Annex 7

WHO guideline on Biopharmaceutics Classification System-based biowaivers

Background

A recommendation was made to the World Health Organization (WHO) Norms and Standards for Pharmaceuticals Team by the group of experts participating at the Joint Meeting on Regulatory Guidance for Multisource Products (1–3 November 2022), as well as by other parties, including the WHO Prequalification Team, to update the WHO Biopharmaceutics Classification System (BCS)-based biowaiver requirements (associated section within the overarching WHO *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability)* (*1*) in order to harmonize those guidelines with those stated in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline M9 on *Biopharmaceutics classification system-based biowaivers*, adopted in November 2019 (*2*).

The *WHO guideline on Biopharmaceutics Classification System-based biowaivers* will supersede the BCS-based biowaiver section of the WHO *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability* (*1*). The purpose of this document is to provide recommendations to support the biopharmaceutics classification of active pharmaceutical ingredients (APIs) and the BCS-based biowaiver of bioequivalence studies for finished pharmaceutical products (FPPs).

Contents

This text is based on the ICH guideline M9: *Biopharmaceutics Classification System-based biowaivers*, November 2019.

1. Introduction

Two finished pharmaceutical products (FPPs) containing the same active moiety of the active pharmaceutical ingredient (API) are considered bioequivalent if their bioavailabilities (rate and extent of API absorption) after administration in the same molar dose lie within acceptable predefined limits. These limits are set to ensure comparable in vivo performance (that is, similarity in terms of safety and efficacy). In in vivo bioequivalence studies, the pivotal pharmacokinetic parameters maximum concentration (C_{max}) and area under the concentration time curve (AUC) are generally used to assess the rate and extent of drug absorption.

The Biopharmaceutics Classification System (BCS)-based biowaiver approach is intended to reduce the need for in vivo bioequivalence studies, as it can provide a surrogate for in vivo bioequivalence. In vivo bioequivalence studies may be exempted if an assumption of equivalence in in vivo performance can be justified by satisfactory in vitro data. The BCS is a scientific approach based on the aqueous solubility and intestinal permeability characteristics of the APIs. The BCS categorizes APIs into one of four BCS classes, as follows:

- class I: high solubility, high permeability
- class II: low solubility, high permeability
- class III: high solubility, low permeability
- class IV: low solubility, low permeability.

This guidance provides recommendations to support the biopharmaceutics classification of APIs and the BCS-based biowaiver of bioequivalence studies for FPPs. The BCS-based biowaiver principles may be applied to bioequivalence purposes not explicitly specified in the guideline, provided they can be supported by a thorough scientific rationale.

2. Scope

BCS-based biowaivers may be used to substantiate in vivo bioequivalence. Examples include the comparison between products used during clinical development through commercialization, post-approval changes, and applications for generic products in accordance with regional regulations.

The BCS-based biowaiver is only applicable to immediate-release, solid orally administered dosage forms or suspensions designed to deliver the API to the systemic circulation. FPPs, having a narrow therapeutic index, are excluded from consideration for a BCS-based biowaiver in this guidance. Fixed-dose combination products are eligible for a BCS-based biowaiver when all APIs

contained in the combination product meet the criteria, as defined in sections 4 and 5 of this guidance.

3. Glossary

The definitions given below apply to the terms used in this document. They have been aligned to the extent possible with the terminology in related WHO guidelines and good practices included in the WHO Quality Assurance of Medicines Terminology Database – List of Terms and related guidelines,[8] but may have different meanings in other contexts.

active pharmaceutical ingredient. Any substance or mixture of substances intended to be used in the manufacture of a pharmaceutical dosage form and that, when so used, becomes an active ingredient of that pharmaceutical dosage form. Such substances are intended to provide pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure and function of the body.

bioavailability. The rate at and extent to which the active moiety is absorbed from a pharmaceutical dosage form and becomes available at the sites of action. Reliable measurements of active pharmaceutical ingredient concentrations at the sites of action are usually not possible. The substance in the systemic circulation, however, is considered to be in equilibrium with the substance at the sites of action. Bioavailability can therefore be defined as the rate at and extent to which the active pharmaceutical ingredient or active moiety is absorbed from a pharmaceutical dosage form and becomes available in the systemic circulation. Based on pharmacokinetic and clinical considerations, it is generally accepted that, in the same subject, an essentially similar plasma concentration time course will result in an essentially similar concentration time course at the sites of action.

bioequivalence. Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and their bioavailabilities, in terms of rate (C_{max} and t_{max}) and extent of absorption (area under the curve), after administration of the same molar dose under the same conditions, are similar to such a degree that their effects can be expected to be essentially the same.

