900 mL phosphate buffered saline pH 6.8 containing 0.03 % ascorbic acid. It is noted that the sum of the peak areas of drospirenone and iso-drospirenone was used for quantification (see below for more information on the isomeration of drospirenone).

Selection of the pH-value and Buffer System

The dissolution characteristics over the entire physiological pH range of 1 to 6.8 were evaluated at 50 rpm using the media shown in Table 1. Figures 1 to 3 illustrate the dissolution profiles on the 3 active ingredients in these media. At pH 1 and 6.8 drug release of drospirenone, EE and LMCA is very fast. At pH 4.5 the dissolution profiles of drospirenone and ethinylestradiol show a plateau at 85 %, only. Because of the low stability of LMCA in aqueous solutions ascorbic acid has been added to the recommended media. According to the sponsor, at all pH values a formation of iso-drospirenone is observed. At a pH value of 1 the isomerization speed of drospirenone is much higher compared to pH 6.8. During the isomerization small amounts of degradation products occur. The sponsor states that both isomers have identical absorption coefficients. Therefore, the sum of the peak areas of drospirenone and iso-drospirenone was used for quantification.

Phosphate buffered saline pH 6.8 (Ph. Eur.) containing 0.03 % ascorbic acid was then chosen as medium for routine testing, because of the low isomerization speed of drospirenone in this medium.

Figure 1. Influence of the pH on the dissolution of drospirenone from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (mean of 12 tablets).

Figure 2. Influence of the pH on the dissolution of ethinylestradiol from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (mean of 12 tablets)

Figure 3. Influence of the pH on the dissolution of levomefolate calcium from Drospirenone + ethinylestradiol + Levomefolate calcium coated tablet (mean of 12 tablets).

Reviewer's Comments

The proposed media is adequate to determine the dissolution of the actives.

Selection of the Dissolution Apparatus

The paddle method (USP apparatus 2) has been selected as it is commonly used for tablets, film-coated tablets or sugar-coated tablets.

Selection of Rotation Speed

The influence of agitation was checked by performing dissolution tests with Drospirenone + EE + LMCA coated tablet at both rotation speeds. Figure 4 to 6 show dissolution profiles of drospirenone, EE and LMCA, respectively.

Figure 4. Influence of the rotation speed on the dissolution of drospirenone from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (mean of 12 tablets).

Figure 5. Influence of the rotation speed on the dissolution of ethinylestradiol from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (mean of 12 tablets).

Figure 6. Influence of the rotation speed on the dissolution of levomefolate from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (mean of 12 tablets).

A rotation speed of 50 rpm was selected as it showed a better discriminating power.

Selection of specified sampling time
During development dissolution profiles with sampling time points after 5, 10, 15, 30, 45 and 60 minutes were recorded. According to the sponsor, for the routine testing a one-point sampling at 30 minutes has been selected as recommended by the relevant FDA guidelines.

Discriminating power of the dissolution method
The discriminating power of the method was evaluated by comparing the dissolution profiles of two different formulations. Drospirenone + EE + LMCA coated tablet contains (b) (4) lactose monohydrate and (b) (4) cellulose microcrystalline and formulation SH T04532AA that contains no lactose monohydrate and (b) (4) % cellulose microcrystalline.

Figures 7 to 9 show that only for drospirenone a significant discrimination between these two different formulations has been achieved, while for EE and LMCA a moderate discrimination is shown. Nevertheless, the dissolution of ethinylestradiol and LMCA fulfills the criteria for a "rapidly dissolving" formulation, since more than (b) (4)% was dissolved within 15 minutes (paddle apparatus, 50 rpm, pH 1 to 6.8).

