Guidance on Bioequivalence Studies for Reproductive Health Medicines

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1. Introduction

In 2006, the World Health Organization (WHO), United Nations Population Fund (UNFPA) and several non-governmental agencies published an Interagency List of Essential Medicines for Reproductive Health[1]. The document represents "an international consensus" on the rational selection of essential reproductive health medicines. It was intended to support decisions regarding the production, quality assurance and national procurement and reimbursement schemes of these medicines. The List was augmented by a guide "Essential Medicines for Reproductive Health: Guiding Principles for Their Inclusion on National Medicines Lists"[2]. This document addresses the principal reproductive health medicines which, also in 2006, were included in the list of products being considered by WHO's Prequalification of Medicines Programme (PQP).

With regard to contraceptives and, in particular, hormonal contraceptives, there is a multitude of products using different combinations of active pharmaceutical ingredients (API), regimens and dosage forms. At its meeting in 2007, WHO's Expert Committee on the Selection and Use of Essential Medicines "noted that the approach to provision of contraceptives was a philosophy of choice and therefore required a wide range of options and that this was in contrast to the principles of drug selection applied for the Model List, i.e. the approach is one of identifying the minimum needed to provide health care". The Committee went on to conclude "that it would take an evidence-based approach to listing contraceptives. The Committee will assess new products on a case-by-case basis using the accepted criteria of comparative efficacy, comparative safety and comparative cost, as well as suitability and acceptability." At that meeting it added a combined injectable contraceptive and an implantable contraceptive to the WHO Model List of Essential Medicines[3]

The current WHO PQP Invitation for Expressions of Interest (EOI) (May 2010), lists the reproductive health (RH) medicines that are being considered for prequalification[4]. The list also includes products that are not on WHO's current Model List of Essential Medicines[5] but which are products that one or more major public sector procurement agency, such as UNFPA, has been purchasing.

Of the 16 products, 12 are hormonal contraceptives and four are obstetric medicines. They include:

Hormonal contraceptives

Combined oral contraceptives (COCs)
- ethinylestradiol + desogestrel, tablet 30 micrograms +150 micrograms
- ethinylestradiol + levonorgestrel, tablet 30 micrograms + 150 micrograms

Progestogen-only pills (POPs)
- levonorgestrel, tablet 30 micrograms
- norgestrel, tablet 75 micrograms
- norethisterone, tablet 350 micrograms

Emergency contraceptive pills (ECPs)
- levonorgestrel, tablet 750 micrograms (pack of two); 1.5 mg (pack of one)

Progestogen-only injectable contraceptives (POCs)

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[1] World Health Organization (2006). The Interagency List of Essential Medicines for Reproductive Health, 2006, WHO, International Planned Parenthood Federation, John Snow Inc, Population Services International, United Nations Population Fund, World Bank. Geneva: World Health Organization. WHO/PSM/PAR/2006.1, WHO/RHR/2006.1. see http://whqlibdoc.who.int/hq/2006/WHO_PSM_PAR_2006.1_eng.pdf

[2] World Health Organization, UNFPA and PATH (2006). Essential Medicines for Reproductive Health: Guiding Principles for Their Inclusion on National Medicines Lists. PATH, Seattle pp104

[3] http://whqlibdoc.who.int/trs/WHO_TRS_946_eng.pdf

[4] http://www.who.int/prequal/info_applicants/eoi/EOI_ReproductiveHealth-V5.pdf

[5] http://whqlibdoc.who.int/hq/2011/a95053_eng.pdf

- medroxyprogesterone acetate, depot injection 150 mg/ml, in 1-ml vial
- norethisterone enanthate, injection 200 mg

Combined injectable contraceptives (CICs)
- medroxyprogesterone acetate + estradiol cypionate, injection 25 mg + 5 mg
- norethisterone enanthate + estradiol valerate, injection 50 mg + 5 mg

Implantable contraceptives
- two-rod levonorgestrel-releasing implant, each rod containing 75 mg of levonorgestrel
- etonogestrel, single implant, 68 mg of etonogestrel

Other reproductive health medicines

- mifepristone, 200 mg tablet
- misoprostol, 200 microgram tablet
- oxytocin, injection 10 IU, 1-ml
- magnesium sulphate, injection 500 mg/ml, in 2-ml and 10 ml ampoule

All the RH medicines on the above list are products that are being produced by manufacturers worldwide as generic or multisource pharmaceutical products. Such generic medicines need to conform to the same appropriate standards of quality, efficacy and safety as those required of the innovator product or other accepted reference product. With regard to efficacy and safety, assurance must be provided that the generic product is therapeutically equivalent and interchangeable with the reference (comparator) product. This may be achieved by demonstrating that the generic product is bioequivalent to the comparator product.

WHO PQP has published a document "Frequently asked questions on the prequalification of medicines for reproductive health" which addresses several questions relating to the need for and conduct of bioequivalence studies on RH medicines[6]. The present document is intended to expand on these questions and provide further guidance on bioequivalence studies.

2. Which products require a bioequivalence study?

WHO PQP will accept a Biopharmaceutics Classification System (BCS) based biowaiver[7] in lieu of undertaking a bioequivalence study for some drugs. In addition, other biowaivers may be granted under certain circumstances. In its guidelines on registration requirements to establish interchangeability of products[8], WHO states that a biowaiver may be possible "*when the pharmaceutical product is to be administered parenterally (e.g. intravenously, subcutaneously or intramuscularly) as an aqueous solution containing the same API in the same molar concentration as the comparator product and the same or similar excipients in comparable concentrations as in the comparator product. Certain excipients (e.g. buffer, preservative and antioxidant) may be different provided it can be shown that the change(s) in these excipients would not affect the safety and/or efficacy of the pharmaceutical product*".

Of the products currently listed above, this applies to oxytocin and magnesium sulphate, which are aqueous solutions administered parenterally. A biowaiver for oily solution injection products containing norethisterone enanthate or a combination of norethisterone enanthate and estradiol valerate is not possible because these products act over a prolonged period of time i.e., they act as a depot formulation.

[6] http://www.who.int/prequal/info_general/documents/FAQ/FAQ_RH-medicines.pdf
[7] WHO Prequalification of Medicines Programme. *General notes on Biopharmaceutics Classification System (BCS)-based biowaiver applications* (October 2012)
[8] WHO Expert Committee on Specifications for Pharmaceutical Preparations, 40th report, Technical Report Series 937, 2006. Annex 7. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability.

