Pharma Metric Labs

BIOEQUIVALENCE STUDY PROTOCOL

BIOEQUIVALENCE STUDY OF 2 MG CYPROTERONE ACETATE AND 0.035 MG ETHINYL ESTRADIOL FILM-COATED TABLET (ELZSA®) MANUFACTURED BY PT. SYDNA FARMA IN COMPARISON WITH DIANE®-35 SUGAR-COATED TABLET MANUFACTURED BY BAYER WEIMAR GMBH, GERMANY FOR BAYER PHARMA AG, GERMANY, IMPORTED BY PT. BAYER INDONESIA, DEPOK, INDONESIA

Protocol No. : 440/STD/PML/2018

Drug Substance	:	**Cyproterone acetate 2 mg and ethinyl estradiol 0.035 mg**
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Study Director	:	**I Gusti Putu Bagus Diana Virgo, Pharm**
Protocol Author	:	**Olivia Valencia, Pharm**
Issue date	:	**12 March 2019**
Version	:	**01**

1. TITLE PAGE

1.1. Study Title

Bioequivalence study of 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol film-coated tablet (**Elzsa®**) manufactured by PT. Sydna Farma in comparison with Diane®-35 sugar-coated tablet manufactured by Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia**.

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1.10. Start and end date of clinical and analytical study

Dates of Clinical Portion:

Screening	:	After protocol approval from NADFC
Period 1	:	After completion of screening process
Period 2	:	Three weeks after Period 1

Dates of Analytical Portion :

Method Validation	:	December 2018
Sample Analysis	:	After completion of Period 1 and 2

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INVESTIGATOR SIGNATURE PAGE

The undersigned hereby confirmed that the protocol have been read and understood, and agreed to abide by the procedures as stipulated. We agreed to conduct the study with reference to The Indonesian Good Clinical Practice Guideline 2016; The Indonesian Bioequivalence Study Guideline BPOM 2015; ASEAN Guidelines for The Conduct of Bioavailability and Bioequivalence Study 2015; EMA Guideline on the Investigation of Bioequivalence 2010; USFDA Guidance for Industry on Bioavailability and Bioequivalence Studies for Orally Administered Drug Products 2003; Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guideline.

Responsibility	Name	Signature and date
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Study Director	I Gusti Putu Bagus Diana Virgo, Pharm	12 Mar 2019
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Statistician	Niken Pratiwi, Pharm	12 Mar 2019
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2. PROTOCOL SYNOPSIS

Drug under Investigation

2 mg cyproterone acetate and 0.035 mg ethinyl estradiol film-coated tablet manufactured by PT. Sydna Farma (**Elzsa®**) and Diane®-35 sugar-coated tablet manufactured by Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia.**

Title

Bioequivalence study of 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol film-coated tablet manufactured by PT. Sydna Farma (**Elzsa®**) in comparison with Diane®-35 sugar-coated tablet manufactured by Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia**.

Protocol Number

406/STD/PML/2018

Issue date

12 March 2019

Version No.

00

Schedule dates

Dates of Clinical Portion:

Screening	:	After protocol approval from Ethics Committee
Period 1	:	After completion of screening process
Period 2	:	Three weeks after Period 1

Dates of Analytical Portion :

Method Validation	:	December 2018
Sample Analysis	:	After completion of Period 1 and 2

Objectives

The objective of this study is to investigate whether 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol film-coated tablet (**Elzsa®**) manufactured by PT. Sydna Farma is bioequivalent to its reference product, Diane®-35 sugar-coated tablet manufactured by Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia**.

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Study design

Randomized, single blind, two period, single dose, cross-over study with three weeks washout period in 24 healthy female subjects under fasting condition.

The overall clinical study requires approximately 8 weeks to be completed. The drug allocation information is limited to the Data Management Officer only.

If the above study results in the conditions below, the study could be continued to the second stage with a minimum 12 subjects addition. The second stage study's pharmacokinetics will be statistically analyzed within 94.12% confidence interval. The conditions are:

1. The first stage study has demonstrated bioequivalence, but the number of subjects is not adequate to fulfill 80% power study calculated from the intra subject CV of AUC_t obtained; OR
2. The first stage study has resulted in non-bioequivalence but the geometric mean ratio of the AUC_t falls within 90-110%, and it is probable to have bioequivalence result if the study is continued to the second stage.

The subjects are enrolled in the study under the following inclusion criteria: healthy female subjects with absence of significant disease or clinically significant abnormal laboratory values on laboratory evaluation, medical history or physical examination during screening, aged between 18 – 55 years, body mass index between 18–25 kg/m^2, have a normal electrocardiogram, blood pressure within normal range (90-120 mmHg for systolic blood pressure and 60-80 mmHg for diastolic blood pressure), heart rate within normal range (60-100 bpm), pass hormone screening related to cyproterone and ethinyl estradiol, and have read the subject information and signed the informed

consent documents. Investigator or Responsible Physician judgment is required to determine subject's eligibility upon clinically insignificant abnormal results.

Exclusion criteria include pregnancy and nursing condition, hypersensitivity to cyproterone acetate and ethinyl estradiol, or other oral contraceptive or other ingredients in the drugs and allergic history, medical condition which might significantly influence the pharmacokinetic of the study drug e.g. chronic gastrointestinal disease, diarrhea, gastric surgery, renal insufficiency, hepatic dysfunction, or cardiovascular disease, history or present of coagulation disorder, thrombophlebitis, and thromboembolic disorder, history of active ulcer, use of any medication one week prior to the study, participation in any clinical study within 3 months prior to the study (< 90 days), donation or lost 300 ml (or more) of blood within 3 months prior to the study, smoke , positive to HIV, HBsAg and HCV tests, history of drug or alcohol abuse within 12 months prior to the study, and likelihood to be non-compliant to the protocol.