Figure 7. Dissolution of drospirenone from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (SH T04532A) and formulation SH T04532AA (mean of 12 tablets)

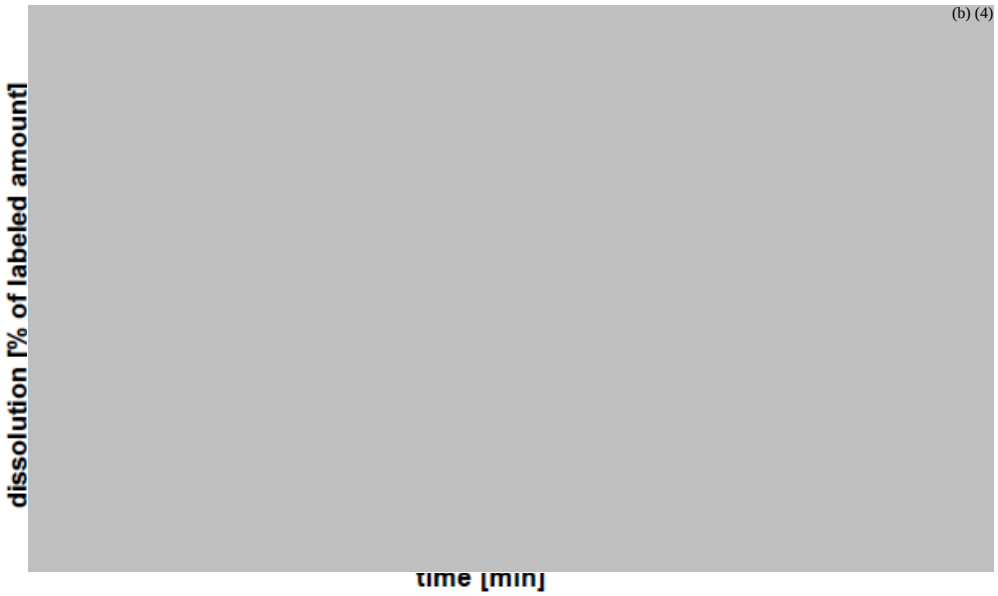


Figure 8. Dissolution of ethinylestradiol from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (SH T04532A) and formulation SH T04532AA (mean of 12 tablets)

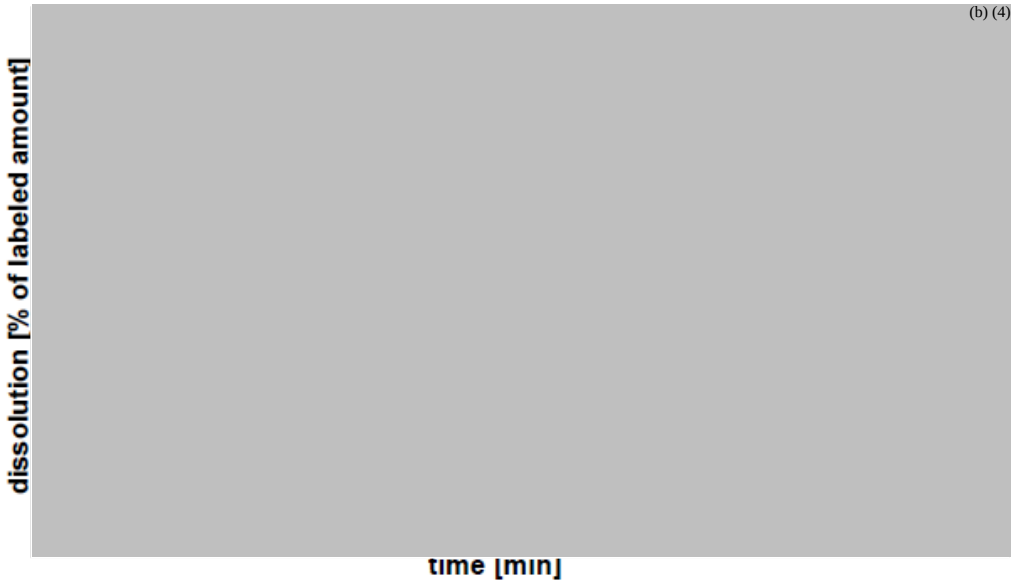


Figure 9. Dissolution of levomefolate from Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet (SH T04532A) and formulation SH T04532AA (mean of 12 tablets).