With regard to the seven orally administered APIs included in the list of reproductive health medicines above, PQP recently undertook a thorough investigation to determine whether any of these APIs may be eligible for a BCS-based biowaiver application[9]. It stated that "*PQP investigated all sources of API aqueous solubility and absorption/permeability data currently available to the programme, including both data available within the programme and data available in scientific literature, in an effort to classify the RH APIs within the BCS framework. While the programme was able to conclude that mifepristone is not eligible for a BCS-based biowaiver, the data available on the other RH APIs was not sufficient to allow for accurate classification, although it appears that some RH APIs, such as norgestrel and levonorgestrel (at the 30 microgram strength), may be eligible if their classification can be confirmed*". PQP has requested additional data from manufacturers to allow it to consider this issue further. Meanwhile, bioequivalence studies are required for products containing these APIs unless the product application includes the data necessary for BCS classification.

3. Design and conduct of bioequivalence studies

There is a significant amount of guidance on the design and conduct of bioequivalence studies. WHO[10] and stringent drug regulatory agencies, such as the United States Food and Drug Administration (USFDA)[11], the European Medicines Agency[12], Health Canada[13] provide full requirements for the conduct of bioequivalence studies. This document is intended to provide the principal requirements for reproductive health medicines.

3.1 Basic principles in the demonstration of bioequivalence

The basic principle underlying pharmacokinetic bioequivalence studies is that if the administration of a multisource product and a comparator product (usually the innovator) produce a similar plasma concentration-time course in the same subject, this will equate to similar concentrations at the site of action and a similar therapeutic outcome.

The plasma concentration-time curve of the API is used to assess the rate and extent of absorption of that substance from a product. A decision on the bioequivalence of the multisource pharmaceutical product and the comparator is based on a comparison of selected pharmacokinetic parameters calculated from those data and preset acceptance limits. The pharmacokinetic parameters to be assessed are:

- AUC, the area under the concentration time curve, which reflects the extent of exposure of the subject to the active substance after administration of a dose;
- Cmax, the maximum plasma concentration or peak exposure; and
- Tmax, the time from administration to maximum plasma concentration which, along with Cmax, represents a measure of the absorption rate of the active substance.

General considerations such as the pharmacokinetics and physico-chemical properties of the API and the formulation itself, should be taken into account in the study design, in addition to the principles of good clinical practice.

[9] http://www.who.int/prequal/info_general/documents/biowaiver/BCS-Classification_RH-APIs.pdf
[10] WHO Expert Committee on Specifications for Pharmaceutical Preparations, 40th report, Technical Report Series 937, 2006. Annex 7. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. Annex 9. Additional guidance for organizations performing in vivo bioequivalence studies.
[11] http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
[12] European Medicines Agency, 2010. Guidance on the investigation of bioequivalence. Doc ref: CPMP/EWP/QWP/1401/98Rev1/Corr.
[13] Guidance Document "Conduct and Analysis of Comparative Bioavailability Studies" Health Canada, 2012 (see http://www.hc-sc.gc.ca/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/bio/gd_standards_ld_normes-eng.pdf

3.2 Good clinical practice

Pharmacokinetic studies are clinical trials and must be carried out in accordance with the provisions and prerequisites for a clinical trial, as outlined in the WHO guidelines for good clinical practice (GCP) for trials on pharmaceutical products[14] or alternatively ICH E6 guideline[15]. The UK Medicines and Healthcare Products Regulatory Agency (MHRA) has recently published a "Good Clinical Practice Guide"[16]. The World Medical Association's Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" has been developed as a statement of ethical principles for medical research involving human subjects[17]. Any study must be conducted in accordance with these principles, including respect for persons, beneficence and non-maleficence.

Moreover, additional information for organizations conducting bioequivalence studies and/or analyzing clinical study samples can be obtained from WHO[18]. This includes information on quality assurance, ethics, informed consent, monitoring and documentation. The bioequivalence study must be approved by an independent ethics committee (or equivalent) before the study is conducted, according to the applicable legislation[19].

3.3 Contract research organizations

While some large multinational companies have in-house capability to implement clinical trials, most studies are outsourced to specialist contract clinical research organizations (CRO).

Care must be taken when selecting a CRO to conduct a clinical trial. CROs which are conducting studies in accordance with GCP can be identified by establishing that they have conducted studies accepted by stringent regulatory authorities[20] (i.e., products were authorized based on the outcomes of these studies) or that they have been successfully inspected by a recognized international body. While it does not have a formal programme for prequalification of CROs, as part of its requirements for prequalification of a product, WHO will usually undertake an inspection of the CRO where a bioequivalence study or other clinical study has been performed. Once a product is prequalified it publishes a WHO Public Inspection Report (WHOPIR) of the findings of the inspection[21].

It is strongly recommended that a manufacturer engages experienced external auditor(s) to conduct an audit of the CRO before signing a study contract. Application of the Declaration of Helsinki should be a key issue addressed in an independent GCP audit. Many CROs are based in lower or middle income countries and it is particularly important that an audit

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[14] WHO Expert Committee on Specifications for Pharmaceutical Preparations, 6th report, Technical Report Series 850, 1995. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products.

[15] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guideline for Good Clinical Practice. E6,1996. See: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf

[16] UK MHRA, 2012, Good Clinical Practice Guide. See: http://www.tsoshop.co.uk/bookstore.asp?FO=1160007&DI=635071&trackid=000039

[17] World Medical Association. See: http://www.wma.net/en/30publications/10policies/b3/

[18] WHO Expert Committee on Specifications for Pharmaceutical Preparations, 40th report, Technical Report Series 937, 2006. Annex 9. Additional guidance for organizations performing in vivo bioequivalence studies.

[19] Operational guidelines for ethics committees that review biomedical research. Geneva, World Health Organization, 2000 (TDR/PRD/ETHICS/2000.1).

[20] *Stringent Drug Regulatory Authority (SRA)* means a regulatory authority which is (a) a member of ICH (as specified on www.ich.org); or (b) an ICH Observer, being the European Free Trade Association (EFTA) as represented by Swiss Medic, Health Canada and World Health Organization (WHO) (as may be updated from time to time); or (c) a regulatory authority associated with an ICH member through a legally binding mutual recognition agreement including Australia, Norway, Iceland and Liechtenstein (as may be updated from time to time).

