Notes on the Design of Bioequivalence Study: Progesterone vaginal ring

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Unit – Medicines Assessment Team (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-seventh Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Geneva, World Health Organization, 2024. WHO Technical Report Series, No. 1052, Annex 8.

Below, additional specific guidance is provided on the invited prolonged release vaginal ring containing progesterone.

Pharmacokinetics of progesterone

Progesterone is extensively metabolized after oral administration during the first pass effect and its half-life is 16.8 ± 2.8 h. The vaginal route of administration circumvents the gastrointestinal and hepatic first pass effect. After the insertion of a vaginal ring, the concentrations of progesterone increase sharply and maximum concentrations are achieved during the first 1 – 3 days, subsequently the plasma concentrations decrease gradually with levels around 10 nmol/L after 16 weeks.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of progesterone contained in vaginal rings, the following guidance with regard to the study design should be taken into account:

Design: A single-dose parallel or crossover design is recommended.

Dose: As the EoI includes progesterone-releasing vaginal rings, containing 2.074 g of micronized progesterone, the bioequivalence study should be conducted with this product.

Fasted/fed: N/A.

Subjects: Healthy, adult, postmenopausal 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 drug is considered to best reflect the biopharmaceutical quality of the product. The data for the parent compound should be used to assess bioequivalence of progesterone.

Sample size: The inter-subject and intra-subject variability of the pharmacokinetic parameters of progesterone (C_{max} and AUC_{0-t}) obtained with progesterone-releasing vaginal ring has not been described in the literature. Therefore, conducting a pilot study is recommended to optimize sampling times and estimate the inter-subject or intra-subject variability of these pharmacokinetic parameters, which is necessary for the calculation of a sufficient sample size for a single dose parallel and cross-over bioequivalence study, respectively.

Washout: N/A for parallel design. For a crossover design, a washout period of one menstrual cycle is recommended.

Blood sampling: The blood sampling should be intensive for the first days after administration to properly characterize the C_{max} of progesterone. For example, samples can be taken pre-dose and 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.5, 4.0, 5.0, 6.0, 9.0, 12.0, 18.0, 24.0, 30.0, 45.0, 60.0, 75.0, 90.0, 105.0 and 120.0 days after insertion.

Analytical considerations: Information currently available indicates that it is possible to measure progesterone in human plasma using LC-MS/MS analytical methodology. 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) to confirm that pre-dose levels are negligible. Otherwise, bioequivalence should be based on progesterone using baseline-corrected data. Baseline progesterone levels at -1, -0.5, and 0 hours before dosing should be measured. The mean of the pre-dose progesterone levels should be used for the baseline adjustment of the post-dose levels. Baseline concentrations should be determined for each dosing period, and baseline corrections should be period specific. If a negative plasma concentration value results after baseline correction, this should be set to 0 prior to calculating the baseline-corrected AUC. See Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information on bioanalytical recommendations. As progesterone is an endogenous compound, note that blank plasma to prepare calibration standards and QC samples should be free of interfering endogenous progesterone (i.e., levels below 20% of the necessary LLOQ).

Statistical considerations: The data for progesterone should meet the following bioequivalence standards in a single-dose parallel or crossover design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}3\ months}$ of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean $AUC_{0\text{-}4\ months}$ 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%.
- The 90% confidence interval of the relative mean $C_{3\ months}$ of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean $C_{4\ months}$, $AUC_{0\text{-}1\ month}$, $AUC_{1\text{-}3\ months}$, $AUC_{1\text{-}4\ months}$ of the test to comparator product should be submitted as supportive information.