Biopharmaceutics Classification System. The Biopharmaceutics Classification System is a scientific framework for classifying active pharmaceutical ingredients

[8] https://www.who.int/publications/m/item/quality-assurance-of-medicines-terminology-database.

based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the pharmaceutical product and the critical examination of the excipients of the pharmaceutical product, the Biopharmaceutics Classification System takes into account the major factors that govern the rate and extent of active pharmaceutical ingredient absorption (exposure) from immediate-release oral solid dosage forms: excipient composition, dissolution, solubility and intestinal permeability.

biowaiver. The regulatory pharmaceutical product approval process whereby the dossier (application) is approved based on evidence of equivalence rather than through in vivo equivalence testing.

comparator product. The comparator product is a pharmaceutical product with which the multisource product is intended to be interchangeable in clinical practice. The comparator product will normally be the innovator product for which efficacy, safety and quality have been established. If the innovator product is no longer marketed in the jurisdiction, the selection principle, as described in *Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products*,[9] should be used to identify a suitable alternative comparator product.

dosage form. The form of the finished pharmaceutical product (for example, tablet, capsule, suspension or suppository).

equivalence requirements. In vivo or in vitro testing requirements for approval of a multisource pharmaceutical product for a marketing authorization.

finished pharmaceutical product. A finished dosage form of a pharmaceutical product that has undergone all stages of manufacture, including packaging in its final container and labelling.

fixed-dose combination product. A finished pharmaceutical product that contains two or more active pharmaceutical ingredients.

generic product. See "multisource pharmaceutical product".

innovator pharmaceutical product. Generally, the innovator pharmaceutical product is that which was first authorized for marketing, on the basis of complete documentation of quality, safety and efficacy.

[9] Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-ninth report. WHO Technical Report Series No. 992, Annex 8. Geneva: World Health Organization; 2015.

interchangeable pharmaceutical product. A product that is therapeutically equivalent to a comparator product and can be interchanged with the comparator in clinical practice.

multisource pharmaceutical product. A pharmaceutically equivalent or pharmaceutically alternative product that may or may not be therapeutically equivalent. Multisource pharmaceutical products that are therapeutically equivalent are interchangeable.

4. Biopharmaceutics classification of the API

BCS-based biowaivers are applicable to FPPs where the APIs exhibit high solubility and either high permeability (BCS class I) or low permeability (BCS class III).

A biowaiver is applicable when the APIs in the test and comparator products are identical. A biowaiver may also be applicable if test and comparator products contain different salts, provided that both belong to BCS class I (high solubility and high permeability). A biowaiver is not applicable when the test product contains an ester, ether, isomer, mixture of isomers, complex or derivative of an API different from that of the comparator product, since these differences may lead to different bioavailabilities not deducible by means of experiments used in the BCS-based biowaiver concept. Prodrugs may be considered for a BCS-based biowaiver when absorbed as the prodrug.

4.1 Solubility

An API is classified as highly soluble if the highest single therapeutic dose is completely soluble in 250 millilitres (mL) or less of aqueous media over the pH range 1.2–6.8 at 37 (± 1) °C.

The applicant is expected to establish experimentally the solubility of the API over the pH range 1.2–6.8 at 37 (± 1) °C . At least three pHs within this range, including buffers at pH 1.2, 4.5 and 6.8, should be evaluated. In addition, solubility at the pH of lowest solubility of the API should be evaluated if it is within the specified pH range.

Solubility should be evaluated by a method appropriate to the properties of the API.

Equilibrium solubility experiments may be performed using a shake flask technique or an alternative method, if justified. Small volumes of solubility media may be employed if the available experimental apparatus will permit it. The pH for each test solution should be measured after the addition of the API and at the end of the equilibrium solubility study to ensure that the solubility measurement is conducted under the specified pH. The experiment should be

conducted over a suitable time frame to reach equilibrium and the pH should be adjusted during this period as necessary.

Alternatively, when an equilibrium solubility study is not feasible due to the high amount of API required for the experiment, or when it is not possible to maintain the pH of the medium with pharmacopoeial buffers, solubility experiments where the highest therapeutic single dose (or a slightly higher amount to avoid recovery problems in the experiments) is examined in a 250 mL volume, or a proportionally smaller amount examined in a proportionally smaller volume of buffer, can be considered (*3*).