Medical history, physical examination, laboratory tests (routine hematology, blood biochemistry and urinalysis), electrocardiograph, pregnancy test and HIV, HBsAg, and HCV tests are carried out to screen the subjects and to obtain eligible subjects who meet the inclusion and exclusion criteria.

Test Drug, Reference Drug, Dose and Mode of Administration:

The reference drug is the commercially available sugar-coated tablet containing 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol, Diane®-35, manufactured by Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia**, reg number: DKI0868204416A1, batch number: 71533A, manufacturing date: March 2017, and expiration date: March 2020.

The test drug is film-coated tablet containing 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol (**Elzsa®**) manufactured by PT. Sydna Farma, batch number: TR20745, manufacturing date: August 2018, and expiration date: August 2020.

A single dose of the drug (either test or reference according to randomization code) will be given orally to the subjects in sitting posture. The drug will be administered with 240 ml of water.

Study Procedures

The subjects are instructed to abstain from taking any medication for at least 1 week before and during the study period. They are not permitted to smoke, consume alcohol, milk, beverages or food containing xanthines, such as tea, coffee, chocolate, cola, or fruit juice for 24 hours prior to the study day and during the entire sampling day until the last blood sample is completely collected.

Subjects are admitted into the study wards in PT. Pharma Metric Labs in the evening before drug administration. The subjects will have dinner and fast overnight from 8 hours prior drug administration, and continued until 4 hours after the administration. Water can be consumed as desired except during the period of 1 hour before and 2 hours after drug administration.

In the following morning, as scheduled, subjects are given a single dose of cyproterone acetate + ethinyl estradiol of either formulation (test or reference) with 240 ml of water in sitting posture. Subjects are then asked to maintain upright position (standing or sitting) for 1 hour after drug administration.

Blood samples for cyproterone acetate and ethinyl estradiol assay (approximately 6 ml) are drawn into tubes containing anticoagulant (EDTA) before drug administration (0 h) and 15, 30, 45 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 15, 24, 36, 48 and 72 hours after drug administration. Whole blood samples

are collected from fresh clean venipuncture using disposable sterile syringe and needle. Collected blood samples are centrifuged at 3000 rpm for 10 minutes using 32-cm rotor diameter centrifuge. Separated plasma is then stored frozen at a maximum temperature of -20°C until assayed.

Standardized meals are served 4 hours after drug administration. A record of the menu served and meal taken by subjects at every study period will be maintained.

After a washout period of three weeks, the study will be repeated in the same manner with the alternate drug.

The subjects' safety is monitored during the entire study. They are under direct supervision by Study Physician at the study site. Vital signs (blood pressure, pulse, respiration rate and body temperature) are monitored and recorded in the Case Report Form.

Analytical Procedures

Plasma concentration of cyproterone acetate and ethinylestradiol will be measured by a validated LC-MS/MS method.

Plasma containing cyproterone acetate and ethinyl estradiol is spiked with internal standard solution and blended well. The drug is then extracted using an organic solvent. The organic layer is transferred to a clean glass tube and evaporated to dryness under nitrogen stream in a water bath. The residue is then reconstituted with organic solvent and an aliquot is injected into the LC-MS/MS system. The LLOQ is 0.4 ng/ml and 4.99 pg/ml for cyproterone acetate and ethinylestradiol, respectively.

Statistical Procedures

The area under the cyproterone acetate and ethinylestradiol concentration versus time curve from 0 to 72 hours (AUC_t) is then calculated using trapezoidal rule. Bioequivalence of the two formulations will be assessed by comparing the AUC_t, and C_{max} of cyproterone acetate and ethinyl estradiol values after ln transformation of the concentration data. The geometric mean ratios (test/reference) of the ln-transformed data and their 90% confidence intervals will be further analyzed with a parametric method (analysis of variance/ANOVA) using Equiv Test® version 2.0 (Statistical Solution Ltd., Saugus. MA, USA), or manual calculation which has been validated to Equiv Test®.

Bioavailability Parameters and Bioequivalence Criteria

The two products are considered bioequivalent when the 90% confidence intervals of the cyproterone acetate and ethinyl estradiol geometric mean ratio between test and reference product fall within the range of 80.00-125.00% for AUC_t and C_{max}.

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4. ABBREVIATION AND DEFINITION OF TERMS

Symbol	Meaning
AE	adverse event
AUC_t	area under the plasma concentration-time curve
BMI	body mass index
CI	confidence interval
C_{max}	maximum observed plasma concentration
CRF	case report form
EMA	European Medicines Agency
Exp	Expiration
fax	facsimile
GCP	good clinical practice
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIV	human immunodeficiency virus
Inf	infinity
k_e	terminal elimination rate constant
L	litre
LC-MS/MS	liquid chromatography tandem with mass spectroscopy
LLOQ	lower limit of quantitation
ln	lon
Mfg	manufacturing
mL	mililitre
ng	nanogram
N.A	not available
NADFC	National Agency of Drug and Food Control
No.	number
QA	quality assurance
SAE	serious adverse event
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
$t_{½}$	half-life, associated with terminal slope
tel	telephone
t_{max}	time to attain maximum observed plasma concentration
US FDA	United States of America Food and Drug Administration

5. INTRODUCTION

The presence of a great number of commercially available formulations with the same active ingredient allows the consumers to choose alternative formulations with competitive price. However, those formulations must be proven bioequivalent to the reference drug. The method of choice to demonstrate the equivalence is to conduct a comparative bioavailability study. If the results demonstrate bioequivalence, one may subsequently claim that the therapeutic quality of the formulation and its reference is similar. The latter means that both the benefits and side effects are similar, and hence the formulations are interchangeable.

The quality of pharmaceutical products is dependent on many factors, such as the quality of the raw material, manufacturing process, quality control, packing material, etc. In order to ensure the safety and efficacy of a pharmaceutical product, it is important for the pharmaceutical industry to produce drugs with high standard, especially for drugs made of unstable raw material, low content tablet, and formulations which need special manufacturing technique (e.g. slow release tablet, suppository, etc).

