Key Output of Programme

- A list of prequalified medicinal products used for treatment of HIV/AIDS, malaria, tuberculosis, influenza, neglected tropical diseases, acute diarrhoea (zinc sulfate), and for reproductive health

- To get a Finished Pharmaceutical Product (FPP) included on the list, a manufacturer provides a comprehensive set of data about the quality, safety and efficacy of its product
  - Quality Assessment
  - Safety & Efficacy Assessment
  - Product Labeling
Safety & Efficacy

- Most FPPs submitted are multisource (generic) products
  - Abbreviated clinical component
  - Safety & efficacy (S&E) based on comparison to a FPP with established S&E

- Pharmaceutical Equivalence
  - Products are pharmaceutically equivalent if they contain the same molar amount of the same API(s) in the same dosage form, if they meet comparable standards, and if they are intended to be administered by the same route.
Pharmaceutical equivalence
– is it enough?
Sometimes, it is ...

- Aqueous solutions
  - Intravenous solutions
  - Intramuscular, subcutaneous solutions
  - Oral solutions
  - Otic or ophthalmic solutions
  - Topical preparations
  - Solutions for nasal administration

- Powders for reconstitution as solution
Pharmaceutical Equivalents

Pharmaceutically equivalent FPPs may differ

Differences in formulation
Excipients, drug particle size, mechanism of release

Differences in manufacture
Equipment, process, site

May result in differences in e.g., disintegration and dissolution, and impact product performance
Sometimes, it is not enough

- Pharmaceutical equivalence by itself does not necessarily imply therapeutic equivalence

- Therapeutic equivalence:
  - Pharmaceutically equivalent
  - Same safety and efficacy profiles after administration of same dose
Products that require studies to determine equivalence ...

- **Solid oral FPPs**
  - immediate- and modified-release FPPs

- **Complex topical formulations**
  - emulsions, suspension, ointments, pastes, foams, gels, sprays, and medical adhesive systems

- **Complex parenteral formulations**
  - depot injections, nasal/inhalational suspension etc
Establishing Equivalence

- Comparative pharmacokinetic studies
  - *In vivo* comparative bioavailability studies
  - Comparison of performance of FPPs based rate and extent of absorption of API from each formulation
    - Area under the concentration-time curve (AUC)
    - Maximal concentration (Cmax)
    - Time to maximal concentration (Tmax)

- Comparative pharmacodynamic studies

- Comparative clinical trials

- Comparative *in vitro* methods
  - Biopharmaceutics Classification System (BCS)-based biowaivers
  - Additional strengths biowaivers
Bioequivalence

- FPPs are bioequivalent if
  - they are pharmaceutically equivalent or pharmaceutical alternatives
  - bioavailabilities (both rate and extent) after administration in the same molar dose are similar to such a degree that their effects can be expected to be essentially the same

- Pharmaceutical alternative
  - Same molar amount of the same API(s) but differ in dosage form (e.g., tablets vs. capsules), and/or chemical form (e.g., different salts, different esters)
  - Deliver the same active moiety by the same route of administration
Establishing Bioequivalence

FPPs being tested

- Comparator product
  - WHO provides recommendations
  - To be discussed shortly

- Test product
  - Biobatch of sufficient size
    - Representative of product proposed for market
    - Support future scale-up
    - Full characterization in dossier
Establishing Bioequivalence

Important PK parameters

AUC: area under the concentration-time curve ⇒ measure of the extent of absorption

Cmax: the observed maximum concentration of a drug ⇒ measure of the rate of absorption

tmax: time at which Cmax is observed ⇒ measure of the rate of absorption
Plasma concentration time profile

\[ C_{\text{max}} \]

\[ T_{\text{max}} \]

\[ \text{AUC} \]
In vivo BE Study
Design

Basic design considerations:

- minimize variability not attributable to formulations
- minimize bias

goal: compare performance 2 formulations
In vivo BE Study Design

- Single-dose administration

- Multiple-dose administration
Preferred Approach

- Single-dose design
In vivo BE Study
Design

• Crossover Design
  – Each subject administered both test and comparator
  – Within-subject comparison
  – Preferred

• Parallel Design
  – Each subject administered test or comparator
  – Between-subject comparison
  – Only recommended for extremely long half-life drugs
  – Consult WHO
Crossover Design

- Blood samples are collected and assayed
  - Before and several times after drug administration. No need after 72 h
- Prior to period 2, pre-dose levels must be <5% of Cmax of 2\textsuperscript{nd} period
- Wash out period must take into account the slow metabolizers
- Minimum wash out: 7 days (1 week)
Drugs with long elimination $t_{1/2}$: Parallel

- Normally wash-out period should not exceed 3-4 weeks
- If a larger wash-out period is necessary a parallel design may be more appropriate
- Variability will be larger, needs higher sample size
  - Parallel design: Total variability (intra+inter)
  - Cross-over: Intra-subject variability
- Sampling: Up to 72 h

Randomization to treatments

Group 1: Treatment A
Group 2: Treatment B
Preferred Approach

- Single-dose administration
- Crossover comparison
Subjects

- Normally healthy volunteers
  - Inclusion / exclusion criteria
  - Randomization

- How many subjects?
  - Required sample size depends on **intra-individual variability** either known through reasonable literature or by means of a pilot study
  - “low” variability: ~ 12 – 26 volunteers
  - “high” variability: ~ can be up to 250 volunteers
Factors affecting the sample size

- The error variance (CV%) of the primary PK parameters
  - Published data
  - Pilot study

- The significance level desired (5%): consumer’s risk

- The statistical power desired (>80%): producer’s risk

- The mean deviation from comparator compatible with BE

- The acceptance criteria: (usually 80-125% or ±20%)
Reasons for a correct calculation of the sample size

- Too many subjects
  - It is unethical to disturb more subjects than necessary
  - Extra subjects at risk and they are not necessary
  - It is an unnecessary waste of some resources ($)

- Too few subjects
  - A study unable to reach its objective is unethical
  - All subjects at risk for nothing
  - All resources ($) is wasted when the study is inconclusive

- Minimum number of subjects: 12
Preferred Approach

- Single-dose administration
- Crossover (within-subject) comparison
- Healthy volunteers (justification for number)
In vivo BE Study Design

- Administration of products under fasted or fed conditions?