The lowest measured solubility over the pH range 1.2–6.8 will be used to classify the API.

A minimum of three replicate determinations at each solubility condition or pH using appropriate pharmacopoeial media is necessary to demonstrate solubility using a suitably validated method.

In addition, adequate stability of the API in the solubility media covering the gastrointestinal transit time should be demonstrated. In cases where the API is not stable, with >10% degradation over the extent of the solubility assessment, solubility cannot be adequately determined, and thus the API cannot be classified. In addition to experimental data, literature data may be provided to substantiate and support solubility determinations, keeping in mind that peer-reviewed articles may not contain the necessary details of the testing to make a judgement regarding the quality of the studies.

4.2 Permeability

The assessment of permeability should preferentially be based on the extent of absorption derived from human pharmacokinetic studies (for example, absolute bioavailability or mass balance).

High permeability can be concluded when the absolute bioavailability is ≥ 85%. High permeability can also be concluded if ≥ 85% of the administered dose is recovered in urine as unchanged (parent drug) or as the sum of parent drug, phase 1 oxidative and phase 2 conjugative metabolites. Regarding metabolites in faeces, only oxidative and conjugative metabolites can be considered. Metabolites produced through reduction or hydrolysis should not be included unless it can be demonstrated that they are not produced prior to absorption (for example, by microbial action within the gastrointestinal tract). An unchanged drug in faeces cannot be counted towards the extent of absorption unless appropriate data support the conclusion that the amount of parent drug in faeces to be accounted for absorbed drug material is from biliary excretion, intestinal secretion or originates from an unstable metabolite (such as glucuronide, sulphate or N-oxide that has been converted back to the parent by the action of microbial organisms).

Human in vivo data derived from published literature (for example, product knowledge and bioavailability studies) may be acceptable, keeping in mind that peer-reviewed articles may not contain the necessary details of the testing to make a judgement regarding the quality of the results.

Permeability can be also assessed by validated and standardized in vitro methods using Caco 2 cells (see Appendix 1). The results from Caco-2 permeability assays should be discussed in the context of available data on human pharmacokinetics. If high permeability is inferred by means of an in vitro cell system, permeability independent of active transport should be proven as outlined in Appendix 1 on Caco 2 cell permeability assay method considerations.

If high permeability is not demonstrated, the API is considered to have low permeability for BCS classification purposes.

4.3 API stability in the gastrointestinal tract

Additional data to document the API's stability in the gastrointestinal tract should be provided if mass balance studies are used to demonstrate high permeability, unless ≥ 85% of the dose is recovered as an unchanged drug in urine. Demonstration of stability in the gastrointestinal tract is required if in vitro Caco-2 studies are used to support high permeability. Stability in the gastrointestinal tract may be documented using pharmacopoeial or simulated gastric and intestinal fluids. Other relevant methods may be used with suitable justification. API solutions should be incubated at 37 °C for a period that is representative of the in vivo contact of the API with these fluids (that is, 1 hour in gastric fluid and 3 hours in intestinal fluid). API concentrations should then be determined using a suitably validated analytical method. Significant degradation (> 10%) of an API precludes BCS high-permeability classification.

5. Eligibility of an FPP for a BCS-based biowaiver

An FPP is eligible for a BCS-based biowaiver provided that the APIs satisfy the criteria regarding solubility and permeability (BCS class I and class III), the FPP is an immediate-release oral dosage form with systemic action, and the FPP is the same dosage form and strength as the comparator product.

FPPs with buccal or sublingual absorption are not eligible for a BCS-based biowaiver application. Furthermore, the BCS-based biowaiver approach is applicable only when the mode of administration includes water. If administration without water is also intended (for example, orodispersible products), a bioequivalence study in which the product is dosed without water should be conducted.

In order for an FPP to qualify for a BCS-based biowaiver, criteria with respect to the composition (excipients) and in vitro dissolution performance of the FPP should be satisfied. The FPP acceptance criteria are described in subsections 5.1 and 5.2 below.