5.1 Pharmacology

Cyproterone acetate blocks the effect of endogenously produced and exogenously administered androgens at the target organs by means of competitive inhibition. This results in a gradual regression of signs of androgenisation, irrespective of whether increased androgen values or increased peripheral sensitivity, are the cause of the disorder. The decrease in androgen concentration at the target organs has an additional therapeutic effect.

Apart from the described antiandrogen effect, cyproterone acetate has also a pronounced progestational action. The combination of ethinylestradiol and cyproterone acetate prevents a possible pregnancy by the inhibition of ovulation, the inspissation of cervical mucus so as to constitute a barrier to sperm, and the rendering of the endometrium unreceptive to implantation.

Dosage of Ethynil Estradiol

Hormone replacement therapy for failure of ovarian development e.g. in patients with gonadal dysgenesis: 10 to 50 micrograms daily, usually on a cyclical basis. Initial estrogen therapy should be followed by combined estrogen/progestogen therapy.

Disorders of menstruation: 20 to 50 micrograms daily from day 5 to day 25 of each cycle. A progestogen is given daily in addition, either throughout the cycle or from days 15 to 25 of the cycle.

If a dose is forgotten it should be taken as soon as it is remembered. If it is nearly time for the next dose then the patient should wait until then. Two doses should not be taken together. Forgetting a dose may increase the likelihood of break-through bleeding and spotting.

5.2 Pharmacokinetic properties

Absorption

Following oral administration cyproterone acetate (CPA) is completely absorbed over a wide dose range. Peak serum concentrations of 15 ng/mL are reached at about 1.6 hours after single ingestion. The absolute bioavailability of cyproterone acetate is unknown. Relative bioavailability was calculated, in a study of eight young women, from a dose-corrected comparison of area under the curves (AUC) of serum levels after 100 mg oral and 300 mg intramuscular depot administration and was found to be 80 + 30% when averaged over all volunteers (range 23% - 119%).

Orally administered ethinylestradiol is rapidly and almost completely absorbed. Peak serum concentrations of about 71 pg/mL are reached within 1-2 hours. Absolute bioavailability, as a result of presystemic conjugation and first pass metabolism, is approximately 60%.

Distribution

Cyproterone acetate is almost exclusively bound to serum albumin. Only 3.5 - 4.0% of the total serum drug concentrations are present as free steroid. The ethinylestradiol-induced increase in sex hormone binding globulin (SHBG) does not influence the serum protein binding of cyproterone acetate. The apparent volume of distribution of cyproterone acetate is about 986 ± 437 L.

Ethinylestradiol is highly but non-specifically bound to serum albumin (approximately 98.5%) and induces an increase in the serum concentrations of SHBG. An apparent volume of distribution of approximately 5 L/kg was determined.

Metabolism

Cyproterone acetate is subject to extensive metabolism. The main metabolite in plasma was identified as 15-hydroxy-CPA which is formed possibly via the cytochrome P450 enzyme CYP3A4. The clearance rate from serum is about 3.6 mL/min/kg.

Ethinylestradiol is subject to presystemic conjugation in both small bowel mucosa and the liver. Ethinylestradiol is primarily metabolized by aromatic hydroxylation but a wide variety of hydroxylated and methylated metabolites are formed, and these are present as free metabolites and as conjugates with glucuronides and sulfate. The metabolic clearance rate is about 5 mL/min/kg.

Elimination

Cyproterone acetate serum levels decrease in two phases which are characterized by half-lives of about 0.8 h and about 2.3 to 3.3 days. Cyproterone acetate is partly excreted in unchanged form. Its metabolites are excreted at a urinary to biliary ratio of about 1:2. The half-life of metabolite excretion is about 1.9 days.

Ethinylestradiol serum levels decrease in two phases, the terminal disposition phase is characterised by half-life of approximately 24 hours. Unchanged drug is not excreted, ethinylestradiol metabolites are excreted at a urinary to biliary ratio of 4:6. The half-life of metabolite excretion is about 1 day.

A bioequivalence study to compare the test product, 2 mg cyproterone acetate/0.035 mg ethinylestradiol fixed-dose tablets and the reference product Diane 35® (2 mg cyproterone acetate/0.035 mg ethinylestradiol fixed-dose tablets, Schering France), was performed. The study design was two-way, open, randomised, cross-over study in 35 female postmenopausal healthy volunteers (aged 35-65 years) after single oral dose. The study was clinically well tolerated. The pharmacokinetic parameters of ethinylestradiol are illustrated in Table I and the parameters of cyproterone acetate in Table II.

Table I. Pharmacokinetic parameters of ethinylestradiol

Pharmacokinetic parameters of ethinylestradiol (n=30)			
	Test product	Diane 35®	90% CI
AUC_{0-t} (pg.h/ml)	2044.34 ± 761.49	2026.55 ± 612.80	92.21 – 104.66
$AUC_{0-\infty}$(pg.h/ml)	2274.67 ± 849.00	2229.15 ± 682.69	93.40 – 106.27
C_{max} (pg/ml)	169.23 ± 52.09	177.49 ± 58.72	91.94 – 100.29
$t_{½}$ (h)	16.07 ± 4.76	16.82 ± 5.39	
t_{max} (h)	2.0 ± 0.75	2.00 ± 0.50	

Table II. Pharmacokinetic parameters of cyproterone acetate

Pharmacokinetic parameters of cyproterone acetate (n=30)			
	Test product	Diane 35®	90% CI
AUC $_{0-t}$ (pg.h/ml)	318206.1 ± 78626.9	327887.3 ± 85063.0	94.08 – 100.15
AUC $_{0-\infty}$ (pg.h/ml)	403149.9 ± 116224.5	428492.9 ± 132888.0	90.70 – 97.75
C_{max} (pg/ml)	27436.24 ± 5872.79	29942.10 ± 7268.07	86.23 – 97.63
$t_{½}$ (h)	103.56 ± 24.84	116.81 ± 38.68	
t_{max} (h)	2.00 ± 0.00	1.50 ± 0.50	

A bioequivalence pilot study to compare the test product, 2 mg cyproterone acetate/0.035 mg ethinylestradiol fixed-dose tablets manufactured by PT. Sydna Farma, and the reference product, Diane 35® fixed-dose tablets manufactured by Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany), was performed in 6 healthy female subjects at PT. Pharma Metric Labs, Jakarta. The pharmacokinetic parameters of ethinylestradiol are illustrated in Table III and the parameters of cyproterone acetate in Table IV.