Setting Dissolution Specifications

The dissolution specification for Yasmin was established based on the results of batches used in clinical trials (Table 3) and on stability batches.

Table 3. Clinical batches of Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet 3.0 mg + 0.03 mg + 0.451 mg

Batch no.	Batch size [kg]	Date of manufacture
WEB7KR	(b) (4)	2006-04-06
WEC1TG		2007-01-07

The results of the release tests (mean) of Drospirenone + EE + LMCA coated tablet 3.0 mg + 0.03 mg + 0.451 mg are given in Table 4 and Table 5, respectively. The individual dissolution rates of Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablet) 3.0 mg + 0.03 mg + 0.451 mg, (n=12) batch no. WEB7KR, are given in Table 6, Table 7, and Table 8.

Table 4. Dissolution rates [%], batch no. WEB7KR

Drug substance	Time [minutes]	V1	V2	V3	V4	V5	V6	Mean ± confidence interval (95 %)	Coefficient of variation
Drospirenone							(b) (4)	93 ± 0.8	0.8
Ethinylestradiol								94 ± 0.9	0.9
Levomefolate calcium								98 ± 0.9	0.8

Table 5. Dissolution rates [%], batch no. WEC1TG

Drug substance	Time [minutes]	V1	V2	V3	V4	V5	V6	Mean ± confidence interval (95 %)	Coefficient of variation
Drospirenone							(b) (4)	95 ± 1.9	1.9
Ethinylestradiol								96 ± 2.0	2.0
Levomefolate calcium								102 ± 6.3	5.9

Table 6. Drosperinone dissolution rates [%], batch no. WEB7KR

Time [minutes]	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	Mean ± confidence interval (95 %)	Coefficient of variation
5												(b) (4)	60 ± 2.3	6.1
10													81 ± 1.3	2.5
15													88 ± 0.7	1.3
30													92 ± 0.7	1.2
45													93 ± 0.6	1.1
60													93 ± 0.8	1.3

Table 7. Ethinylestradiol dissolution rates [%], batch no. WEB7KR

Time [minutes]	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	Mean ± confidence interval (95 %)	Coefficient of variation
5												(b) (4)	83 ± 3.1	6.0
10													92 ± 1.1	1.9
15													93 ± 0.7	1.2
30													93 ± 1.2	2.0
45													93 ± 0.7	1.2
60													94 ± 0.8	1.4

Table 8. Levomefolate dissolution rates [%], batch no. WEB7KR

Time [minutes]	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	Mean ± confidence interval (95 %)	Coefficient of variation
5	(b) (4)												87 ± 4.2	7.7
10													98 ± 1.0	1.6
15													99 ± 1.0	1.6
30													99 ± 1.0	1.6
45													99 ± 1.2	1.9
60													99 ± 1.0	1.6

Reviewer's Comments

Tables 6 through 8 show that more than (b) (4) *of the three active ingredients dissolve in less than 15 min. Therefore, this reviewer proposes the following acceptance criteria:*

Specification
NLT Q= (b) (4) at 15 min for Drospirenone,, Ethinyl Estradiol, and levomefolate

Long-term stability data of 3 production batches of Drospirenone + Ethinylestradiol + Levomefolate calcium coated tablets at storage conditions of 25 °C/60 % RH, 30 °C/75 % RH, and 40 °C/75 % RH were presented covering up to now a storage period of 12 months. The dissolution at 30 min was higher than (b) (4) *at all conditions up to 12 months. In the majority of cases, the dissolution at 30 min for all active ingredients was higher than* (b) (4) *at stage 1 (refer to P.8.3.01-02 submitted 11/16/09).*