5.1 Excipients

Ideally, the composition of the test product should mimic that of the comparator product. However, where excipient differences exist, they should be assessed for their potential to affect in vivo absorption. This should include consideration of the API properties as well as excipient effects. To be eligible for a BCS-based biowaiver, the applicant should justify why the proposed excipient differences will not affect the absorption profile of the API under consideration (that is, rate and extent of absorption, using a mechanistic and risk-based approach). The decision tree for performing such an assessment is outlined in Figures 1 and 2 in Appendix 2.

The possible effects of excipients on aspects of in vivo absorption such as solubility, gastrointestinal motility, transit time and intestinal permeability, including transporter mechanisms, should be considered. Excipients that may affect absorption include sugar alcohols, such as mannitol, sorbitol and surfactants (for example, sodium lauryl sulfate). The risk that a given excipient will affect the absorption of an API should be assessed mechanistically by considering:

- the amount of excipient used;
- the mechanism by which the excipient may affect absorption;
- absorption properties (rate, extent and mechanism of absorption) of the API.

The amount of excipients that may affect absorption in the test and comparator formulations should be addressed during product development, such that excipient changes are kept to a minimum. Small amounts included in the tablet coating, or levels below documented thresholds of effect for the specific API, are of less concern.

By definition, BCS class I APIs are highly absorbed and have neither solubility- nor permeability-limited absorption. Therefore, they generally represent a low-risk group of compounds in terms of the potential for excipients to affect absorption, compared to other BCS classes. Consideration of excipient effects for BCS class I-containing FPPs should focus on potential changes in the rate or extent of absorption. For example, if it is known that the API has high permeability due to active uptake, excipients that can inhibit uptake transporters are likely to be of concern. For BCS class I APIs that exhibit slow absorption, the potential for a given excipient to increase absorption rate should also be

considered. These excipients that may affect absorption should be considered as detailed in Fig. 1, Appendix 2.

For BCS class I APIs, qualitative and quantitative differences in excipients are permitted, except for excipients that may affect absorption, which should be qualitatively the same and quantitatively similar (that is, within ± 10% of the amount of excipient in the comparator product). Additionally, the cumulative difference for excipients that may affect absorption should be within ± 10%.

BCS class III APIs are considered to be more susceptible to the effects of excipients. These APIs are not considered highly permeable, and may have site specific absorption, so there are a greater number of mechanisms through which excipients can affect their absorption than for BCS class I APIs. For BCS class III APIs, all of the excipients should be qualitatively the same and quantitatively similar (except for film coating or capsule shell excipients). Excipients that may affect absorption should be qualitatively the same and quantitatively similar (that is, within ± 10% of the amount of excipient in the comparator product), and the cumulative difference for these excipients should be within ± 10%. The acceptable differences in excipients are defined in Table A7.1. Examples of acceptable differences in excipients are shown in Appendix 2. Differences in colorants and flavouring may be permitted when these constitute very small amounts of the formulation. For the types of excipients not listed in Table A7.1, the same rule should be applied as for the excipients that may affect absorption.

It is known that in some cases the absolute amount of an excipient present in the gastrointestinal tract is relevant to whether that excipient will exert an effect on absorption, for example, an effect on relevant transporters. Since the allowable differences for BCS class III APIs defined in Table A7.1 are based on percentage difference relative to core weight (w/w), it is possible for absolute amounts of excipients in two formulations to differ significantly while still maintaining proportionality within the limits expressed in Table A7.1. Control over differences in absolute amount of excipients where it is known that effects on absorption can be observed (for example, amounts of surfactants) is provided in Table A7.1; however, possible effects of other excipients is not controlled. Therefore, to control for possible excipient effects based on absolute amount differences between products, the total core weight of the proposed product should not deviate by more than 20% from the total core weight of the comparator product.

It is recognized that there are limitations to the application of Table A7.1 (for example, difficulty in determining the film coat weight for the comparator product). Table A7.1 is provided as a target to give clarity to applicants. Deviations from this will require appropriate justification, based on the principles described above.

Table A7.1
Criteria expected to demonstrate quantitative similarity for products containing BCS class III APIs

Within the context of quantitative similarity, differences in excipients for FPPs containing BCS class III APIs should not exceed the following targets:	
Excipient class	Percentage of the amount of excipient in the comparator
Excipients that may affect absorption Per excipient: Sum of differences:	 10% 10%
	Percentage difference relative to core weight[a] (w/w)
Major excipients types: Filler Disintegrant Starch Other Binder Lubricant Stearates Other Glidant Talc Other	 10% 6% 2% 1% 0.5% 2% 2% 0.2%
Total % change permitted for all excipients (including excipients that may affect absorption):	10%

[a] Core does not include tablet film coat or capsule shell.