Table III. Pharmacokinetic parameters of ethinylestradiol of the bioequivalence pilot study

Pharmacokinetic Parameter		Test Drug (Lydia®) (n = 6) mean (SD)	Reference Drug Diane®-35 (n = 6) mean (SD)
Primary	C_{max} (pg.mL^{-1})	107.45 (60.00)	86.37 (37.26)
	AUC_t (h.pg.mL^{-1})	1020.47 (411.03)	987.75 (479.76)
Secondary	AUC_{inf} (h.pg.mL^{-1})	1133.58 (431.60)	1098.46 (592.95)
	T_{max} (h)	1.17 (0.38)	1.83 (0.41)
	$T_{½}$ (h)	11.34 (9.04)	9.11 (5.66)

Table IV. Pharmacokinetic parameters of cyproterone acetate of the bioequivalence pilot study

Pharmacokinetic Parameter		Test Drug (Lydia®) (n = 6) mean (SD)	Reference Drug Diane®-35 (n = 6) mean (SD)
Primary	C_{max} (ng.mL^{-1})	18.87 (2.55)	16.47(3.21)
	AUC_t (h.ng.mL^{-1})	119.54 (41.35)	116.13 (40.84)
Secondary	AUC_{inf} (h.ng.mL^{-1})	127.26 (45.23)	122.32 (42.91)
	T_{max} (h)	1.58 (0.20)	1.67 (0.41)
	$T_{½}$ (h)	7.41(3.81)	6.25 (3.66)

5.3 Side-effects

Gastrointestinal	:	Nausea, abdominal pain, vomiting, diarrhea
Metabolism and nutrition	:	Fluid retention
Psychriatric	:	Altered mood, decreased libido, increased libido
Nervous system	:	Headache, migraine
Skin and subcutanoeous tissue	:	Rash, urticaria, erythema nodosum
Reproductive system and breast	:	Breast pain, breast hypertrophy, vaginal discharge, breast discharge
Imune system	:	Hypersensitivity
Investigations	:	Increased weight, decreased weight

The risk of venous thromboembolism (VTE) occurring with these medicines is low and well known, and warnings are included in their product information to alert patients and prescribers to the risks.

There have been no reports of serious deleterious effects from overdose. On the basis of general experience with combined oral contraceptives, symptoms that may occur in case of overdose of active tablets are: nausea, vomiting and, in young girls, slight vaginal bleeding.

6. OBJECTIVES

The primary objective of this study is to find out whether 2 mg cyproterone acetate and 0.035 mg ethinyl estradiol film-coated tablet (**Elzsa®**) manufactured by PT. Sydna Farma is bioequivalent to its reference product, Diane®-35 sugar-coated tablet manufactured by Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia**.

7. PRODUCT INFORMATION

7.1 Test product:

Name	:	**Elzsa®** film-coated tablet
Active ingredient	:	Cyproterone acetate and ethinyl estradiol
Dosage form	:	Film-coated tablet
Strength	:	2 mg cyproterone acetate + 0.035 mg ethinylestradiol
Manufacturer	:	PT. Sydna Farma
Reg No.	:	N.A.
Batch No	:	TR20745
Mfg Date	:	August 2018
Exp. Date	:	August 2020
Dose	:	1 film-coated tablet (administered with approximately 240 ml water)
Batch size	:	200,000 film-coated tablets

7.2 Reference / comparator product

Name	:	Diane®-35
Active ingredient	:	Cyproterone acetate and ethinyl estradiol
Dosage form	:	Sugar-coated tablet
Strength	:	2 mg cyproterone acetate + 0.035 mg ethinylestradiol
Manufacturer	:	Bayer Weimar GmbH, Germany for Bayer Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia**
Reg No.	:	DKI0868204416A1
Batch No	:	71533A
Mfg Date	:	March 2017
Exp. Date	:	March 2020
Dose	:	1 sugar-coated tablet (administered with approximately 240 ml water)

The test batch or lot must be manufactured according to cGMP and originated from a batch of industrial scale. If this is not feasible, a small production batch or pilot batch can be used provided not smaller than 10% of the industrial scale batch, or 100,000 units (whichever is larger).

7.3 Pharmaceutical equivalence data

The pharmaceutical equivalence data will be provided by sponsor. The difference of the assay between test and reference product should not be more than 5%.

7.4 Comparation of dissolution profiles

The result of the comparative dissolution test will be provided by sponsor.

7.5 Letter of Confirmation

Letter of confirmation will be provided by sponsor. This letter confirms that the formula of the product which will be commercially marketed is the same as the formula of the test drug used in the bioequivalence study.

8. INVESTIGATIONAL PLAN

8.1. Study design

Randomized, single blind, two period, single dose, cross-over study with three weeks washout period in 24 healthy female subjects under fasting condition.

The overall clinical study requires approximately 8 weeks to be completed. The drug allocation information is limited to the Data Management Officer only.