- Fasted conditions
  - Study conducted under fasted conditions the norm
  - Comparator product labeling (SPC)
    • Specifies fasted conditions
    • Does not specify fasted/fed for administration
    • States that either fasted or fed administration

- Fed conditions
  - If specified in comparator product labeling (SPC)
**In vivo BE Study Design**

Administration of products under fasted or fed conditions?

**Fed conditions**
- If specified in comparator product labeling (SPC)
- Type of meal to be consumed
  - high-fat, high-calorie meal
  - “standard” or typical breakfast

**Administration under both fasted and fed conditions**
- Not generally necessary for immediate-release products
- Required for modified-release products
Preferred Approach

- Single-dose administration
- Crossover (within-subject) comparison
- Healthy volunteers

Administration with or without food
- Fasted study is the norm
- Labeling of the comparator product is the guide
  - Bioavailability / pharmacokinetics
  - Adverse events

Consultation with Programme encouraged
Sampling Times

- Need a sufficient number of samples to properly characterize the concentration-time profile
  - 19 – 21 samples is typical

- Frequent sampling around expected Tmax

- Long enough such that $\frac{AUC_T}{AUC_1} > 0.8$
  - For long half-life drugs, $AUC_{0-72h}$ is adequate

- If Tmax is early e.g., at 1 h, rapid sampling will be necessary
  - For example, pre-dose, 15, 30, 45, 60, 75, 90, 120 min, 2.5h, 3h, etc.

- Cmax should not occur in first sampling time
Typical *in vivo* BE Design

- Single-dose administration
- Cross-over (within-subject) comparison
- Healthy volunteers
- Administration with or without food
  - Fasted study is the norm
- Adequate sampling protocol
- Thoroughly validated bioanalytical method (to be discussed)
- Data from parent compound used for BE decision
- Statistical analysis of concentration vs. time data
The primary concern in BE assessment is to limit the risk of a false declaration of equivalence.

Statistical analysis of the BE trial should demonstrate that a clinically significant difference in bioavailability between the multisource product and the comparator product is unlikely.

The statistical procedures should be specified in the protocol before the data collection starts.
The statistical method for testing PK BE 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 and by carrying out two one-sided tests at the 5% level of significance.

To establish PK BE, the calculated confidence interval should fall within a preset bioequivalence limit.
Statistical considerations

**BE Limits**

- The concept of the ±20% difference is the basis of BE limits (goal posts)

- If the concentration dependent data were linear, the BE limits would be 80-120%

- On the log scale, the BE limits are 80-125%

- The 90%CI must fit entirely within specified BE limits e.g. 80-125%
Acceptance criteria

- Single-dose, two-way crossover study
- Average bioequivalence
- AUC: 90% Confidence Interval (CI) within 80.0-125.0%
- Cmax: 90% CI within 80.0-125.0%
Acceptance criteria

![Graph showing acceptance criteria with points A, B, C, and D on the relative bioavailability axis.]}
## International Comparison

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>AUC 90% CI Criteria</th>
<th>Cmax 90% CI Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada (most drugs)</td>
<td>80 – 125%</td>
<td>none (point estimate only)</td>
</tr>
<tr>
<td>Europe &amp; USA</td>
<td>80 – 125%</td>
<td>80 – 125%</td>
</tr>
<tr>
<td>South Africa (most drugs)</td>
<td>80 – 125%</td>
<td>75 – 133% (or broader if justified)</td>
</tr>
<tr>
<td>Japan (some drugs)</td>
<td>80 – 125%</td>
<td>Some drugs wider than 80 – 125%</td>
</tr>
<tr>
<td>Worldwide</td>
<td>80 – 125%</td>
<td>Generally 80 – 125%</td>
</tr>
</tbody>
</table>
Special study designs

- **Highly variable drugs (HVD)**
  - When the intra-subject variability (ANOVA CV) > 30%
  - Reference-scaled average bioequivalence
  - Scaling allows for widening of acceptance criteria based on intra-subject CV observed for comparator product

- **Group sequential two-stage designs**
  - When the intra-subject variability is unknown
  - The study is conducted in two stages. The first group of subjects allows for an estimate of the variability to be calculated. This determines the size of the second group
  - The overall Type I error rate should be protected (5%)
Acceptance range in other regions

- 80-125% for the 90% CI is the conventional acceptance range

- 80-125% for the 90% CI for AUC and Cmax in FDA
  - Except scaling for HVD based on intra-subject variability of the reference
  - Except narrowing for NTID based on intra-subject variability of the reference

- 80-125% for 90 CI% of AUC and for PE of Cmax in Canada
  - 90-111% for 90% CI of AUC and 80-125% for CI of Cmax in Canada

- 80-125% for 90% CI of AUC and Cmax normally in EU
  - Except scaling for Cmax in HVD based on intra-subject variability
  - Narrowing to 90-111 in AUC and/or Cmax in NTI drugs
Statistical Analysis: log-transformation

- All concentration-dependent pharmacokinetic parameters (e.g. AUC and Cmax) should be log-transformed using either common logarithms to the base 10 or natural logarithms.