[21] WHO Public Inspection Reports of CROs. See: http://www.who.int/prequal/

addresses the process of informed consent and the issue of remuneration. Socio-cultural norms, gender issues and literacy level can provide barriers to the consent process as well as impacting on the recruitment process.

An audit should not be restricted to GCP but there should also be an audit of the analytical facility, whether in-house or independent, for its adherence to Good Laboratory Practice (GLP)[22,23]. It should also be certified under ISO17025[24] for the analytes to be measured. (see section 3.10 on analytical methods). WHO PQP will normally require an inspection of the CRO when reviewing the study to ensure acceptability.

When finalizing the agreement with a CRO, the company should agree on a timeline and monitoring plan. This may require contracting an independent clinical trial monitor.

3.4 Study design

The design of the study should aim to eliminate bias and, to the extent possible, minimize variability unrelated to formulation effects. Test conditions should reduce variability within and between subjects. The study should be standardized with regard to diet, fluid intake, exercise, posture and intake of other medicinal or non-medicinal products.

In cases where two formulations are being compared, a randomised, two-period, two-sequence single dose crossover design is the standard, which is most frequently recommended. In such studies, the subjects receive the multisource product and the comparator in a randomized order, one in each of the two study periods.

Measurable drug concentrations at the start of the second period which may interfere with the second period should be prevented. Hence, the treatment periods should be separated by a wash-out period that is sufficient to ensure that drug concentrations are below the lower limit of bioanalytical quantification in the subjects at the beginning of the second period. Normally at least 5 elimination half-lives are necessary to achieve this.

The crossover design applies to all the oral solid dosage reproductive health medicines currently being considered for prequalification. However, it does not apply to the long-acting injectable contraceptives and contraceptive implants. For injectable contraceptives that require bioequivalence studies, since the products have a long release profile and the active substances have a long apparent half-life, a parallel study design should be applied.

Prior to embarking on a bioequivalence study, it is strongly recommended that companies preparing for submission for WHO prequalification provide a final protocol to WHO for review and advice on any issues that may impact on the assessment of the study results.

3.5 Comparator product

For applications for prequalification, WHO has identified comparator products which must be used in bioequivalence studies[25].

These comparator products must be purchased from the market of an ICH or ICH-associated country[26]. Innovator products obtained from local markets that are not ICH or ICH-associated country markets are not acceptable. There are pharmaceutical distribution companies in the USA, Europe, and other ICH-associated countries that are licensed to sell pharmaceutical products to companies for scientific study. Many national drug regulatory

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[22] WHO TDR. Handbook for Good Laboratory Practice (GLP). World Health Organization, Geneva, pp243 http://www.who.int/prequal/info_general/documents/GLP/glp-handbook.pdf

[23] OECD Series on Principles of Good Laboratory Practice (GLP) and Compliance Monitoring. See: http://www.oecd.org/chemicalsafety/testing/oecdseriesonprinciplesofgoodlaboratorypracticeglpandcompliancemonitoring.htm

[24] ISO/EC 17025:2005 General requirements for the competence of testing and calibration laboratories. International Standards Organization, Geneva

[25] http://www.who.int/prequal/info_applicants/info_for_applicants_BE_comparator.htm

[26] See Footnote 19.

agencies have information requirements for products that are being imported for clinical trials and most CROs have experience in dealing with these issues. However, if a national authority will not allow the import of the necessary comparator product, consideration must be given to conducting the study at a CRO located elsewhere.

The dossier submitted for prequalification must state the country of origin of the comparator product together with the lot number and expiry date of the product, as well as results of pharmaceutical analysis to prove pharmaceutical equivalence. Further, in order to establish the origin of the comparator product, the applicant must present all of the following documents:

- Copy of the comparator product labelling (snap-shot of the box). The name of the product, name and address of the manufacturer (marketing authorization holder), batch number, and expiry date should be clearly visible on the labelling.
- Copy of the invoice from the distributor or company from which the comparator product was purchased. The address of the distributor must be clearly visible on the invoice.
- Documentation verifying the method of shipment and storage conditions of the comparator product from the time of purchase to the time of study initiation.
- A signed statement certifying the authenticity of the above documents and that the comparator product was purchased from the specified national market. The certification should be signed by the company executive responsible for the application to PQP.

3.6 Generic product

The generic or test product used in the study should be representative of the product to be marketed. Composition and quality characteristics (including stability) and manufacturing methods (including equipment and procedures) should be the same as those to be used in the future routine production runs.

The batch size of the multisource product used in the bioequivalence study should be normally 100,000 units or at least $1/10^{th}$ of production scale, whichever is greater, unless otherwise justified. In case of a production batch smaller than 100,000 units, a full production batch will be required. If the product is subjected to further scale-up, this should be properly validated.

Potency and *in vitro* dissolution characteristics of the multisource and the comparator pharmaceutical products should be substantiated prior to performance of the bioequivalence study. The difference in content of the active pharmaceutical ingredient of the comparator and the multisource product should be less than 5%. In exceptional cases where this difference is more than 5% and a suitable batch of the comparator product cannot be found, content correction may be accepted. This should be specified in the protocol and justified by inclusion of the results from the assay of the test and reference products in the protocol.

3.7 Study subjects

The number of subjects to be included in a bioequivalence study depends on the inter-subject variability of the pharmacokinetic variables, the desired significance level (5%) and statistical power, and the applied 90% confidence intervals of 80-125% for the ratio of the population geometric means (test/reference) for the parameters AUC and Cmax under consideration. The number of subjects to be included should be based on an appropriate sample size calculation.

It is accepted that the number of evaluable subjects in a bioequivalence study should not be less than 12. However, for all the products under consideration there is considerable inter-subject variability and even using a crossover design, where every subject is their own control, there is a necessity for 24-36 subjects based on the intra-subject variability. In the case of depot medroxyprogesterone acetate, 60 subjects per arm are required.

Subjects should be between 18-55 years, preferably have a Body Mass Index between 18.5 and 30 kg/m^2. Many of the reproductive health medicines are potent steroid hormones for the control of fertility in women, the studies should therefore be undertaken in healthy female subjects.

Subjects should have no history of alcohol or drug abuse. Inclusion/exclusion criteria should be clearly stated in the protocol.