BCS-based biowaivers are applicable to fixed-dose combination products that are the same dosage form and strength. Fixed-dose combination product formulations containing only BCS class I APIs should meet criteria regarding excipients for a BCS class I API. Fixed-dose combination product formulations containing only BCS class III APIs, or BCS class I and BCS class III APIs, should meet criteria regarding excipients for a BCS class III API.

5.2 In vitro dissolution

When applying the BCS-based biowaiver approach, comparative in vitro dissolution tests should be conducted using one batch representative of the proposed commercial manufacturing process for the test product relative to the comparator product. The test product should originate from a batch of at least one tenth of production scale or 100 000 units, whichever is greater, unless otherwise justified. During a (clinical) development phase, smaller batch sizes may be acceptable, if justified. The API content or potency of the comparator product should be close to the label claim, and the difference in API content or potency between the test and comparator products should be not more than 5%. The comparative in vitro dissolution tests should use pharmacopoeial apparatus and suitably validated analytical methods.

The following conditions should be employed in the comparative dissolution studies to characterize the dissolution profile of the product.

- Apparatus: paddle or basket.
- Volume of dissolution medium: 900 mL or less (it is recommended to use the volume selected for the quality control test).
- Temperature of the dissolution medium: 37 (± 1) °C.
- Agitation: paddle apparatus – 50 revolutions per minute (rpm); basket apparatus – 100 rpm.
- At least 12 units of comparator and test product should be used for each dissolution profile determination.
- Media: three buffers: pH 1.2, pH 4.5, and pH 6.8. Pharmacopoeial buffers should be employed. Additional investigation may be required at the pH of minimum solubility (if different from the buffers above).
- Organic solvents are not acceptable and no surfactants should be added.
- The sampling intervals employed in dissolution studies should be short for a scientifically sound comparison of the performance of the test and comparator products (for example, 5, 10, 15, 20 and 30 minutes).
- Samples should be filtered during collection, unless in situ detection methods are used. For this purpose, filters should be employed in line, at the end of the sampling probe, or both during sample collection.
- The pH of each dissolution medium should be maintained throughout the test. The pH of each dissolution medium should be

measured at the beginning (prior to introduction of the testing unit) and at the end of each dissolution test.

- For gelatin capsules, or tablets with gelatin coatings where cross-linking has been demonstrated, the use of enzymes may be acceptable, if appropriately justified.

Dissolution profiles for the test and comparator products should be generated in the same laboratory by the same staff at the same time using the same equipment. Compilation of historical data is not acceptable.

When high variability or coning is observed in the paddle apparatus at 50 rpm for both comparator and test products, the use of the basket apparatus at 100 rpm is recommended. Additionally, alternative methods (such as the use of sinkers or other appropriately justified approaches) may be considered to overcome issues such as coning, if scientifically substantiated. All experimental results should be provided.

To qualify for a BCS-based biowaiver for BCS class I APIs, both the test product and comparator product should display either very rapid (≥ 85% for the mean percent dissolved in ≤ 15 minutes) in vitro dissolution characteristics, or rapid (≥ 85% for the mean percent dissolved in ≤ 30 minutes) and similar in vitro dissolution characteristics (that is, based on f2 comparison), under all of the defined conditions. In cases where one product has rapid dissolution and the other has very rapid dissolution, similarity of the profiles should be demonstrated as below.

For the comparison of dissolution profiles, where applicable, the similarity factor (f2) should be estimated by using the following formula:

$$f2 = 50 \cdot \log \{[1 + (1/n)\Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \cdot 100\}.$$

In this equation f2 is the similarity factor, n is the number of time points, R_t is the mean percent comparator API dissolved at time t after initiation of the study, and T_t is the mean percent test API dissolved at time t after initiation of the study.

The evaluation of the f2 is based on the following conditions.

- A minimum of three time points (zero excluded).
- The time points should be the same for the two products.
- Mean of the individual values for every time point for each product.
- Not more than one mean value of ≥ 85% dissolved for either of the products.
- To allow the use of mean data, the coefficient of variation (%CV) should not be more than 20% at early time points (up to 10 minutes) and should not be more than 10% at other time points.