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22574	ORIG-1	BAYER CORP PHARMACEUTICAL DIV	YASMIN PLUS (DEOSPIRENONE ETHINYL ESTRAD

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

/s/

SANDRA SUAREZ
07/07/2010

PATRICK J MARROUM
07/07/2010

NDA/BLA Number: 22-574 **Applicant: Bayer** **Stamp Date: 11/16/2009**

Drug Name: Drospirenone 3 mg, ethinyl estradiol 0.03 mg, levomefolate calcium 0.451 mg (Yasmin + Metafolin) **NDA/BLA Type: Original**

On **initial** overview of the NDA/BLA application for RTF:

	Content Parameter	Yes	No	Comment
Criteria for Refusal to File (RTF)				
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	x		
2	Has the applicant provided metabolism and drug-drug interaction information?	x		Literature data
Criteria for Assessing Quality of an NDA				
	Data			
3	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g. CDISC)?	x		
4	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			N/A. Genotyping for polymorphism of 5, 10-Methylenetetrahydrofolate reductase (MTHFR) 677 C>T was performed in studies A39814 and A43598. Individual genotype data were not submitted. The Pharmacogenomics reviewer Dr. Li Zhang has indicated that a request for this data is not needed at this time.
	Studies and Analyses			
5	Has the applicant made an appropriate attempt to determine the reasonable dose individualization strategy for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	x		
6	Did the applicant follow the scientific advice provided regarding matters related to dose selection?			N/A
7	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted in a format as described in the Exposure-Response guidance?			N/A
8	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			N/A
9	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is			N/A

	indeed effective?			
10	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			N/A
11	Is the appropriate pharmacokinetic information submitted?	x		
12	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x		
	General			
13	On its face, is the clinical pharmacology and biopharmaceutical section of the NDA organized in a manner to allow substantive review to begin?	x		
14	Is the clinical pharmacology and biopharmaceutical section of the NDA indexed and paginated in a manner to allow substantive review to begin?	x		
15	On its face, is the clinical pharmacology and biopharmaceutical section of the NDA legible so that a substantive review can begin?	x		
16	Are the clinical pharmacology and biopharmaceutical studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x		
17	Was the translation from another language important or needed for publication?		x	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes_____

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- Please see Comments for Sponsor section of at the end of the filing memo.

Doanh Tran, R.Ph., Ph.D

Reviewing Clinical Pharmacologist Date

Myong Jin Kim, Pharm.D.

Team Leader/Supervisor Date

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	**22-574**	**Brand Name**	**Yasmin Plus (pending)**
OCP Division	**DCP3**	**Generic Name**	**Drospirenone 3 mg, ethinyl estradiol 0.03 mg, levomefolate calcium 0.451 mg**
Medical Division	**DRUP**	**Drug Class(es)**	**Hormone, vitamin**
OCP Reviewer	**Doanh Tran, R.Ph., Ph.D**	**Indication(s)**	**Prevention of pregnancy** (b) (4) **Improvement in folate status in women who elect to use an oral contraceptive**
OCP Team Leader	**Myong Jin Kim, Pharm. D.**	**Dosage Form**	**Tablet**
		Dosing Regimen	**1 tablet daily for 28 consecutive days. The last 7 days of each cycle use tablets containing only the 0.451 mg levomefolate calcium.**
Date of Submission	**11/16/2009**	**Route of Administration**	**Oral**
Estimated Due Date of OCP Review	**7/1/2010**	**Sponsor**	**Bayer HealthCare**
PDUFA Due Date	**9/16/2010**	**Priority Classification**	**Standard**
Division Due Date	**7/16/2010**		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	**Number of studies submitted**	**Number of studies reviewed**	**Critical Comments If any**
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				

fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:	x	2		A39814, A43598
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:	x			A47012
Data sparse:	x			A47012
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:	x	2		A27410, A28575
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References		109		references to support ADME, DDI, PD
Total Number of Studies		113		