- The choice of common or natural logs should be consistent and should be stated in the study report.
Logarithmically transformed, concentration-dependent pharmacokinetic parameters should be analysed using analysis of variance (ANOVA)

Usually the ANOVA model includes the formulation, period, sequence or carry-over and subject factors

Parametric methods, i.e. those based on normal distribution theory, are recommended for the analysis of log-transformed bioequivalence measures
Statistical Analysis: in log scale

- The general approach is to construct a 90% confidence interval for the quantity $\mu_T - \mu_R$ and to reach a conclusion of pharmacokinetic equivalence if this confidence interval is within the stated limits.

- The nature of parametric confidence intervals means that this is equivalent to carrying out two one-sided tests of the hypothesis at the 5% level of significance.

- The antilogs of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the multisource and comparator products.
Statistical Analysis: outliers

- Methods for identifying and handling of possible outlier data should be specified in the protocol.

- Medical or pharmacokinetic explanations for such observations should be sought and discussed.

- As outliers may be indicative of product failure, post hoc deletion of outlier values is generally discouraged.

- An approach to dealing with data containing outliers is to apply distribution-free (non-parametric), statistical methods:
  - No longer valid or acceptable
  - Non-parametric methods ignore outlier values and it is equivalent to remove them.
Fixed-dose combination products

- The study design should follow the same general principles as described in previous sections.
- The multisource FDC product should be compared with the pharmaceutically equivalent comparator FDC product.
- In certain cases (e.g. when no comparator FDC product is available on the market) separate products administered in free combination can be used as a comparator.
Fixed-dose combination products

- Sampling times should be chosen to enable the pharmacokinetic parameters of all APIs to be adequately assessed.

- The bioanalytical method should be validated on respect to all compounds measured

- Statistical analyses should be performed with pharmacokinetic data collected on all active ingredients; the 90% confidence intervals of test/comparator ratio of all active ingredients should be within acceptance limits.
Bioanalytical Methods

- Two excellent sources:
  - Guideline on bioanalytical method validation (EMA, 2011)
  - Bioanalytical Method Validation – Draft (USFDA, 2013)

- These guidelines focus on the validation of the bioanalytical methods generating quantitative concentration data used for pharmacokinetic and toxicokinetic parameter determinations.

- Guidance and criteria are given on the application of these validated methods in the routine analysis of study samples from animal and human studies.
Bioanalytical Methods

- **Measurement of drug concentrations** in biological matrices (such as serum, plasma, blood, urine, and saliva) is an important aspect of medicinal product development.

- **Such data may be required to support applications for**
  - new actives substances and
  - generics as well as
  - variations to authorised drug products.

- **The results of**
  - animal toxicokinetic studies and
  - of clinical trials,
  - including bioequivalence studies are used to make critical decisions supporting the safety and efficacy of a medicinal drug substance or product.
Bioanalytical Methods

- It is therefore paramount that the applied bioanalytical methods used are well characterised, fully validated and documented to a satisfactory standard in order to yield reliable results.

- Acceptance criteria wider than those defined in these guidelines may be used in special situations.

- This should be prospectively defined based on the intended use of the method.
Bioanalytical Methods

- This guideline provides recommendations for the validation of bioanalytical methods applied to measure drug concentrations in biological matrices obtained in animal toxicokinetic studies and all phases of clinical trials.

- In addition, specific aspects for the analysis of study samples will be addressed.

- Furthermore, this guideline will describe when partial validation or cross validation should be carried out in addition to the full validation of an analytical method.

- Methods used for determining quantitative concentrations of biomarkers used in assessing pharmacodynamic endpoints are out of the scope of this guideline.
Full validation of an analytical method

- A full method validation should be performed for any analytical method whether new or based upon literature.

- The main objective of method validation is to demonstrate the reliability of a particular method for the determination of an analyte concentration in a specific biological matrix, such as blood, serum, plasma, urine, or saliva.

- Moreover, if an anticoagulant is used, validation should be performed using the same anticoagulant as for the study samples.

- Generally, a full validation should be performed for each species concerned.
Main characteristics of a bioanalytical method

- Characteristics that are essential to ensure the acceptability of the performance and the reliability of analytical results are:
  - Selectivity,
  - Lower limit of quantification,
  - Response function and calibration range,
  - Accuracy,
  - Precision,
  - Matrix effects,
  - Stability of the analyte(s) in the biological matrix and
  - Stability of the analyte(s) and of the internal standard in the stock and working solutions under the entire period of storage and processing conditions.
Several analytes

- Usually one analyte or drug has to be determined, but on occasions it may be appropriate to measure more than one analyte.

- This may involve two different drugs, but can also involve a parent drug with its metabolites, or the enantiomers or isomers of a drug.

- In these cases the principles of validation and analysis apply to all analytes of interest.