If the above study results in the conditions below, the study could be continued to the second stage with a minimum 12 subjects addition. The second stage study's pharmacokinetics will be statistically analyzed within 94.12% confidence interval. The conditions are:

1. The first stage study has demonstrated bioequivalence, but the number of subjects is not adequate to fulfill 80% power study calculated from the intra subject CV of AUC_t obtained; OR
2. The first stage study has resulted in non-bioequivalence but the geometric mean ratio of the AUC_t falls within 90-110%, and it is probable to have bioequivalence result if the study is continued to the second stage.

8.1.1. Subject screening and selection

Subject screening and selection take place prior to the sampling period. Informed consent must be obtained from each subject before the screening process. Participation must be voluntary. All subjects will be informed of possible side effects and be advised that they are free to withdraw from the study at any stage. A subject information sheet and consent form in the subjects' first language will be provided.

The investigator informs all the subjects about the details of the study, i.e. the objective and the procedure, as well as the study rules to be followed during the study and the possible adverse effects of the drug.

Medical history, physical examination, laboratory tests (routine hematology, blood biochemistry and urinalysis), electrocardiograph, pregnancy test and HIV, HBsAg, and HCV tests are carried out to screen the subjects and to obtain eligible subjects who meet the inclusion and exclusion criteria.

The Study Coordinator or Study Physician takes subjects' demography data which include full name, sex, date of birth, age, address, race, height, weight, and smoking and alcohol consumption habit.

The medical history taken includes systemic review of allergies, family history, cramps or pain legs history and surgical history. Prior to physical examination and laboratory tests, the hormonal screening will be conducted to ensure that there is no interference with endogenous substance baseline levels.

The physical examination includes overall appearance, eyes, ears, nose, throat, head and neck, heart, lung, abdomen (including liver and spleen), lymph nodes, skin, musculoskeletal, and nervous system examination. The responsible physician will also examine if there is any sign of varices. The vital signs will also be measured, which include blood pressure, pulse, respiration rate and body temperature.

The laboratory tests cover complete hematology (hemoglobin, leucocyte, white blood cell differential counts, erythrocyte, platelet, hematocrite, and erythrocyte sedimentation rate), blood chemistry (alanine aminotransferase/ALT/SGPT, aspartate aminotransferase/AST/ SGOT, alkaline phosphatase, bilirubin, blood glucose level, ureum, and creatinine), and urinalysis (density, pH, leucocyte, nitrite, albumin/protein, glucose, ketone (acetone), urobilinogen, bilirubin, and blood count). A total of 15 mL of blood will be taken for the laboratory test.

Pregnancy test is carried out in screening process and on the day of sampling just before the drug administration. Women of childbearing potential are advised to take the necessary precaution to prevent pregnancy and to report to the Investigator or Study Physician if they suspect pregnancy.

The subjects are required to meet the inclusion and exclusion criteria. The screening data of all subjects will be evaluated and subjects with any current or past medical condition which may significantly affect the study results and assessment will be excluded from the study. Investigator or Responsible Physician judgment is required to determine subject's eligibility upon clinically insignificant abnormal results.

Inclusion criteria

The inclusion criteria are healthy female subjects who/with:

- have read the subject information and signed informed consent documents
- age 18 – 55 years
- body mass index between 18–25 kg/m^2
- have a normal electrocardiogram
- blood pressure within normal range (90-120 mmHg for systolic blood pressure and 60-80 mmHg for diastolic blood pressure)
- heart rate within normal range (60-100 bpm)
- with absence of significant disease or clinically significant abnormal laboratory values on laboratory evaluation, medical history or physical examination during screening
- pass hormone screening related to cyproterone acetate and ethinyl estradiol

Exclusion criteria

Any of the following criteria will exclude the subject from the study:

- those who are pregnant and/or nursing woman.
- those with history of hypersensitivity to cyproterone acetate and ethinyl estradiol or other oral contraception or other ingredients in the drugs or a history of serious allergic reaction to any drug, significant allergic disease, allergic reaction, or active allergic.
- those with a history or presence medical condition which might significantly influence the pharmacokinetic of the study drug, e.g. chronic gastrointestinal disease, diarrhea, gastric surgery, renal insufficiency, hepatic dysfunction or cardiovascular disease.
- those with a history or presence of any coagulation disorder or clinically significant hematology abnormalities, thrombophlebitis, and thromboembolic disorder.
- those who are using any medication (prescription or non-prescription drug, food supplement, herbal medicine, oral contraception, anti-platelet drug), particularly the medication known to affect the pharmacokinetic of the study drug, within one week prior to the drug administration day.
- those who have participated in any clinical study within 3 months prior to the study (< 90 days).
- those who have donated or lost 300 ml (or more) of blood within 3 months prior to the study.
- those who smoke.
- those who are positive to HIV, HBsAg, and HCV tests (to be kept confidential).
- those with a history of drug or alcohol abuse within 12 months prior to screening for this study.
- those who are unlikely to comply with the protocol, e.g uncooperative attitude, inability to return for follow up visits, poor venous access.

8.1.2. Restriction

The subjects are instructed to abstain from taking any medication for at least one week before and during the study period unless it is medically necessary. If ingestion of any drug during the study is considered necessary, the Investigator has to be consulted and the drug has to be recorded (also its dose and time of administration) as a concomitant medication.

They are not permitted to smoke, consume alcohol, milk, beverages or food containing xanthines, such as tea, coffee, chocolate, cola, or fruit juice for 24 hours prior to the study day and the entire sampling day until the last blood sample is completely collected. The subjects have to avoid severe physical exertion during sampling hours.

On the evening before the study day, Study Physician will interview the subjects for the restriction compliance.

8.1.3. Standardization of study condition

Subjects stay at the study site one night before and during the sampling period for at least 24 hours after drug administration. Subjects are admitted to the study wards at PT. Pharma Metric Labs in the evening before the study day. Subjects are instructed to fast from 8 hours before until 4 hours after drug administration. Food intake during the study period will be standardized for all subjects. The menu served and meal taken by subjects at every study period should also be recorded. Water can be consumed as desired except during the period of 1 hour before and 2 hours after drug administration.

