Notes on the Design of Bioequivalence Study: Ethinylestradiol / Levonorgestrel

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team – Medicines (PQT/MED) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.

The current notes should be read and followed in line with the general guidelines of submission of documentation for WHO prequalification. In particular, please consult the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability" in: *Fifty-first Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Geneva, World Health Organization, 2017. WHO Technical Report Series, No. 1003, Annex 6.

Below, additional specific guidance is provided on the invited immediate release products containing ethinylestradiol and levonorgestrel.

Pharmacokinetics of ethinylestradiol and levonorgestrel

Orally administered ethinylestradiol is rapidly and completely absorbed. Peak serum concentrations are reached within 1.0 – 2.0 hours. Ethinylestradiol serum levels decrease in two phases, the terminal disposition phase is characterized by a half-life of approximately 15 - 24 hours.

Levonorgestrel is rapidly and completely absorbed. Maximum concentrations are reached just 1.0 hour after ingestion. The elimination has two phases with half-lives of 0.5 and 20 – 60 h.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of ethinylestradiol and levonorgestrel the following guidance with regard to the study design should be taken into account:

Design: A single-dose crossover design is recommended.

Dose: As the EoI includes ethinylestradiol / levonorgestrel 30 / 150 micrograms tablets, the bioequivalence study should be conducted with this strength.

Fasted/fed: As ethinylestradiol / levonorgestrel can be taken with or without food, a fasted state study is recommended.

Subjects: Healthy adult female subjects should be recruited. It is not necessary to include patients in the bioequivalence study.

Parent or metabolite data for assessment of bioequivalence: The parent drugs are considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence for ethinylestradiol and levonorgestrel should be based on the determination of the parent compounds.

Sample size: Levonorgestrel C_{max} seems to be moderately variable (20% approx.). These data may facilitate the calculation of a sufficient sample size for a cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of levonorgestrel in healthy volunteers of 20 – 60 hours, a washout period of 21 – 28 days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive for the first hours after administration to properly characterize the C_{max} of ethinylestradiol and levonorgestrel. It is not necessary to take blood samples beyond 72 hours for the characterization of the pharmacokinetics of immediate release products containing drugs with long half-lives. For example, samples can be taken pre-dose and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours.

Analytical considerations: Information currently available indicates that it is possible to measure ethinylestradiol and levonorgestrel and in human plasma using LC-MS/MS analytical methodology (e.g., LLOQ= 1 pg/ml and 25 pg/ml, respectively). The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the C_{max} in most profiles of each formulation (test or comparator).

Statistical considerations: The data for ethinylestradiol and levonorgestrel should meet the following bioequivalence standards in a single-dose crossover design study:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to comparator product should be within 80.00 – 125.00%
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80.00 – 125.00%.