Accuracy, precision, stability in presence of the other analyte
Establishing Equivalence

Different approaches for establishing equivalence

- Standard: in vivo BE studies
- PD studies
- Clinical studies
- In vitro methods
Biopharmaceutics Classification System

- BCS originally explored with the aim of granting biowaivers for scale-up and post-approval changes (SUPAC)

- Biowaiver may be considered when
  - An *in vivo* bioavailability and/or bioequivalence is considered not necessary for FPP approval
    - *In vivo* studies can be expensive and time consuming
  - Under certain circumstances, a dissolution test could be used as a basis for the decision on equivalent product performance

- More recently, further uses of BCS have been explored
Rationale

The theory is that the oral availability of an API from a FPP can be expected to range from being

– heavily dependent on the formulation and method of manufacture of the pharmaceutical product (e.g., Class II or IV APIs); to
– Being mostly dependent on the permeability properties of the API (e.g., Class III APIs)
Rationale

- Requirement for *in vivo* bioequivalence testing may be waived under certain conditions
  - Solubility of API
  - Permeability of API
  - Uncomplicated API
    - Not narrow therapeutic range
    - No known bioavailability problems
  - Immediate-release FPP
  - Acceptable dissolution characteristics of FPP

- Minimizing risk of inappropriate BE decision
Biopharmaceutics Classification System (BCS)

- Classification system for APIs
  - Aqueous solubility
  - Intestinal permeability

API classification according to BCS

<table>
<thead>
<tr>
<th>BCS Classification</th>
<th>Solubility</th>
<th>Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS class I</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>BCS class II</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>BCS class III</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>BCS class IV</td>
<td>low</td>
<td>low</td>
</tr>
</tbody>
</table>
**BCS-based Biowaiver guidance**


  **Annex 7:** Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability


  - FDA has a new draft guidance

BCS-based Biowaiver

- Eligibility for BCS-based Biowaiver
  - General Notes on Biopharmaceutics Classification System (BCS)-based Biowaiver Applications

- Requirements for BCS-based Biowaiver
  - General Notes on BCS-based Biowaiver Applications
  - Biowaiver Application Form: Biopharmaceutics Classification System (BCS)

- http://apps.who.int/prequal/info_applicants/info_for_applicants_BE_implementation.htm
BCS-based Biowaiver: Two step process

1. Classification of the API
   a. Aqueous solubility
   b. Absorption / permeability

2. FPP evaluation
   • Conventional, immediate-release products
   • Comparison to the comparator product
     a. Comparison of formulations (excipients)
     b. Comparative dissolution profiles (CDP)
Step 1

Classification of the API
Classification criteria

High solubility:

- The highest dose is completely soluble in 250 ml or less of aqueous solution at pH 1.2 – 6.8 (37°C)

250 ml: derived from typical BE study protocols that prescribe the administration of a FPP to fasting human volunteers with a glass (approximately 250 ml) water
## pH in the gastrointestinal tract

<table>
<thead>
<tr>
<th>site</th>
<th>fasted pH</th>
<th>fed pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>stomach</td>
<td>1.4 – 2.1</td>
<td>4.3 – 5.4</td>
</tr>
<tr>
<td>small intestine:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>duodenum</td>
<td>4.9 – 6.4</td>
<td>4.2 – 6.1</td>
</tr>
<tr>
<td>jejunum</td>
<td>4.4 – 6.6</td>
<td>5.2 – 6.2</td>
</tr>
<tr>
<td>ileum</td>
<td>6.5 – 7.4</td>
<td>6.8 – 7.5</td>
</tr>
<tr>
<td>large intestine:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cecum</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>upper colon</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>lower colon</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>
High solubility:

◆ The highest dose is completely soluble in 250 ml or less of aqueous solution at pH 1.2 – 6.8 (37°C)

◆ A solubility profile should be developed
  ◆ At a minimum, solubility should be determined at pH 1.2, 4.5, 6.8, and at pKa if within range

◆ Dose solubility volume (DSV) = dose (mg) / solubility (mg/mL)
  e.g., highest dose = 500mg, solubility (37°C) at pH 4.5 = 31.2 mg/mL
  DSV = 500/31.2 = 16.03 mL
  16.03mL < 250mL so highly soluble at pH 4.5
## Solubility classification for biowaiver eligibility: Based on highest strength or highest dose?

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Parameter for solubility classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>Highest therapeutic dose</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>Highest strength</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Highest therapeutic dose</td>
</tr>
<tr>
<td>Singapore</td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td></td>
</tr>
<tr>
<td>South Korea</td>
<td>Highest strength</td>
</tr>
<tr>
<td>USA</td>
<td></td>
</tr>
<tr>
<td>WHO PQTm</td>
<td>Highest therapeutic dose</td>
</tr>
</tbody>
</table>
Highly permeable:

- An API is considered **HIGHLY PERMEABLE** when the **extent of absorption** in humans is determined to be > 85% of an administered dose, based on a mass balance determination or in comparison to an intravenous reference dose, in the absence of evidence suggesting instability in the gastrointestinal tract.

Intestinal membrane permeability may be determined by *in vitro* or *in vivo* methods that can predict extent of drug absorption in humans.
Highly permeable:

- EU guidance: linear and complete *absorption* reduces the possibility of an IR FPP influencing the bioavailability (absorption >85%).