Two dissolution profiles are considered similar when the f2 value is ≥ 50. When both test and comparator products demonstrate that ≥ 85% of the labelled amount of the API is dissolved in 15 minutes, comparison with an f2 test is unnecessary and the dissolution profiles are considered similar. When the %CV for the mean data is too high based on the requirements listed above, f2 calculation is considered unreliable. In such cases, an alternative method for the assessment of similarity in dissolution profiles, such as the bootstrap 90% confidence interval of expected f2, should be employed in keeping with regional expectations for dissolution similarity assessment.

To qualify for a BCS-based biowaiver for BCS class III APIs, both the test product and comparator product should display very rapid (≥ 85% for the mean percent dissolved in ≤ 15 minutes) in vitro dissolution characteristics under the defined conditions.

For fixed-dose combination product formulations, dissolution profiles should meet the criteria for all APIs in the fixed-dose combination product. Fixed-dose combination product formulations containing only BCS class I APIs should meet dissolution criteria for a BCS class I API. Fixed-dose combination product formulations containing only BCS class III APIs should meet dissolution criteria for a BCS class III API. For fixed-dose combination products containing both BCS class I and BCS class III APIs, the dissolution criteria for the applicable BCS class for each component should be applied.

For products with more than one strength, the BCS approach should be applied for each strength. It is required that test and comparator product dissolution profiles are compared at each strength.

6. Documentation

The applicant should provide complete information on the critical quality attributes of the test APIs and FPP and as much information as possible for the comparator product, including polymorphic form and enantiomeric purity; and any information on bioavailability or bioequivalence problems with the APIs or FPP, including literature surveys and applicant-derived studies. All study protocols and reports should be provided. Information on validated test methods should be appropriately detailed according to current regulatory guidance and policies.

The reporting format should include tabular and graphical presentations showing individual and mean results and summary statistics.

The report should include all excipients and their qualitative and, where appropriate, quantitative differences between the test and comparator products.

A full description of the analytical methods employed, including validation and qualification of the analytical parameters, should be provided. A detailed description of all test methods and media, including test and comparator

batch information (unit dose (strength and assay), batch number, manufacturing date, batch size and, where known, expiry date) should also be provided. The dissolution report should include a thorough description of experimental settings and analytical methods, including information on the dissolution conditions such as apparatus, de-aeration, filtration during sampling and volume.

In addition, complete information with full description of the methods applied should be provided for the Caco-2 cell permeability assay method, if applicable (see Appendix 1).

References

1. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-first report. WHO Technical Report Series No. 1003, Annex 6. Geneva: World Health Organization; 2017 (https://www.who.int/publications/m/item/annex-6-trs-1003 accessed 4 February 2024).
2. Biopharmaceutics classification system-based biowaivers. ICH harmonised guideline M9, current step 4 version, November 2019. Geneva: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2019 (https://database.ich.org/sites/default/files/M9_Guideline_Step4_2019_1116.pdf, accessed 4 February 2024).
3. Protocol to conduct equilibrium solubility experiments for the purpose of Biopharmaceutics Classification System-based classification of active pharmaceutical ingredients for biowaiver. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-third report. WHO Technical Report Series No. 1019, Annex 4. Geneva: World Health Organization; 2019 (https://www.who.int/publications/m/item/annex-4-trs-1019 accessed 5 February 2024).

Appendix 1

Caco-2 cell permeability assay method considerations

Permeability assays employing cultured Caco-2 epithelial cell monolayers derived from a human colon adenocarcinoma cell line are widely used to estimate intestinal drug absorption in humans. Caco-2 cells undergo spontaneous morphological and biochemical enterocytic differentiation and express cell polarity with an apical brush border, tight intercellular junctions and several active transporters as in the small intestine. Due to a potential for low or absent expression of efflux (for example, P-gp, BCRP, MRP2) and uptake (for example, PepT1, OATP2B1, MCT1) transporters, the use of Caco-2 cell assays as the sole data in support of high permeability for BCS classification is limited to passively transported drugs (see "Assay considerations" below).

Method validation

The suitability of the Caco-2 cell assays for Biopharmaceutics Classification System (BCS) permeability determination should be demonstrated by establishing a rank-order relationship between experimental permeability values and the extent of drug absorption in human subjects using model drugs of zero, low (< 50%), moderate (50–84%), and high (≥ 85%) permeability. A sufficient number of model drugs are recommended for the validation to characterize high, moderate and low permeability (a minimum of five for each), plus a zero-permeability marker; examples are provided in Table 1. Further, a sufficient number (minimum of three) of cell assay replicates should be employed to provide a reliable estimate of drug permeability. The established relationship should permit differentiation between low , moderate- and high-permeability drugs.