For bioequivalence studies with a parallel study design, special attention should be paid to standardize treatment groups as much as possible with regard to variables which may affect the pharmacokinetics of the active substance. This is necessary to reduce any bias that could be introduced due to differences in the study groups for the inter-subject comparison.

3.8 Study standardization

Bioequivalence studies should be standardized to lower the variability not attributable to formulation effects. In addition to standardization of exercise, posture and intake of other medicinal or non-medicinal products before and during the study, food intake and time of dosing should also be standardized.

A study conducted under fasting conditions should be undertaken when the innovator's Summary of Product Characteristics (SmPC) states that the product should be taken under fasting conditions or without regard to meal intake, or if food is not mentioned in the posology for the comparator product.

For a study with a fasting design, a fasting period of 8-10 hours before intake of the investigational products is normally applied. Free access of water is allowed up to one hour before administration. The study products should be taken with at least 150 ml of water. About two hours after administration, access to water is again allowed and about four hours after administration of the study products, a standard meal is served.

3.9 Sampling times

Blood samples should be taken frequently to obtain a reliable estimation of Cmax and AUC. Sampling points should include a pre-dose sample. Samples should be taken more frequently around the estimated Tmax. For estimation of AUC(0-t), plasma sampling should be long enough to provide a reliable estimation of the extent of exposure, which is achieved if AUC(0-t) covers at least 80% of AUC(0-∞). At least three to four samples are needed during the terminal log-linear phase of the concentration-time profile in order to reliably estimate the terminal rate constant.

For the orally administered reproductive health medicines, it is not necessary to collect samples after 72h, as AUC(0-72h) is considered sufficient for a reliable estimation of the extent of absorption. If samples are collected for 72 hours after drug administration, it is not necessary for AUC(0-t) to cover at least 80% of AUC(0-∞).

3.10 Dose

Most of the reproductive health medicine formulations listed are manufactured at a single dosage strength, which is the strength that should be used to evaluate bioequivalence.

In case the application concerns several strengths and extrapolation of the results obtained in the bioequivalence study for one strength to the other strengths is requested, the selection of the strength or dose to be administered depends on the pharmacokinetics of the active substance. For products showing linear pharmacokinetics, normally the highest strength of a series of strengths should be used.

The results obtained in the bioequivalence study for one strength may be extrapolated to another strength where all of the following criteria have been fulfilled:

- the pharmaceutical products are manufactured by the same manufacturing process;
- the qualitative composition of the different strengths is the same;

- the composition of the strengths are quantitatively proportional, i.e. all active and inactive ingredients are in exactly the same proportions in the different strengths;
- or the strengths contain a low amount of API (up to 10 mg per dosage unit), and the total weight of the dosage form remains nearly the same for all strengths (within 10% of the total weight) with the amount of being filler changed to account for the change in amount of active substance; and
- appropriate *in vitro* dissolution data at a pH of 1.2, 4.5 and 6.8 showing comparable dissolution between the different strengths.

As an example, this applies to the levonorgestrel emergency contraceptive where there are two strengths manufactured: 750µg and 1.5mg. If dossiers for both products are submitted for prequalification and the products meet the above criteria, the bioequivalence study need only be undertaken on the 1.5mg formulation.

Misoprostol provides a slightly different situation; it is being used for multiple obstetric indications, several of which are included in WHO's Model List of Essential Medicines[27]. These indications use different dosages and may be administered by different routes (oral, vaginal, sublingual, buccal) – dosage guidelines are clearly described in a table produced by FIGO based on guidelines developed by WHO and FIGO[28]. Although information requirements for all of the indications is provided, the focus below is on the use of misoprostol for the prevention of post-partum haemorrhage which requires a single dose of 600µg of misoprostol orally. Some companies have undertaken studies on 400µg misoprostol administered orally for the original indication of prevention of gastric ulcers associated with NSAIDs. A bioequivalence study conducted using a dose of 400 µg (2x200 µg) could be submitted as a part of an application for a 200µg product that will be indicated for use at a dose of 600µg.

3.11 Analytical methods

The analytical part of bioequivalence trials should be performed in accordance with the principles of Good Clinical Practice (GCP), see section 3.3. Prior to beginning the bioequivalence study, the analytical method should have been validated and proven to be accurate and precise for analysis of the analyte over the range of concentrations anticipated (see specific considerations in section 5)[29] [30].

For validation, specificity, accuracy, precision, the lower limit of quantitation, the response function (calibration curve performance) and stability of the analyte under the designated storage conditions have to be demonstrated. Validation procedures, methodology and acceptance criteria should be specified in the analytical protocol, and/or the SOP. The lower limit of quantitation of a bioanalytical method should be no higher than 5% of the lowest expected Cmax, since this is level at which pre-dose concentrations should be detectable.

All experiments used to support claims or draw conclusions about the validity of the method should be described in a report (method validation report) that is included in the submission to a regulatory authority.

During analysis of subject samples, within study validation should be carried out using quality control samples in each analytical run. Acceptance criteria should be predetermined and in accordance with normally applied criteria. Reanalysis of subject samples should be

[27] World Health Organization. Model List of Essential Drugs, 17th List, March 2011, See: http://whqlibdoc.who.int/hq/2011/a95053_eng.pdf
[28] http://www.misoprostol.org/File/guidelines.php
[29] USFDA. Guidance for Industry: Bioanalytical Method Validation. See: http://www.fda.gov/cder/guidance/index.htm.
[30] European Medicines Agency, 2011. Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009. See: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf

defined in the study protocol and/or SOP. Normally reanalysis of subject samples because of a pharmacokinetic reason is not acceptable. This is especially important for bioequivalence studies, as this may bias the outcome of such a study.

3.12 Parameters to be assessed

Pharmacokinetic parameters

For the single-dose bioequivalence studies of the solid oral dosage forms of the listed reproductive health medicines, the following parameters should be measured or calculated:

- AUC(0-72h) /AUC(0-t)
- Cmax
- AUC(0-∞)
- Tmax
- T½ (elimination half-life)

AUC (0-t)/AUC(0-72h) and Cmax are the pivotal parameters for which bioequivalence should be proven. AUC(0-∞), Tmax and T½ are considered supportive data.

In the case of misoprostol, with a very short T½, calculation of the AUC for 0-6h is normally sufficient and in the case of the injectable contraceptive medroxyprogesterone acetate, the AUC should be calculated for both its period of dosage in normal use, AUC(0-90days) and for the 140 days for which blood levels were measured, AUC(0-140days), as specified in the protocol.