Filability and QBR comments

	"X" if yes	**Comments**
Application filable?	**x**	
Comments sent to firm?		
QBR questions (key issues to be considered)		**1. Is Yasmin + Metafolin tablet bioequivalent to Yasmin tablet with respect to the PK of drospirenone and ethinyl estradiol?** **2. Is Yasmin + Metafolin tablet bioequivalent to Metafolin only tablet with respect to the PK of L-5-methyltetrahydrofolate?** **3. Are the labeling proposals for ADME and drug interactions adequately supported by the available data?** **4. Does the single dose BE study provide adequate evidence that levomefolate calcium 0.451 mg does not alter the PK of drospirenone and ethinyl estradiol?**
Other comments or information not included above		
Primary reviewer Signature and Date		
Secondary reviewer Signature and Date		

Filing Memo

Clinical Pharmacology Review

NDA: 22-574

Compound: Drospirenone (DRSP) 3 mg, ethinyl estradiol (EE) 0.03 mg, levomefolate calcium 0.451 mg

Sponsor: Bayer

Date: 12/11/2009

Reviewer: Doanh Tran

Background: Yasmin (DRSP 3 mg and EE 0.03 mg) is an approved oral contraceptive (OC) product (NDA 21-098). The sponsor has developed a new product that contains DRSP 3 mg and EE 0.03 mg as well as 0.451 mg levomefolate calcium (Metafolin®), a crystalline synthetic derivative of the naturally occurring predominant form of folate (i.e., L-5-methyl-tetrahydrofolate or L-5-MTHF). Yasmin + Metafolin consists of 21 tablets each containing 3 mg of DRSP, 0.03 mg of EE, and 0.451 mg of Metafolin, and 7 tablets containing 0.451 mg of Metafolin only. Both Yasmin + Metafolin and Metafolin only tablets are formulated as immediate release tablets. The dosage of Yasmin + Metafolin is one hormone-containing tablet daily for 21 consecutive days followed by one Metafolin only tablet daily for 7 days per treatment cycle. The proposed additional indication for this product is as follows: (b) (4)

The NDA is supported mainly by 2 bioequivalence (BE) studies (studies A27410 and A28575), and 2 pharmacodynamic (PD) studies (studies A39814 and A43598). The endpoints for the PD studies were plasma folate, red blood cell folate, and plasma homocysteine concentrations. Additional supporting data include a PK/PD population analysis using data from the above 4 studies, a relative bioavailability analysis of a literature report comparing L-5-MTHF and folic acid (Langenohl et al. 2003), and 109 literature references related to absorption, distribution, metabolism, and excretion (ADME) properties, drug interaction, and PD of levomefolate calcium. The above information is provided via cross reference to information previously submitted in the Yaz + Metafolin NDA 22-532 (submitted on 8/21/2009).

Bioavailability: Bioavailability of Yasmin + Metafolin tablets was evaluated in the single dose, crossover BE study A27410 (protocol number 309662). This study evaluated plasma DRSP and EE concentrations and serum L-5-MTHF concentrations. This study also provided BE bridging information for EE and DRSP between Yasmin and Yasmin + Metafolin tablets. Additionally, BE analysis was carried out comparing baseline-corrected and baseline-uncorrected L-5-MTHF between Yasmin + Metafolin and Metafolin only tablets. Results provided by sponsor indicate that for these BE analyses, the 90% confidence intervals for the ratios of geometric means for C_{max} and AUC were

within the 80 – 125% range. Summary of calculated PK parameters are shown in tables 1 – 4 below.