- FDA guidance: absolute bioavailability >90%
Biopharmaceutics Classification System

- Biopharmaceutics Classification System (BCS)
  - Classification system for APIs
    - Aqueous solubility
    - Intestinal permeability

- API classification according to BCS

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</tr>
<tr>
<td>BCS class IV</td>
<td>low</td>
<td>low</td>
</tr>
</tbody>
</table>
Eligibility of an API for a BCS-based biowaiver

1. Classification within BCS
   1. Class I and III APIs are eligible

2. Risk assessment
   1. Narrow therapeutic index (NTI)
   2. Critical use(?)
## International Comparison

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>BCS Class eligible for BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>I &amp; III</td>
</tr>
<tr>
<td>USA</td>
<td>I (I &amp; III proposed)</td>
</tr>
<tr>
<td>Canada</td>
<td>I &amp; III</td>
</tr>
<tr>
<td>China</td>
<td>I</td>
</tr>
<tr>
<td>Singapore / ASEAN</td>
<td></td>
</tr>
<tr>
<td>South Korea</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>I (only specified APIs)</td>
</tr>
<tr>
<td>Japan</td>
<td>None at this time</td>
</tr>
<tr>
<td>WHO PQTm</td>
<td>I &amp; III</td>
</tr>
</tbody>
</table>
Current Situation

- Two-pronged approach for biowaiver eligibility
  - APIs identified as Class I or III by PQTm
    - Programme has reviewed existing information on the solubility, bioavailability, and dissolution data of the invited medicines
    - APIs have been identified as eligible for a BCS-based biowaiver application
    - Data for classification not required as part of application
  - Applicants can submit solubility and absorption/permeability data to aid in API classification as part of a biowaiver application
Current Situation

- Medicines for HIV/AIDS and related diseases
  - Abacavir sulfate (Class III)
  - Emtricitabine (Class I)
  - Lamivudine (Class III)
  - Stavudine (Class I)
  - Zidovudine (Class I)

- Related
  - Fluconazole polymorphs II & III (Class I)

- NTD treatments
  - Diethylcarbamazine (Class III*)

- Anti-tuberculosis medicines
  - Ethambutol (Class III)
  - Isoniazid (Class III)
  - Levofloxacin (Class I)
  - Moxifloxacin HCl (Class I)
  - Ofloxacin (Class I)
  - Pyrazinamide (Class III)
  - Linezolide (Class I)
Current Situation

- A biowaiver request can be made for monocomponent or fixed-dose combination (FDC) products containing eligible APIs.

- Monocomponent or FDC FPPs containing other APIs must be supported with *in vivo* BE data.
Step 2

Evaluation of FPP
FPP evaluation

- Selection of comparator product
  - To be discussed
  - Same requirements as comparator for in vivo study

- Biobatch reflective of proposed commercial product

- Two key elements
  - Comparison of formulations (excipients)
  - Comparative dissolution profiles (CDP)
Comparative Dissolution

One component of the evaluation of an FPP for a biowaiver
What is dissolution testing (IR products)?

It measures the portion (%) of the API

1. that has been released from tablets/capsules matrix and
2. that has dissolved in the dissolution medium during controlled testing conditions within a defined period

In simple terms:

– The tablet/capsule thus first disintegrates
– Then the API will be able to dissolve
– Slow disintegration ➜ slow dissolution
Emtricitabine capsules

Disintegration of shell

Continuous UV detection – fiber optic

Dissolution of API

Source: Chinese Pharmacopoeial Commission development report
Apparatus

Apparatus 1
basket

Apparatus 2
paddle
Single point dissolution test

- Simplest form of dissolution
  - One sample is withdrawn from the dissolution medium per vessel
    - Through an in-line or end-of-sampling probe filter
  - at a pre-determined time point and
  - the sample is analysed for the % API(s) dissolved
    - UV/VIS or HPLC most common

- Result is given as e.g.
  - 93 % of label claim in 30 minutes (range: 89 – 97 %)
  - No decimal is required
Multi-point dissolution

In multipoint dissolution

- multiple (≥ 3) samples are withdrawn from the dissolution medium per vessel during dissolution testing
- at pre-determined time points (intervals) and
- each sample is analysed for the % API dissolved

A graph of % API dissolved against time

= the dissolution profile
Multi-point dissolution
Example of dissolution profile

ACTIVE INGREDIENT: CLARITHROMYCIN
MEDIUM: PHOSPHATE BUFFER pH 6.8

Dissolution (%)

WITHDRAWAL TIME IN MINUTES

Clarithromycin 250 mg tablets
Comparative dissolution testing
The principle and basic requirements

- Comparison of 2 or more products or batches containing the same API
  - by means of multipoint dissolution (comparing profiles)

1. The strength of products / batches may OR may not be the same depending on purpose of test

2. The dissolution conditions must be the same, e.g.
   - Apparatus, rotation speed, medium, volume & temperature

3. Samples are taken at the same time points for data comparison
Comparative dissolution testing example

ACTIVE INGREDIENT: CLARITHROMYCIN
MEDIUM: PHOSPHATE BUFFER pH 6.8

Dissolution (%) vs. Withdrawal time in minutes for:
- PRODUCT B 500 mg (triangle)
- PRODUCT B 250 mg (square)
Comparative dissolution testing

When are dissolution profiles similar?