8.1.4. Drug administration

At approximately 1 hour before drug administration, the subjects undergo physical examination for baseline vital sign data (body temperature, blood pressure, heart rate/pulse, and respiration rate). Female subjects of child-bearing age must undergo urinary pregnancy test. The data will be recorded in the CRFs.

As scheduled, starting at 07:00 in the morning of the sampling day, subjects are given a single dose of cyproterone acetate and ethinyl estradiol of either formulation (test or reference) with 240 ml of water. Subjects administer the drug products in sitting posture. Subjects are asked to maintain upright position (standing or sitting) for 1 hour after drug administration.

8.1.5. Pharmacokinetic sampling

Blood samples for cyproterone acetate and ethinylestradiol assay (approximately 6 ml) are drawn into tubes containing anticoagulant (EDTA) before drug administration (0 h) and 15, 30, 45 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 15, 24, 36, 48 and 72 hours after drug administration. Whole blood samples are collected from fresh clean venipuncture using disposable sterile syringe and needle.

The predose sample is collected within an hour before the first drug dosing SN01. A total volume of 216 ml of blood will be collected from each subject subject (2 periods x 18 sampling points x 6 mL per sampling points) of 108 mL per period. This volume of blood was used for pharmacokinetic analysis.

Collected blood samples are centrifuged at 3000 rpm for 10 mins using centrifuge with 32-cm rotor diameter, plasma is separated and stored frozen at a maximum temperature of -20°C until assayed.

8.1.6. Labeling of the samples

The blood collection tubes are labeled with the study number, study period, subject number and sample code, e.g. P1, T1, SN01, 440 is period 1, sample 1, subject 01, study number 440.

8.1.7. Subject monitoring

During the study period all subjects are under direct supervision by Study Physician at the study site. A brief clinical history interview and physical examination must be conducted a day before the study, and repeated again before patient discharge.

Vital signs (blood pressure, pulse, respiration rate and body temperature) are also monitored and recorded in the CRF. The baseline vital signs should be measured prior to drug administration, and subsequently at 1, 3, 5, 9, 15, 24, 36, 48 and 72 hours after drug administration. These vital signs can be measured before or after blood collection. If the vital signs are outside the normal limit, the physician will take action needed to normalize the condition or withdraw the subjects from the study. Any adverse events which may occur during the study are monitored and should also be recorded in the CRF.

8.1.8. Removal of Subject from assessment

Subjects may withdraw, at their own request, from the study at any stage. If the subject discontinues from the study at any time after being assigned to a subject number, the CRF must be completed accordingly. Subjects may be discontinued from the study for any of the following reasons:

a. Subjects wish to withdraw from the study, irrespective of the reason,

b. Adverse event of the study drug,
c. Illness requiring medication that interfere with the pharmacokinetics of the study drug,
d. Protocol violation by the subjects that interfere with the pharmacokinetics of the study drug,
e. Pregnancy test is positive
f. Subject vomited on or before 2x median of t_{max} under BE study of immediate release drug, or vomited after dosing and within blood sampling period under BE study of modified release drug,
g. Subject experienced diarrhea within blood sampling period.

The Investigator is free to discontinue any subject participation in the study if in her opinion, continued participation is not in the subject interest or may jeopardize the subject health. The subject must be withdrawn from further participation in the study if they develop a serious adverse event that may be related to drug administration.

8.2. Study Treatments

8.2.1. Drug accountability

The test drug with the certificate of analysis and the reference drug are supplied by the sponsor. The certificate of analysis contains product description, results of test as per protocol specifications/pharmacopoeia. In vitro dissolution data is desirable if it is available. The drug is stored in a locked drug storage area. The Responsible Pharmacist is accountable for the study drug. The dispensing and inventory records are kept in the study site file. This record is available for inspection by the sponsor or the regulatory personnel. The sponsor will have to supply a sufficient number of study drugs.

At the end of the study, all unused medications (test and reference drugs) will be returned to the sponsor or destroyed if authorized by the sponsor.

8.2.2. Coding / labeling of the drug used in the study

The label of the study drug is as following:

No. of study protocol	:	440/STD/PML/2018
Reference Drug	:	Diane®-35
Manufactured by	:	Bayer Weimar GmbH, Germany for Pharma AG, Germany, **imported by PT. Bayer Indonesia, Depok, Indonesia**
Batch Number	:	71533A
Manufacturing date	:	March 2017
Expiration date	:	March 2020
Mode of administration	:	per oral (po)
Storage Condition	:	Store below 25°C
		CAUTION
		FOR CLINICAL STUDY ONLY

No. of study protocol	:	440/STD/PML/2018
Test Drug	:	**Elzsa®** film-coated tablet
Manufactured by	:	PT. Sydna Farma
Batch Number	:	TR20745
Manufacturing date	:	August 2018
Expiration date	:	August 2020
Mode of administration	:	per oral (po)
Storage Condition	:	Store below 25°C
		CAUTION
		FOR CLINICAL STUDY ONLY

8.2.3. Blinding

The drug allocation information is limited to the Data Management Officer only. The rest of the study team members are blinded to the treatment sequence.

8.2.4. Randomization

The randomization is performed using block randomization, and the Test-Reference and Reference-Test sequences are balanced. The randomization code is provided by the Data Management Officer, sealed and stored in a locked place, and is limited to the Data Management Officer only.

The code breaking (decoding) will be carried out after all blood samples are completely analyzed and just before the statistical analysis. The sponsor's representative will open the randomization code.

8.2.5. Drug administration

On the sampling day as per schedule, subjects are given a single dose of cyproterone acetate and ethinyl estradiol of either formulation (test or reference) with 240 ml of water.

8.2.6. Washout period

Washout period is determined based on the half-life of the study drug (analyte). At least 5 times elimination half-life is necessary. The wash out period for this study is three weeks. After a washout period, the study is repeated in the same manner with the alternate drug.