The method of calculating AUC-values should be specified. For estimation of AUC, a non-compartmental-method should be applied, and AUC should be calculated using a pre-defined method such as the linear/log trapezoidal integration method.

Pro-drugs

The concentration of the drug compound in the formulated product should be used for estimation of bioequivalence. However, where the administered drug substance is an inactive pro-drug which is rapidly converted *in vivo*, as in the cases of lynestrenol, desogestrel and misoprostol, bioequivalence is based on the measurement of the active compound, norethisterone, etonogestrel (3-ketodesogestrel) and misoprostol acid, respectively.

Statistical analysis

The statistical method for testing pharmacokinetic bioequivalence is based upon the determination of the 90% confidence interval around the ratio of the log-transformed population means (multisource/comparator) for the pharmacokinetic parameters under consideration, which is equivalent to carrying out two one-sided tests at the 5% level of significance. The pharmacokinetic parameters under consideration should be analysed using ANOVA and the data should be transformed prior to analysis using a logarithmic transformation.

The 90% confidence interval for the ratio of the multisource/comparator product should be contained within the acceptance interval of 80-125%.

A statistical test for unequal carry-over is not considered necessary. Carry-over effect can be assessed by evaluation of the absence of positive pre-dose samples in the second period.

For the fixed dose combinations, levonorgestrel + ethinyl estradiol and desogestrel + ethinyl estradiol, bioequivalence evaluation should be assessed for both active substances.

3.13 Subject accountability

All subjects included in the study for which evaluable data is available for both treatment periods should be included in the analysis of bioequivalence and statistics. In principle any reason for exclusion is valid provided it is specified in the protocol and the decision to

exclude is made before data analysis. Acceptable reasons for exclusion include, for instance, vomiting and diarrhoea. Exclusion based on statistical analysis or for pharmacokinetic reasons is not considered acceptable.

4. Some general issues relating to hormonal contraceptives

4.1 Progestogens

As stated in the introduction, there are a multitude of products using different combinations of APIs, regimens and dosage forms. It is also of interest to note that all the hormonal contraceptive products listed contain APIs and use regimens that were developed many years ago. In some cases, this makes it difficult to obtain original pharmacokinetic data based on current study design and analytical methodology.

With regard to the oral dosage forms, Combined Oral Contraceptives (COCs), Progestogen-Only Pills (POPs) and Emergency Contraceptive Pills (ECPs), the progestogens used are often classified by generation. Products marketed today may contain the following progestogens:

- First generation: norethindrone (norethisterone, first marketed in 1957).
- Second generation: norgestrel, levonorgestrel (the active enantiomer of norgestrel, used extensively for more than 40 years).
- Third generation: desogestrel, gestodene, norgestimate.
- Fourth generation: dienogest, drospirenone, nestorone, nomegestrol acetate and trimegestone.

The continuing development of progestogens obviously has a justification in improving safety but the driving force for a new progestogen is to replace one coming off patent. Interestingly, there has been little effort to change from ethinyl estradiol as the principal synthetic estrogen to use for 'the pill', so the changes have been primarily with the progestogen. When there have been gaps in patent protection before a new progestogen has become available, another approach has been the development of different administration regimens, such as the biphasic and triphasic preparations, which provide different doses of progestogen and estrogen at different times of the monthly cycle. There is no evidence to show that they have any advantage over standard monophasic preparations and WHO has stated that "There is no justification at present to recommend multiphasic OCs in preference to monophasic OCs"[31]. Hence none of these products appear on the current EOI.

For the injectable POCs and Combined Injectable Contraceptives (CICs), the most common progestogen used, medroxy-progesterone acetate (MPA) was developed in the late 1950s; while norethisterone enanthate (NET-EN) became available in the 1960s. There are other old progestogens used in some regions but they have been inadequately studied in terms of safety and efficacy.

4.2 Estrogens

For the COCs, ethinyl estradiol was synthesized by Schering in 1938 and has been the principal estrogen used in combined oral contraceptives for the past 40 years. There is now limited use of estradiol esters in COCs, although the estradiol esters used in the CICs, estradiol cypionate (E2C) and estradiol valerate (E2V) have been available since the 1930s.

4.3 Placebos

For COCs and CICs, products were designed to allow the women to experience a vaginal bleeding episode that mimicked the normal menstrual cycle. This bleeding episode is a consequence of estrogen withdrawal. As such, the active tablets in COCs are administered for 21 days and some formulations are provided in 21-day packs. However, in order to assist

[31] http://www.who.int/rhl/fertility/contraception/dscom/en/index.html

restarting treatment each month after a break of 7 days, many formulations are provided as 28-day packs which contain 7 placebo pills containing either lactose or ferrous fumarate.

No additional safety and efficacy (clinical) data are required for the placebo products - only quality (chemistry and manufacturing) information would be required for those tablets. Placebo tablets must be designed to ensure that they have appropriate ingredients, process, controls, specifications and stability, and other requirements that conform to acceptable quality standards and cGMP for oral solid dosage forms.

Some 30 years ago, USAID requested its principal supplier to add 7 tablets of ferrous fumarate (60 or 75mg) to packs of combined oral contraceptives on the basis that anaemia is common in women in developing countries. This is a practice that has continued in the award of certain public sector tenders. It has been accepted that the addition of 7 tablets of 60mg or 75mg of ferrous fumarate instead of placebo tablets represents an iron supplement and not a therapeutic dose.

The use of a compound such as ferrous fumarate is not included in the current EOI but, as it would be considered a supplement in such products, quality information, in line with that described above for a placebo, would be required.

4.4 Tablet coating

Oral contraceptives can be sugar-coated, film-coated or uncoated. It is not a requirement that a product employ the same non-functional coat as the appropriate comparator product *e.g.,* the comparator may be a sugar-coated tablet while the product under development can be a film-coated tablet. The use of a different non-functional coat may impact the dissolution characteristics of a product relative to the comparator, however, this is not considered to be important if *in vivo* bioequivalence is demonstrated for the two products.