Read more: Generic guideline, Appendix 1

– Recommendations for conducting and assessing comparative dissolution profiles
Comparative dissolution testing  
Profile similarity determination

1. If both the test and reference product show \( \geq 85\% \) dissolution within 15 minutes,
   - the profiles are considered to be similar
     • No calculations are required

   If this is not the case, apply point 2 (next point)

2. **Calculate the f2 value (similarity factor):**
   - If \( f_2 \geq 50 \)
     • the profiles are regarded similar
     • No decimal required (\( f_2 = 49.51 \equiv 50 \))
Comparative dissolution testing
Similarity factor $f_2$

$$f_2 = 50 \cdot \log \left\{ \left[ 1 + \left( \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right) \right]^{-0.5} \cdot 100 \right\}$$

- $n =$ number of time points
- $R_t =$ % API dissolved of reference product at time point $x$
- $T_t =$ % API dissolved of test product at time point $x$

- Minimum of 3 time points (zero excluded)
- 12 units (one / vessel) for each batch
- Only one measurement should be considered after the reference product has reached 85 % dissolution (or asymptote is reached)
- **RSD:** $\leq 20\%$ at early time point & $\leq 10\%$ at later time points (apply with some discretion)
Typical mistakes

Often manufacturers include the following points in the f2 calculation

- **Time zero** in the f2 calculation
  - % dissolved = 0 at t = 0 minutes

- **Points beyond** the reference product reaches **85%**
  - It is not according to the “rules”

- What is the problem with including these points?
  - **The f2 value will increase** – may lead to false positive f2
Comparative dissolution testing
Similarity factor $f_2$

Take note - **apply WHO requirement in PQP**:

- Unfortunate differences between WHO, FDA and EMEA guidelines on determination of “dissolution last point” for $f_2$ calculations:

<table>
<thead>
<tr>
<th>Source</th>
<th>Only one measurement (of both products) should be considered after:</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA (2000)</td>
<td>BOTH the reference AND test products have reached 85 % dissolution (or asymptote is reached)</td>
</tr>
<tr>
<td>WHO (2006)</td>
<td>the REFERENCE product has reached 85 % dissolution (or asymptote is reached)</td>
</tr>
<tr>
<td>EMEA (2010)</td>
<td>ANY ONE of the reference OR test product has reached 85 % dissolution (or asymptote is reached)</td>
</tr>
</tbody>
</table>
### Example 1

**Determination of similarity of profiles**

#### Example 1-A

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% API dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tablet A (Ref)</td>
</tr>
<tr>
<td>10</td>
<td>87</td>
</tr>
<tr>
<td>15</td>
<td>96</td>
</tr>
<tr>
<td>20</td>
<td>99</td>
</tr>
<tr>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>101</td>
</tr>
<tr>
<td>60</td>
<td>101</td>
</tr>
</tbody>
</table>

**f2 required?** Yes

**f2 (n = ?)**

#### Example 1-B

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% API dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tablet D (Ref)</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>72</td>
</tr>
<tr>
<td>20</td>
<td>85</td>
</tr>
<tr>
<td>30</td>
<td>97</td>
</tr>
<tr>
<td>45</td>
<td>102</td>
</tr>
<tr>
<td>60</td>
<td>102</td>
</tr>
</tbody>
</table>

**f2 required?** No, ≥ 85% in 15 min

**f2 (n = N/A ?)**
Example 1
Determination of similarity of profiles (cont.)

<table>
<thead>
<tr>
<th>Example 1-C</th>
<th>Example 1-D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time (min)</strong></td>
<td><strong>% API dissolved</strong></td>
</tr>
<tr>
<td></td>
<td>Tablet X (Ref)</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>45</td>
<td>80</td>
</tr>
<tr>
<td>60</td>
<td>95</td>
</tr>
</tbody>
</table>

f2 required? Yes
f2 (n = ?)
Sampling intervals

Why must samples be taken at short intervals for profile comparison?

To prevent false positive results

Test: Let us take previous example 1-D and omit some points
Applicants like to give data collected at: 15, 30, 45 and 60 minutes

### Example 1-D (acceptable intervals)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% API dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tablet D (Ref)</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>30</td>
<td>83</td>
</tr>
<tr>
<td>45</td>
<td>98</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

### Example 1-D (poor intervals)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% API dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tablet X (Ref)</td>
</tr>
<tr>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>30</td>
<td>83</td>
</tr>
<tr>
<td>45</td>
<td>98</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

f2 required? \( f_2 \) required? \( f_2 \)

\( f_2 (n = ?) \) \( f_2 (n = ?) \)
# Comparative dissolution testing

## Dissolution conditions (study design)

| **Apparatus (choice)** | • Paddle, 75 (or 50) rpm or  
<table>
<thead>
<tr>
<th></th>
<th>• Basket, 100 rpm</th>
</tr>
</thead>
</table>
| **Dissolution media (All three media for full comparison)** | 1. pH 6.8 phosphate buffer  
|                        | 2. pH 4.5 acetate buffer |
|                        | 3. Buffer pH 1.2 or 0.1 M HCl |
|                        | 4. Release medium (if different) |
| **Volume of media**    | 900 ml or less |
| **Temperature**        | $37 \degree C \pm 0.5 \degree C$ |
| **Sampling points**    | 5, 10, 15, 20, 30, 45, (60, 120) min. (short intervals) |
| **Units (vessels)**    | 12 |
Comparative dissolution testing
Comparison of products / batches

When are the dissolution properties of two products (batches) regarded similar?

When their dissolution profiles are similar
  - in all media (not so simple for Class 2 and 4 APIs)

• Statements of instability or insolubility are not acceptable unless demonstrated / justified (literature also acceptable)
  • Assessor must query unjustified statements like this
Some practical matters
Evaluators should be aware of

1. **Coning** (heap formation) in dissolution vessel

2. **Dissolution results > than assay** ??
   - Do not query too easily

3. **Filtration** of dissolution samples
Practical matters
Coning / Heap formation

Coning (heap formation) in dissolution vessel

- With paddle speed = 50 rpm
- This may slow down (suppress) the dissolution
- Affects only some products
- WHO avoids this by paddle 75 rpm in BE guide & Ph.Int.
  - Thus avoiding possible product-to-product variable due to hydrodynamics
Practical matters
Coning / Heap formation

- Coning sometimes seen at lower paddle speed
- The drug / tablet is covered by excipient heap
FPP evaluation
(Back to it!)