8.3. Clinical and Safety Records

8.3.1. Adverse events (AEs) monitoring

The subjects' safety is monitored during the entire study. Information regarding adverse events of the study drugs is obtained from physical examination and/or complaint from the subjects. All subjects are instructed to report the occurrence of any discomfort feeling to the Study Physician. An adverse event is any undesirable event associated with the use of drug, whether or not considered drug related. They include any side effect, injury, toxicity, or hypersensitivity reactions, also any undesirable clinical or laboratory change, which does not commonly occur in the subject.

All adverse events, regardless of severity, will be followed up by the Investigator until they are resolved in a satisfactory manner.

8.3.2. Classification of Adverse event (AE) intensity

The intensity of an adverse event is classified according to the following criteria:

Mild : awareness of the signs and symptoms but is easily tolerated

Moderate : sufficient discomfort to cause interference with normal activities

Severe : incapacitating with inability to perform normal activities.

The intensity of any AE which occurs during the study will be documented in the CRFs.

8.3.3. Classification of Adverse event (AE) causality

The causal relationship of an adverse event is classified to the following criteria:

Causality term	Assessment criteria, all points should be reasonably complied with
Definite / certain	• Event with plausible time relationship with drug intake • Cannot be explained by disease or other drug • Event definite pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or recognized pharmacological phenomenon)
Probable/likely	• Event with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs
Possible	• Event with reasonable time relationship to drug intake • Could also be explained by other disease or other drug • Information on drug withdrawal may be lacking or unclear
Unlikely	• Event with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations
Unrelated	• Where there is sufficient information to conclude no causal relationship

8.3.4. Serious adverse event (SAE) reporting

A serious adverse event is any adverse event that is fatal, is life-threatening, requires or prolongs hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly/birth defect, or other medically important events which may not life threatening/cause death/ hospitalized but may jeopardize the patient or may require intervention to prevent one of the outcomes above.

Life-threatening SAE means that the patient is at immediate risk of death from the events as it occurred. It does not include an event that, has it occurred in a more serious form, may have caused death.

Requires hospitalization SAE is defined as hospital admission if required for treatment of the adverse event.

In the event of a SAE, for reporting purposes, Principal Investigator or Study Director may be required to break the individual randomization code of the related subject. Record of this decode will be maintained.

If any SAE occurs, the Investigator will notify the sponsor within 24 hours and to Ethic Committee within 3 calendar days. The sponsor's responsibility is to report a SAE to the regulatory authority after the investigator's note has been delivered within 15 calendar days.

Any SAE will be reported to:

Nony Shilviani
PT. Sydna Farma
Jl. RC Veteran No. 89, Bintaro
Jakarta 12330
Indonesia
Tel : +62 21 736 3738
Fax : +62 21 736 3739
Email : nony.shilviani@sydna-farma.com

The Investigator is responsible to assure the care or hospitalization of the subjects suffering from study-related adverse events, and the cost for the treatment will be covered by the sponsor.

8.4. Pharmacokinetics parameter

The area under the cyproterone acetate and ethinyl estradiol concentration versus time curve from 0 to 72 hours (AUC_t) is calculated using trapezoidal method.

The total area under the curve from administration to infinity (AUC_{inf}) is calculated as the sum of AUC_t and the residual area (C_t divided by k_e, where C_t is the last measured concentration and k_e is the apparent terminal elimination rate). k_e is estimated by log-linear regression from at least 3 concentrations at the terminal portion of the log-transformed concentration plot. The $t_{½}$ is calculated by dividing 0.693 by k_e.

The following equation is used for calculation:

$AUC_{inf} = AUC_t + C_t/k_e$

$t_{½} = 0.693/k_e$

The peak plasma concentration (C_{max}) and the time to attain peak (t_{max}) are determined by inspection of the individual plasma concentration-time profiles of the drug (the observed values).

8.5. Statistical evaluation

Bioequivalence of the two drug formulations is assessed by calculating individual AUC_t and C_{max} values. The mean ratio (test/reference) of the ln-transformed data and their 90% confidence intervals is analyzed with a parametric method (analysis of variance/ANOVA) using Equiv Test® version 2.0 (Statistical Solution Ltd., Saugus. MA, USA) or manual calculation which has been validated to Equiv Test®

<u>90% *confidence intervals*</u> (90% CIs) are calculated using the following equation :

(90% CI) $_{diff.}$ = *difference* $\pm$ $t_{0.1\ (n-2)}$ x $SE_{diff.}$

difference = mean of ln T – mean of ln R

n = number of subject

$\alpha = 0.05$

SE diff = $(½MS_{residual} \times (1/nTR + 1/nRT))^{½}$

(90% CI) ratio = anti ln $(90\% CI)_{diff} \times 100\%$

The two products are considered bioequivalent when the 90% confidence interval of the cyproterone acetate and ethinyl estradiol geometric mean ratio between test and reference product falls within the range of 80.00 - 125.00% for AUC_t and C_{max}.

If a pre-dose concentration of cyproterone acetate and ethinyl estradiol is detected, the subject's data can be included in the pharmacokinetic and statistical analysis without adjustment, if the pre-dose concentration is equal to or less than 5% of the C_{max} value of the corresponding period. If the pre-dose concentration is greater than 5% of the C_{max} value, the subject's data will be dropped from all pharmacokinetic and statistical evaluations of cyproterone acetate and ethinyl estradiol.

Subjects who lack of any measurable concentrations, or have only very low plasma concentrations of cyproterone acetate and ethinyl estradiol for the reference medicinal product, will be excluded from statistical analysis. According to EMA Guidance (CPMP/EWP/QWP/1401/98 Rev.1/Corr**), a subject is considered to have very low plasma concentrations if its AUC is less than 5% of reference medicinal product geometric mean, calculated without inclusion data from the outlying subject. Data (concentrations and pharmacokinetic parameters) from these excluded subjects will be presented but excluded from descriptive statistics. If the study is continued to the second stage, the 94.12% confidence intervals (94.12% CIs) are calculated using $\alpha = 0.0294$.