It is important to note that manufacturers must use a suitable dissolution method and information in the quality dossier should include multi-point dissolution profiles for the lot used in bioequivalence studies in three media across the physiological pH range. Recommendations for conducting and assessing comparative dissolution profiles are to be found in WHO TRS970.[32]

5. Specific considerations for the bioequivalence of RH medicines

5.1 Levonorgestrel

Orally administered levonorgestrel is rapidly and almost completely absorbed with a bioavailability of almost 100% and is not impacted by a first pass effect of the liver. Levonorgestrel in serum is primarily protein bound, 50% to albumin and 47.5% to sex hormone binding globulin (SHBG). Metabolites of levonorgestrel are not considered to be pharmacologically active and are excreted in urine and faeces.

Levonorgestrel is one of the most widely used progestogen in the COC levonorgestrel 150µg and ethinyl estradiol 30µg and in the POP levonorgestrel 30µg. Since these are products that have been available for 40 years, pharmacokinetic data are summarized in SmPCs. For the COC, a Cmax of approx. 3 ng/ml is reached in serum just one hour after ingestion. The serum concentrations fall in 2 phases with a half-life of around 0.5 hours and an elimination half-life of 20 hours[33]. For the 30µg POP, peak serum concentrations are around 0.8ng/ml, reached about 1 hour after ingestion[34]

[32] WHO Expert Committee on Specifications for Pharmaceutical Preparations. 2012. Technical Report Series 970. Annex 4. Guidelines on submission of documentation for a multisource (generic) finished pharmaceutical product for the WHO Prequalification of Medicines Programme: quality part. Appendix 1 Recommendations for conducting and assessing comparative dissolution profiles.
[33] http://www.medicines.org.uk/emc/medicine/1828/SPC/Microgynon+30+ED#PHARMACOKINETIC_PROPS
[34] http://www.bayerresources.com.au/resources/uploads/PI/file9397.pdf

However, in recent years there has been significant use of a single high dose, 1.5mg, of levonorgestrel, either as two tablets of 750µg or a single tablet of 1.5mg, for emergency contraception (ECPs). One SmPC states that following ingestion of one tablet of 750µg, maximum drug serum levels of 14.1±7.7ng/ml at a Tmax of 1.6±0.7h. The elimination half-life is 24.4+5.3 hours[35,36]. With a single 1.5mg tablet, a Cmax of 20ng/ml with a Tmax of 1.4h has been observed[37]

The numbers of subjects to be included in a cross-over study should be based upon the within-subject variability for Cmax and AUC. In practice, it has been found that bioequivalence could be concluded based upon results from 28 subjects.

Blood sampling up to about 72 h after administration should be sufficient to obtain a reliable estimation of the extent of absorption. For the lower doses of 150µg in the COC and 30µg in the POP, a possible sampling scheme could be: 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours. With an elimination half-life of about 20h after single dose administration, a washout period of 10-14 days is sufficient. Considering a Cmax of about 800pg/ml for the POP, the analytical method should have a limit of quantitation of at most approximately 40pg/ml (5% Cmax level).

Given the difference in doses, for the ECPs with a total dose of 1.5mg, a possible sampling scheme could be: pre-dose and at 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210 minutes, 4, 6, 9, 12, 24, 48 and 72 hours after dosing. Although the elimination half-life is 20-30h after single dose administration, given the significantly higher dose, a washout period of 28-35 days maybe preferred. Considering a Cmax around 20ng/ml, the analytical method should theoretically have a limit of quantitation of at most 1ng/ml (5% Cmax level), although in practice, a method of greater sensitivity is likely to be used (see previous paragraph).

5.2 Desogestrel

Desogestrel is rapidly and almost completely absorbed and converted into etonogestrel (3-ketodesogestrel), its biologically active metabolite. Following oral administration, the relative bioavailability of desogestrel, as measured by serum levels of etonogestrel, is approximately 84%.

In the third cycle of use after a single desogestrel and ethinyl estradiol tablet, maximum concentrations of 3-keto-desogestrel of 2.81±1.20ng/mL (mean±SD) are reached at 1.4±0.8 hours. The kinetics of 3-ketodesogestrel are non-linear due to an increase in binding of 3-keto-desogestrel to sex hormone-binding globulin in the cycle, attributed to increased sex hormone-binding globulin levels which are induced by the daily administration of ethinyl estradiol. The elimination half-life for 3-keto-desogestrel is approximately 38±20 hours at steady state. Metabolites of 3-ketodesogestrel are not known to have any pharmacological effects, and are further converted into sulphates and glucuronides.[38]

Blood sampling up to 72h after administration should be sufficient to obtain a reliable estimation of the extent of absorption. A possible sampling scheme could be: 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours. With an elimination half-life of about 38h after single dose administration, a washout period of 10-14 days is sufficient. Considering a Cmax of about 2ng/ml, the analytical method should have a limit of quantitation of at most approximately 100pg/ml (5% Cmax level).

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[35] Patient Information leaflet for Plan-B, Duramed
http://www.accessdata.fda.gov/drugsatfda_docs/label/2006/021045s011lbl.pdf

[36] Kook K, Gabelnick H, Duncan G. Pharmacokinetics of levonorgestrel 0.75mg tablets. Contraception. 2002. 66:73-76

[37] Devoto L et al. Pharmacokinetics and endometrial tissue levels of levonorgestrel after administration of a single 1.5-mg dose by the oral and vaginal route. Fert. Steril. 2005, 84:46-51

[38] http://www.rxlist.com/apri-drug/clinical-pharmacology.htm

5.3 Ethinyl estradiol

Ethinyl estradiol is rapidly and completely absorbed from the gastrointestinal tract, it undergoes extensive first-pass metabolism, and its absolute bioavailability is approximately 40%-60%. After single oral administration, one study has shown a Cmax of 33pg/ml at a Tmax of 1.25±2.25h[39]. T½ was 18.2±13.7h. Following repeated oral administration, the serum concentration of ethinyl estradiol is increased by approximately 30%-60%, reaching a steady-state level during the second half of each treatment cycle. Ethinyl estradiol has numerous metabolites, excreted as free compounds and glucuronide and sulphate conjugates[39].

Blood sampling up to about 72 h after administration should be sufficient to obtain a reliable estimation of the extent of absorption. A possible sampling scheme could be: predose, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours. With an elimination half-life of about 26h after single dose administration, a washout period of 10-14 days is sufficient. Considering a Cmax of about 33pg/ml, the analytical method should have a limit of quantitation of at most approximately 5pg/ml (5% Cmax level).