- Selection of comparator product
  - To be discussed

- Biobatch reflective of proposed commercial product

- Two key elements
  - Comparison of formulations (excipients)
  - Comparative dissolution profiles (CDP)
Dissolution test conditions

● Comparative *in vitro* dissolution
  – Comparative testing should ensure the similarity of the test and comparator product in three different pH media considered relevant for absorption from the GI tract
  – Comparative *in vitro* dissolution testing should be conducted in at least three aqueous media of pH 1.2, 4.5, and 6.8
    • Volume of media: 900 mL
    • Temperature of media: $37 \pm 1^\circ C$
    • Agitation: paddle apparatus at 75 rpm or basket apparatus at 100 rpm
    • Replicates: 12 units
    • Sampling schedule: e.g., 5, 10, 15, 20, 30, and 45 minutes
    • Surfactants not permitted
Dissolution Definitions

- **‘Very rapidly’ dissolving FPPs**
  - Not less than 85% of the labeled amount is released within 15 minutes or less from the test and comparator product
  - In this case, profile comparison is not needed

- **‘Rapidly’ dissolving FPPs**
  - Not less than 85% of the labeled amount is released within 30 minutes or less from the test and comparator product
  - Profile comparison (e.g., f2 testing) required
FPP comparison
Class I APIs

● Excipients
  – Should employ well known excipients in usual amounts
  – Beneficial to contain similar amounts of the same excipients
  – Critical excipients (e.g., mannitol, sorbitol, surfactants), if present, should not differ qualitatively or quantitatively

● Comparative *in vitro* dissolution
  – Products should be similarly rapidly dissolving
    • NLT 85% in 30 minutes for both products
    • f2 profile comparison (unless 85% in 15 minutes for both FPPs)
FPP comparison
Class III APIs

- APIs are highly soluble but limitations to absorption due to various reasons

- Excipients
  - Qualitatively the same excipients
  - Quantitatively very similar (as per Level 1 change according to SUPAC)

- Comparative *in vitro* dissolution
  - NLT 85% dissolved within 15 minutes for both products
Considerations

- BCS-based biowaivers for some FDCs difficult
  - FDC comparator not available

- FDCs must include only Class I or III APIs to be eligible e.g., rifampicin containing FPPs are not eligible for a BCS-based biowaiver

- Identification of API eligibility based on solubility, permeability, safety and related properties
  - This does not imply that the comparator product(s) will be very rapidly or rapidly dissolving
  - Very rapidly or rapidly dissolving properties are not required to make an in vivo bioequivalence comparison
Considerations

- The comparative *in vitro* dissolution data is the equivalence data
  - Fully developed protocol and operating procedures
  - Complete documentation
  - Biowaiver Application Form: Biopharmaceutics Classification System
  - Monitoring, auditing, inspection
BCS-based biowaiver
Fictional example

- Refer to handout

- Step 1: Classification of API
  - Two-pronged approach for PQTm
    - 1. PQP identifies eligible APIs
      - Solubility data in dossier should corroborate classification
    - 2. Applicant provides classification data
BCS-based biowaiver
Fictional example

- **Step 2: FPP evaluation**
  - Biobatch assessment
  - Comparative assessment of formulation
    - Proposed product
    - WHO comparator product
  - Comparative dissolution profiles
    - At a minimum, at pH 1.2, 4.5, and 6.8

- **Conclusion of assessment**
  - Biowaiver granted?
  - Next steps
Additional strengths biowaivers

- Waiver of requirement to conduct *in vivo* BE studies with each strength of a product line

- *In vivo* data available for one strength
  - Usually highest strength
  - Linear pharmacokinetics

- Similarity of formulations
  - Proportionality

- Similarity of dissolution characteristics
Similarity of formulations

- Annex 7 of TRS 992 defines proportionally similar formulations as:
  - All active and inactive ingredients are in exactly the same proportion in the different strengths
    - e.g., 50 mg tablet has exactly half of all ingredients of 100 mg tablet and twice that of 25 mg tablet
  - For a high potency API (amount of API is low; up to 10 mg per dosage unit)
    - Total weight of FPP remains the same (within ± 10%)
    - Same inactive ingredients, change obtained by altering API with corresponding change to the highest percentage excipient
Similarity of dissolution characteristics

- Comparative *in vitro* dissolution testing
  - Comparative testing should ensure the similarity of the different strengths in three different pH media considered relevant for absorption from the GI tract
  - Comparison of different strengths within product line
  - Not comparison to comparator product
    - Comparison to comparator may be supportive in some cases *e.g.*, Class IV API
Comparative \textit{in vitro} dissolution

**Immediate-release FPPs**
- Comparative testing should be conducted in at least three media of pH 1.2, 4.5, and 6.8
- 12 units
- Paddle apparatus at 75 rpm or basket apparatus at 100 rpm
- Use of surfactants discouraged
- If both strengths release >85% in 15 minutes, further profile comparison unnecessary
- Otherwise, profile comparison required
  - f2 testing
Comparator (Reference) Products

- A FPP with which the multi-source product is intended to be interchangeable in clinical practice

- The selection of the comparator product is usually made at the national level by the drug regulatory authority

- A different set of circumstances apply to comparator selection for Prequalification Programme (PQP)
Comparator (Reference) Products

Example of how a national RA can select a comparator:

- choose innovator for which quality, safety and efficacy has been established from national market (nationally authorised innovator)
- choose WHO comparator product from the comparator list (WHO comparator product)
- choose innovator product from well-regulated country (ICH et al. innovator)
- if no innovator comparator is available, a generic market leader can be chosen
Comparator (Reference) Products

Selection of a comparator for a single national market:

Difficult to translate when other countries are at stake

National comparator may be the national market leader

No problem in that market

but others!?
Differentiate between use for single market or many countries!