Table 1: Mean PK parameters of DRSP after single oral administration of two different tablet formulations containing 0.03 mg EE and 3 mg DRSP with 0.451 mg Metafolin (Yasmin Plus, SH T04532A) and without Metafolin (Yasmin, SH T0470FA)

Treatment	Parameter Cmax (ng/mL)	AUC (ng/mLxh)	AUC(0-72h) (ng/mLxh)	AUC(0-tlast) (ng/mLxh)
Yasmin (N=43)	27.9 (21.0%)	457 (20.1%)	371 (18.1%)	442 (19.4%)
Yasmin Plus (N=41)	27.2 (22.6%)	449 (23.6%) (N=40)	366 (19.8%)	438 (22.9%)

Table 2: Mean PK parameters of EE after single oral administration of two different tablet formulations containing 0.03 mg EE and 3 mg DRSP with 0.451 mg Metafolin (Yasmin Plus, SH T04532A) and without Metafolin (Yasmin, SH T0470FA)

Treatment	Parameter Cmax (pg/mL)	AUC (pg/mLxh)	AUC(0-tlast) (pg/mLxh)
Yasmin (N=43)	61.0 (30.9%)	712 (28.1%) (N=34)	639 (31.0%)
Yasmin Plus (N=41)	60.4 (27.1%)	705 (27.8%) (N=32)	637 (29.1%)

Table 3: Mean PK parameters of L-5-methyl-THF after single oral administration of two different tablet formulations containing 0.03 mg EE and 3 mg DRSP with 0.451 mg Metafolin (Yasmin Plus, SH T04532A) and one tablet containing 0.451 mg Metafolin (SH T04532C) (baseline uncorrected values)

Treatment	Parameter (baseline uncorrected) Cmax (nmol/L)	AUC(0-tlast) (nmol/Lxh)
Yasmin Plus (N=41)	65.2 (30.7%)	393 (32.8%)
Metafolin (N=43)	61.8 (29.2%)	390 (33.2%)

Table 4: Mean PK parameters of L-5-methyl-THF after single oral administration of two different tablet formulations containing 0.03 mg EE and 3 mg DRSP with 0.451 mg Metafolin (Yasmin Plus, SH T04532A) and one tablet containing 0.451 mg Metafolin (SH T04532C) (baseline corrected values)

Treatment	Parameter (baseline corrected) Cmax (nmol/L)	AUC(0-tlast) (nmol/Lxh)
Yasmin Plus (N=41)	51.7 (30.6%)	236 (26.3%)
Metafolin (N=43)	48.7 (30.4%)	239 (26.5%)

Absorption: After oral administration of levomefolate calcium, absorption was rapid with the median T_{max} for L-5-MTHF of 0.5 hour (range 0.5 to 1.5 hour). Mean L-5-MTHF C_{max} of about 50 nmol/L (in study A27410) and about 44 nmol/L (in study A28575) above baseline were observed following single administration of 0.451 mg levomefolate calcium. The sponsor's assessment of the literature data indicates that the pharmacokinetics after single oral administration of equimolar doses of L-5-MTHF and folic acid are comparable (N.B. 0.451 mg levomefolate calcium is equimolar to 0.4 mg folic acid).

Distribution, Metabolism, and Excretion: These data are primarily based on literature reports. The sponsor's conclusions are quoted below. This filing review does not consider the validity of these conclusions.

"Biphasic kinetics is reported for folates with a fast- and a slow-turnover pool. The fast-turnover pool has a half-life of hours and probably reflect[s] newly absorbed folate which is consistent with the terminal half-life of approximately 4 - 5 hours after single oral administration of 0.451 mg Metafolin. The slow-turnover pool reflects turnover of folate polyglutamate and has a mean residence time of greater than or equal to 100 days. Exogenous folate and an enterohepatic folate cycle help to maintain a constant supply of L-5-methyltetrahydrofolate.

L-methyltetrahydrofolate is the predominant folate in the circulation and therefore the folate form normally transported into peripheral tissues to be used for cellular folate metabolism. There are three physiological mechanisms for the transport and the uptake of L-5- methyltetrahydrofolate by various cell types: two carrier-mediated, active transport mechanisms (the reduced folate carrier and the folate receptor), and passive diffusion."