5.4 Medroxyprogesterone acetate

There are no modern pharmacokinetic data in the published literature. There are two review articles, one stating that MPA administered by intramuscular injection has a half-life of 40 to 50 days[40]. The other says that intramuscular MPA is released slowly; a 150 mg dose is first detectable in the blood 30 minutes after injection, plateauing at 1.0 ng/mL for three months, followed by a gradual, tapering decline that lasts up to nine months in some women. Ovulation usually resumes when blood levels of MPA fall below 0.1 ng/ml[41].

Information quoted as part of a package insert states “Following a single 150 mg IM dose of Depo-provera CI in eight women between the ages of 28 and 36 years old, medroxyprogesterone acetate concentrations, measured by an extracted radioimmunoassay procedure, increase for approximately 3 weeks to reach peak plasma concentrations of 1 to 7ng/mL. The concentration of medroxyprogesterone acetate decreases exponentially until it becomes undetectable (< 100pg/mL) between 120 to 200 days following injection. The apparent half-life for medroxyprogesterone acetate following IM administration of Depo-Provera is approximately 50 days. Most medroxyprogesterone acetate metabolites are excreted in the urine as glucuronide conjugates.”[42]

A more recent study was submitted to the USFDA as part of an Abbreviated New Drug Application[43]. The study was a single-dose, fasting, parallel study with 124 normal female volunteers receiving a dose of 150mg by injection in the gluteal muscle. It showed a Cmax of 4.49 ng/ml with a coefficient of variation (CV) of 58.7% for the reference product (ref), Depo-Provera, and a Cmax of 4.84ng/ml with a CV of 85.8% for the test product (test). The Tmax ref was 6.76 days with a CV of 127.8% and the Tmax test was 4.93 days with a CV of 120.4%. The T½ ref was 36.05 days with a CV of 60.6% and the T½ test was 44.03 days with a CV of 66.1%.

This study shows the large inter-subject variability and the reason why such a large study is required to demonstrate bioequivalence. It did show that bioequivalence can be achieved with 60 subjects/arm. Blood sampling needs to be undertaken up to 140days after the day of injection. A possible sampling scheme could be: pre-injection, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4,

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[39] Junior EA et al. Bioequivalence of two oral contraceptive drugs Containing ethinylestradiol and gestodene in healthy female volunteers. J Bioequiv Availab 2010, 2:125-130

[40] Schindler AE et al. (2003), Classification and pharmacology of progestins. Maturitas.46 Supp1:7-16

[41] Mishell DR (1996). "Pharmacokinetics of depot medroxyprogesterone acetate contraception". JRM **41** (5 Suppl): 381–390

[42] http://www.rxlist.com/depo-provera-drug/clinical-pharmacology.htm

[43] Center for Drug Evaluation and Research, USFDA, Bioequivalence review, ANDA76-533, 2004 – obtained under the FOI Act

4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 14, 16, 18, 21, 24, 28, 35, 49, 63, 77, 91, 105, 119, 140 days.

A Cmax of 4-5ng/ml could be expected, although, given the high inter-subject variability, this could be as low as 800pg/ml; and since the level of MPA considered necessary to exert a contraceptive effect is estimated as being as low as 200 pg/ml, rather than using an analytical method with a limit of quantitation of 200-250pg/ml (5% Cmax level), a LC/MS/MS analytical method should be used aiming for a limit of quantitation of 50pg/ml.

5.5 Mifepristone

Mifepristone is used for the termination of pregnancy. Up to 63 days after establishment of pregnancy, a single dose of 200mg is administered orally followed 24-48h later by 800µg of misoprostol administered vaginally or sub-lingually. The misoprostol regimen is modified for later gestation. This is discussed in WHO's recently updated document on safe abortion[44]. Although some companies market the drug at a dose of 600mg, evidence is that the optimal dose is 200mg, a dose used across north-west Europe. This is supported by the fact that at doses of 100-800mg, Cmax does not differ significantly; probably due to saturation of the serum binding capacity of the α1-acid glycoprotein for mifepristone[45]. A study quoted in the same paper gave a Cmax of 9.30±2.22µmol/l and a Tmax of 1.71±0.54 hours in 9 subjects receiving 200mg of mifepristone although a later dose response study[46] gave significantly lower levels.

An unpublished study[47] on a group of 64 subjects receiving a single oral dose of 200mg of the mifepristone reference product, Mifegyne, and a test product orally, showed a Cmax ref of 3.83µmol/l with a SD of 1.50µmol/l and a Cmax test of 3.94µmol/l with a SD of 2.51µmol/l. The Tmax ref was 1.4h with a SD of 0.8h and the Tmax test was 1.9h with a SD of 3.9h.

Blood sampling up to about 72h after administration should be sufficient to obtain a reliable estimation of the AUC. A possible sampling scheme could be: predose, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours. With an elimination half-life of about 26h after single dose administration, a washout period of 10-14 days is sufficient. Considering a Cmax of about 1.3µg/ml, the analytical method should have a limit of quantitation of at most approximately 65ng/ml (5% Cmax level).

5.6 Misoprostol

As stated in section 3, misoprostol is being used for several obstetric indications. Although information related to all indications is provided below, the focus of the information provided is related to the use of misoprostol for the prevention of post-partum haemorrhage which requires a single dose of 600µg of misoprostol orally. Some companies may have undertaken studies on 400µg misoprostol administered orally for the original indication of prevention of gastric ulcers associated with NSAIDs. A bioequivalence study conducted using a dose of 400µg (2x200µg) could be submitted as a part of an application for a 200µg product that will be indicated for use at a dose of 600µg.

A study was submitted to the USFDA as part of an Abbreviated New Drug Application[48]. It was a fasting, cross-over study with 36 normal female volunteers receiving an oral dose of 400µg of the reference product (ref), Cytotec, and for the test product (test) orally. It showed a Cmax ref of 600pg/ml with a CV of 38.7% and a Cmax test of 550pg/ml with a CV of 41.4%. The Tmax ref was 19.78min with a CV of 43.3% and the Tmax test was 23.56min

[44] World Health Organization (2012). Safe abortion: technical and policy guidance for health systems, Second edition, pp1-132.
[45] Sitruk-Ware R, Spitz IM. / Contraception 68 (2003) 409–420
[46] Heikinheimo O, Kekkonen R. Dose-response relationships of RU486. Ann Med 1993;25:71–6.
[47] P Hall, personal communication, 2013
[48] Center for Drug Evaluation and Research, USFDA, Bioequivalence review, ANDA76-095, 2002 – obtained under the FOI Act

with a CV of 74.9%. The T½ ref was 27.14 min with a CV of 12.6% and the T½ test was 26.94 min with a CV of 46.1%.