EMA:

Austria  France  Latvia  Poland
Belgium  Germany  Liechtenstein  Portugal
Cyprus  Greece  Lithuania  Slovak Republic
Czech Republic  Hungary  Luxemburg  Slovenia
Denmark  Iceland  Malta  Spain
Estonia  Ireland  The Netherlands  Sweden
Finland  Italy  Norway  United Kingdom

For an abridged application claiming essential similarity to a reference product, application to numerous Member States based on bioequivalence with a reference product from one Member State can be made.
Comparator (Reference) Products

Comparator products should be obtained from a well regulated market with stringent regulatory authority i.e., from countries participating in the International Council on Harmonization (ICH).

Countries officially participating in ICH
- ICH members: European Union, Japan, USA, Canada, and Switzerland
- Other countries associated with ICH (through legally binding mutual recognition agreements) include Australia, Norway, Iceland and Liechtenstein.
Comparator lists

- List of acceptable comparator products for each treatment area on WHO PQTm website

- [http://apps.who.int/prequal/info_applicants/info_for_applicants_BE_comparator.htm](http://apps.who.int/prequal/info_applicants/info_for_applicants_BE_comparator.htm)

- There are instances when a comparator is not available in the ICH region
  - *e.g.*, Terizidone 300mg
    - Terivalidin 250 mg (Sanofi-Aventis, South Africa)
  - *e.g.*, Artesunate + Amodiaquine 100 mg + 270 mg FDC
    - Coarsucam (Sanofi-Aventis)
### Recommended comparator products: anti-tuberculosis medicines

<table>
<thead>
<tr>
<th>Invited medicinal products</th>
<th>Recommended comparator product (Strength, Manufacturer)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single ingredient first-line anti-tuberculosis medicines</strong></td>
<td></td>
</tr>
<tr>
<td>Ethambutol, 100 mg tablet and 200 mg, 275 mg and 400 mg tablet/capsule, 25 mg/ml oral solution</td>
<td>Myambutol (400 mg tablet, Riemser Arzneimittel or Teofarma) Ethambutol hydrochloride (100, 400 mg tablet, West Ward, US^2)</td>
</tr>
<tr>
<td>Isoniazid, 50 mg, 100 mg and 150 mg tablet and 300 mg tablet/capsule</td>
<td>Isozid (100 mg tablet, Fatol) Isoniazid (100 mg, 300 mg tablet, Sandoz, US^2)</td>
</tr>
<tr>
<td>Pyrazinamide, 150 mg tablet and 250 mg and 400 mg tablet/capsule, 30 mg/ml oral syrup</td>
<td>Pyrazinamide Lederle (500 mg tablet, Riemser Arzneimittel) Pyrazinamide (500 mg tablet, Dava Pharm Inc, US^2)</td>
</tr>
<tr>
<td>Rifampicin, 150 mg and 300 mg capsule</td>
<td>Rimactane (150 mg, 300 mg tablet, Novartis or Sandoz) Rifadin (150 mg, 300 mg capsule, Sanofi-Aventis) Rifampicin (150mg, 300 mg, Sandoz, NL)</td>
</tr>
<tr>
<td>Streptomycin, 0.75 g and 1 g powder for solution for injection (vial)</td>
<td>Streptomycin (1g/2.5ml injection, Pfizer, US^2)</td>
</tr>
<tr>
<td><strong>Fixed-dose combination products of first-line anti-tuberculosis medicines:</strong></td>
<td></td>
</tr>
<tr>
<td>Isoniazid + Rifampicin, 75 mg + 150mg, 150 mg + 150 mg, and 150 mg + 300 mg tablet/capsule</td>
<td>Rifinah (rifampicin 300 mg + isoniazid 150 mg tablet, Sanofi-Aventis), Rifamate (rifampicin 300 mg + isoniazid 150 mg capsule, Sanofi-Aventis, US^2)</td>
</tr>
</tbody>
</table>

For other invited fixed-dose combination products of anti-tuberculosis medicines, use appropriate combination of the recommended single ingredient comparator products.
Comparator (Reference) Products

Information Requirements

Within the submitted dossier, the country of origin of the comparator product should be reported together with lot number and expiry date, as well as results of pharmaceutical analysis to prove pharmaceutical equivalence. Further, in order to prove the origin of the comparator product the applicant must present all of the following documents:

1. Copy of the comparator product labelling. The name of the product, name and address of the manufacturer, batch number, and expiry date should be clearly visible on the labelling.
2. 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.
3. Documentation verifying the method of shipment and storage conditions of the comparator product from the time of purchase to the time of study initiation.
4. 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 or equivalent responsible for the application to the Prequalification Programme.
Summary

- Study design considerations for *in vivo* bioequivalence studies

- *In vitro* approaches for establishing bioequivalence
  - BCS-based biowaivers
  - Additional strengths biowaivers

- Key elements for comparative dissolution testing

- Selection of comparator products
Thank you for your attention!