"When comparing 0.451 mg Metafolin with 0.4 mg folic acid, a similar pattern of other important circulating folates (i.e. folic acid, 5-formyl-THF/10-formyl-THF, THF, and 5,10- methylene-THF) were found. The incorporation of L-5-methyl-THF into the cellular folate metabolism is preceded by the conversion to L-tetrahydrofolate via the methionine synthase reaction before effective polyglutamylation and tissue retention is achieved. Folate coenzymes are involved in three major interrelated metabolic cycles in the cytosol of cells. These cycles are required for the synthesis of thymidylate and purines, precursors for DNA and RNA synthesis, and for the synthesis of methionine from homocysteine and the interconversion of serine and glycine."

"The elimination of L-5-methyl-THF from the body occurs by urinary excretion of intact folates and catabolic products and fecal excretion."

Drug-drug interactions: Literature reports related to the effects of folate on other drugs as well as effects of other drugs on folate status were provided.

Specific population: No studies were conducted.

Pharmacodynamics: Direct effect of levomefolate calcium on prevention of neural tube defects (NTD) was not evaluated. The effect of 24 weeks administration of 0.451 mg levomefolate calcium on plasma folate, red blood cell folate, and plasma homocysteine concentrations were compared to no folate in study A43598 (Yaz + Metafolin versus Yaz, protocol 310662) and to 0.4 mg/day folic acid in study A39814 (Yasmin + Metafolin versus Yasmin and 0.4 mg/day folic acid, protocol 309763). The sponsor also provided a population PK/PD report evaluating the pharmacokinetics of folate in plasma and red blood cells.

Clinical vs. to-be-marketed formulations: The sponsor stated that all 4 clinical studies were performed with the proposed to-be-marketed product. Throughout the whole clinical development the tablet composition and manufacturing processes were not changed.

Yaz and Yasmin products used in the BE studies A27410 and A28575 were approved products. Yaz and Yasmin tablets used in the PD studies A43598 and A39814 were in the color of the new Yaz + Metafolin and Yasmin + Metafolin tablets, respectively. Release dissolution testing results were provided, which showed that they met the release dissolution specification.

Method validation: Plasma DRSP and EE, and serum L-5-MTHF concentrations from BE studies A27410 and A28575 were measured using LC/MS/MS methods. Total folate in plasma and whole blood concentration from PD studies A43598 and A39814 were measured using a microbiological assay with Lactobacillus casei. Method validation reports were submitted in the NDA.

Folate metabolite concentrations from study A39814 were measured using a semi-quantitative assay.

Recommendation:

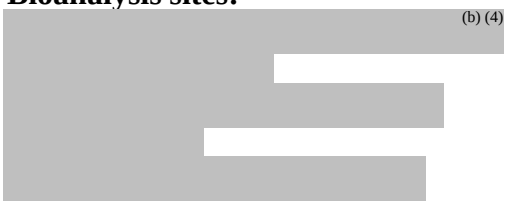
The Office of Clinical Pharmacology/Division of Clinical Pharmacology 3 finds that the Human Pharmacokinetics and Bioavailability section for NDA 22-574 is fileable.

Since bioequivalence study A27410 (protocol 309662) provides the primary link between the new Yasmin + Metafolin product and the approved Yasmin for safety and efficacy of drospirenone and ethinyl estradiol, this reviewer recommends that a Division of Scientific Investigation (DSI) consult be sent for inspection of the clinical study site and select bioanalysis sites listed below that were used for this study.

Clinical study site:
Principle investigators:
A. Port (up to 12/31/2006)
U. Eydeler (from 1/1/2007)

Other investigators:

Bioanalysis sites:



Comments for Sponsor:

- None

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22574	ORIG-1	BAYER CORP PHARMACEUTICAL DIV	YASMIN PLUS (DEOSPIRENONE ETHINYL ESTRAD

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

/s/

DOANH C TRAN
01/04/2010

MYONG JIN KIM
01/06/2010