8.6. Assay methodology and validation

8.6.1. Assay method description

Plasma concentration of cyproterone acetate and ethinyl estradiol is measured using a validated LC-MS/MS method. Plasma is first spiked with internal standard solution, then further mixed using vortex. The drug is then extracted with organic solvent. The organic layer is transferred to a clean glass tube and evaporated to dry under nitrogen stream in a water bath. The residue is then reconstituted with organic solvent and an aliquot is injected into the LC-MS/MS system. The LLOQ is 0.4 ng/ml and 4.99 pg/ml for cyproterone acetate and ethinylestradiol, respectively.

8.6.2. Instruments

Blood samples and standards are transferred or taken using Soccorex multipippette. The standard is weighed using a Mettler Toledo XP 26DR micro analytical balance. A Hettich Rotofix 32A centrifuge is used to separate plasma and the supernatant liquid mixture. A Caliper TurboVap LV dwell that applies nitrogen is used to evaporate the solvent.

The LC-MS/MS system included an Acquity™ solvent delivery manager, Acquity™ sample manager and TQD™ MS/MS from Waters Corp. Data acquisition, peak integration and calibration were performed by Mass Lynx software from Waters Corp. Its separation is performed on a C18 column and protected by suitable guard column.

The chromatographic system was as follows:

Column	: Waters Acquity BEH C_{18} 1.7 µm, 2.1 x 50 mm
Mobile phase	: Acetonitrile : 0.1% Formic acid in H_2O (80:20)
Flow rate	: 0.3 mL/minute

8.6.3. Quantification

The calculation of drug level is performed using cyproterone acetate and ethinyl estradiol calibration curve based on the peak ratios of the analyte versus internal standard used at the specified concentrations (1 blank, 1 zero, and 6-8 nonzero). The result of the calibration curve is a linear regression line (statistically calculated).

8.6.4. Validation

The method validation is performed according to international guideline. The assay method is validated to demonstrate adequate selectivity, linearity, accuracy and precision, carry-over, matrix effect, dilution integrity, LLOQ, anticoagulant effect and stability.

8.7. Quality assurance

The Quality Assurance (QA) department reviews BA/BE study conduct at regular intervals and maintain written and signed records of findings and problems related to the study. Any problem found during review, which may affect the integrity of the study data, will be brought to the attention of the Principal Investigator.

The QA department or sponsor representative will assure that no deviations from the approved protocols or standard operating procedures without proper authorization and documentation. The QA department will review the analytical report and the bioequivalence study report to assure that those reports accurately reflects the raw data.

8.8. Others

8.8.1. Clinical records

All data generated during the study is directly entered into the CRF accurately. All entries must be legibly recorded in blue ink, while corrections are made by crossing the wrong entry with a single line and writing the correct entry. All corrections must be initialed and dated.

Data from analytical laboratory are transcribed into appropriate forms and recorded. Chromatograms are treated as raw data, and data derived from statistical and pharmacokinetic analysis are archived. Personnel involved in the study, monitors from sponsor, or regulatory representatives have access to the source data. All data are liable for quality assurance audit. Copies of all pertinent information (data) are retained by PT. Pharma Metric Labs for at least 5 years after study completion. Additional considerations are made for complying with the applicable local laws and guidelines.

8.8.2. Documents necessary to start the study

The following documents are necessary before starting the study:

- Study protocol,
- Information for subjects and informed consent,
- Case Report Form,
- Ethics Committee/Institutional Review Board written approval of the study protocol & information for subjects,
- Study drug certificate of analysis, comparative dissolution profiles and letter of confirmation,
- NADFC written approval of the study protocol
- Clinical Trial Import License (if required)

8.8.3. Ethics approval and amendments

The protocol, the informed consent statement, and advertisement are reviewed by the Independent Ethics Committee of the Medical Faculty, University of Indonesia. Any modification of the protocol which may have any impact on the conduct of the study, potential benefit of the study, or may affect subjects' safety, including changes of the study objective, study design, study population, sample size, study procedure, or significant administrative aspects will require a formal amendment to the protocol. Such amendment has to be agreed upon by the sponsor, the responsible investigator and the Ethics Committee prior to implementation.

Minor administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way of the study is conducted. These minor administrative changes will be agreed upon by the sponsor and the responsible investigator and will be documented in a memorandum. The Ethics Committee may be notified of the minor administrative changes at the discretion of the responsible investigator.

8.8.4. Protocol Deviation

All of the protocol deviation in this study will be recorded and reported to the sponsor and to the Ethics Committee. The sponsor will report the deviation to NADFC.

8.8.5. Informed consent

An informed consent statement will be read and signed by each subject. The subjects are provided with a copy of the signed informed consent statement. They may withdraw from the study at anytime regardless the reason. Verification of the signed informed consent is noted in the CRF.

8.8.6. Subjects compensation

The subject will be compensated for their participation in the study.

8.8.7. Termination (discontinuation) of the study

The investigators have the right to terminate the study for safety reason at any time in reference to section 8.1.8.

8.8.8. Confidentiality

Subjects' medical information including the results of HIV, HBsAg, and HCV, is confidential and may not be disclosed to third parties other than:

a. Subjects itself
b. Monitor from the sponsor
c. Regulatory authority
d. Ethics committee members
e. Quality assurance personnel, Study Physician and Study Coordinator

8.8.9. Investigator obligation

Investigators agree to conduct the study in compliance with all stipulation of this protocol and in accordance with GCP and GLP. Investigators further agree to ensure that all associates assisting in the conduct of the study are informed of their obligations.

The results from this study may be published by the Investigators with written approval from the Sponsor.

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