Blood sampling up to 6h after administration is sufficient to obtain a reliable estimation of the AUC. A possible sampling scheme could be: predose, 5, 10, 15, 20, 30, 40, 50, 60, 90 minutes, 3, 4, 6 hours. With a rapid elimination half-life, a washout period of 3 days is sufficient. Considering a Cmax of about 600pg/ml, the analytical method should have a limit of quantitation of at most approximately 30pg/ml (5% Cmax level).

The current WHO treatment guidelines recommend misoprostol for a range of therapeutic indications, employing a variety of routes of administration as follows:

- In settings where oxytocin is unavailable:
    - Prevention of postpartum hemorrhage (PPH): oral misoprostol 600 µg.
    - Treatment of postpartum hemorrhage (PPH): sublingual misoprostol 800 µg.
- Spontaneous and Induced Abortion: oral, vaginal, buccal, or sublingual misoprostol (at different doses and regimens depending on factors such as gestational age at the time of administration); and
- For the induction of labour: vaginal misoprostol 25µg.

To maximize a product's utility for treatment programmes, it would be beneficial if prequalified misoprostol products include all of the above noted indications. However, the bioequivalence between the proposed and comparator products demonstrated following oral administration as discussed above cannot be extrapolated to the other routes of administration. In order to obtain the full range of indications for a prequalified product, the following data would be required in addition to the study employing oral administration as described above:

- Data from a single-dose, crossover bioequivalence study employing sublingual administration. Proof of bioequivalence in this study would be considered sufficient information to grant indications employing sublingual and buccal routes of administration.
- Pharmacokinetic data (not necessarily a bioequivalence study) showing that, following vaginal administration, the proposed product produces in vivo misoprostol concentrations with a mean maximal concentration (Cmax) of at least 200 pg/mL (normalized for a 800 ug dose) and an extent of absorption (AUC) that exceeds that observed following oral administration of the product (on a dose normalized basis).
- Further, additional dissolution data will be needed in order to accept the product for the indication of "induction of labour" due to the required administration of fractional doses.

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Table1. Summary of bioequivalence study requirements for reproductive health medicines

	Study design*	No of subjects	Blood sampling	Analyte	PK parameters	Acceptance**
Medroxy-progesterone acetate (MPA), 150mg/ml injection	Randomized, single dose, open-labelled and parallel study in non-fasting subjects.	60 per arm	Pre-injection, 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 14, 16, 18, 21, 24, 28, 35, 49, 63, 77, 91, 105, 119, 140 days after drug administration.	Medroxyprogesterone acetate in plasma or serum, determined by LC-MS/MS.	$AUC_{0\text{-}90d}$, $AUC_{0\text{-}140d}$ & $AUC_{0\text{-}\infty}$, C_{max}, T_{max}, $T_{½}$	$AUC_{0\text{-}90d}$, $AUC_{0\text{-}140d}$, C_{max}
Levonorgestrel tablet, 750µg or 1.5mg	Randomized, single blind, two-period, cross-over study in fasting subjects. Wash-out period, 28-35 days.	28	Pre-dose administration, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210 minutes, 4, 6, 9, 12, 24, 48 and 72 hours after drug administration.	Levonorgestrel in plasma or serum, determined by LC-MS/MS.	$AUC_{0\text{-}72h}$, C_{max}, T_{max}, $T_{½}$	$AUC_{0\text{-}72h}$, C_{max}
Levonorgestrel tablet, 30µg	Randomized, single blind, two-period, cross-over study in fasting subjects. Wash-out period, 10-14 days.	28	Pre-dose administration, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours after drug administration.	Levonorgestrel in plasma or serum, determined by LC-MS/MS.	$AUC_{0\text{-}72h}$, C_{max}, T_{max}, $T_{½}$	$AUC_{0\text{-}72h}$, C_{max}
Levonorgestrel tablet, 150µg & ethinyl estradiol, 30µg tablet	Randomized, single blind, two-period, cross-over study in fasting subjects. Wash-out period, 10-14 days.	28	Pre-dose administration, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours after drug administration.	Levonorgestrel and ethinyl estradiol in plasma or serum, determined by LC-MS/MS.	$AUC_{0\text{-}72h}$, C_{max}, T_{max}, $T_{½}$	$AUC_{0\text{-}72h}$, C_{max}
Desogestrel tablet, 150µg & ethinyl estradiol, 30µg tablet	Randomized, single blind, two-period, cross-over study in fasting subjects. Wash-out period, 10-14 days.	28	Pre-dose administration, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours after drug administration.	Etonogestrel and ethinyl estradiol in plasma or serum, determined by LC-MS/MS.	$AUC_{0\text{-}72h}$, C_{max}, T_{max}, $T_{½}$	$AUC_{0\text{-}72h}$, C_{max}
Mifepristone tablet, 200mg	Randomized, single blind, two-period, cross-over study in fasting subjects. Wash-out period, 10-14 days.	28	Pre-dose administration, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 minutes, 3, 4, 6, 9, 12, 24, 48 and 72 hours after drug administration.	Mifepristone in plasma or serum, determined by LC-MS/MS.	$AUC_{0\text{-}72h}$, C_{max}, T_{max}, $T_{½}$	$AUC_{0\text{-}72h}$, C_{max}
Misoprostol, 600µg orally (for prevention of post-partum haemorrhage)	Randomized, single blind, two-period, cross-over study in fasting subjects. Wash-out period, 3 days.	36	Pre-dose administration, 5, 10, 15, 20, 30, 40, 50, 60, 90 minutes, 3, 4, 6 hours after drug administration.	Misoprostol acid in plasma or serum, determined by LC-MS/MS.	$AUC_{0\text{-}t}$, C_{max}, T_{max}, $T_{½}$	$AUC_{0\text{-}t}$, C_{max}

*For comparator products, see http://www.who.int/prequal/info_applicants/BE/Comparator-RH2012-20March.pdf

**Acceptance limits for all products: the 90% confidence interval must be within 80-125%