Caco-2 cell monolayer integrity should be confirmed by comparing transepithelial electrical resistance (TEER) measures or other suitable indicators, prior to and after an experiment.

In addition, cell monolayer integrity should be demonstrated by means of compounds with proven zero permeability (refer to Table 1).

Reporting of the method validation should include a list of the selected model drugs along with data on extent of absorption in humans (mean, standard deviation, coefficient of variation) used to establish suitability of the method, permeability values for each model drug (mean, standard deviation, coefficient of variation), permeability class of each model drug, and a plot of the extent of absorption as a function of permeability (mean ± standard deviation or 95%

confidence interval), with identification of the high-permeability class boundary and selected high-permeability model drug used to classify the test API.

In addition, a description of the study method, drug concentrations in the donor fluid, description of the analytical method and equation used to calculate permeability should be provided. Additionally, information on efflux potential (for example, bidirectional transport data) should be provided for a known substrate.

Table 1
Examples of model APIs for permeability assay method validation

Group	API
High permeability ($f_a \geq 85\%$)	Antipyrine Caffeine Ketoprofen Naproxen Theophylline Metoprolol Propranolol Carbamazepine Phenytoin Disopyramide Minoxidil
Moderate permeability ($f_a = 50–84\%$)	Chlorpheniramine Creatinine Terbutaline Hydrochlorothiazide Enalapril Furosemide Metformin Amiloride Atenolol Ranitidine
Low permeability ($f_a < 50\%$)	Famotidine Nadolol Sulpiride Lisinopril Acyclovir Foscarnet Mannitol Chlorothiazide Polyethylene glycol 400 Enalaprilat

Table 1 *continued*

Group	API
Zero permeability	FITC-Dextran Polyethylene glycol 4000 Lucifer yellow Inulin Lactulose
Efflux substrates	Digoxin Paclitaxel Quinidine Vinblastine

Assay considerations

Passive transport of the test compound should be demonstrated. This may be verified using a suitable assay system that expresses known efflux transporters, such as by demonstrating independence of measured in vitro permeability on initial drug concentration, for example, 0.01, 0.1 and 1 times the highest strength dissolved in 250 mL, or on transport direction (efflux ratio, such as ratio of apparent permeability (Papp) between the basolateral-to-apical and apical-to-basolateral directions < 2 for the selected drug concentrations).

$$\text{Efflux ratio} = P_{appBL \rightarrow AP} / P_{appAP \rightarrow BL}.$$

Functional expression of efflux transporters should be verified by using bidirectional transport studies demonstrating asymmetric permeability of selected efflux transporter substrates (for example, digoxin, vinblastine or rhodamine 123, at non-saturating concentrations).

The test API concentrations used in the permeability studies should be justified. A validated Caco-2 method used for drug permeability determinations should employ conditions established during the validation and include a moderate-permeability and a high-permeability model drug in the donor fluid along with the test drug as internal standards to demonstrate consistency of the method. The choice of internal standards should be based on compatibility with the test drug (that is, they should not exhibit any significant physical, chemical or permeation interactions). The permeability of the internal standards may be determined following evaluation of the test drug in the same monolayers or monolayers in the same plate, when it is not feasible to include internal standards in the same cell culture well as the test drug permeability evaluation. The permeability values of the internal standards should be consistent between different tests, including those conducted during method validation. Acceptance

criteria should be set for the internal standards and model efflux drug. Mean drug and internal standards recovery at the end of the test should be assessed. For recoveries < 80%, a mass balance evaluation should be conducted including measurement of the residual amount of drug in the cell monolayer and testing apparatus.

Evaluation of the test drug permeability for BCS classification may be facilitated by selection of a high-permeability internal standard with permeability in close proximity to the moderate- or high-permeability class boundary. The test drug is considered highly permeable when its permeability value is equal to or greater than that of the selected internal standard with high permeability.

Information to support high permeability of a test drug (mean, standard deviation, coefficient of variation) should include permeability data on the test API, the internal standards, in vitro gastrointestinal stability information, and data supporting passive transport mechanism.

Appendix 2

Further information on the assessment of excipient differences

Fig. 1
Biopharmaceutics Classification System class I active pharmaceutical ingredients

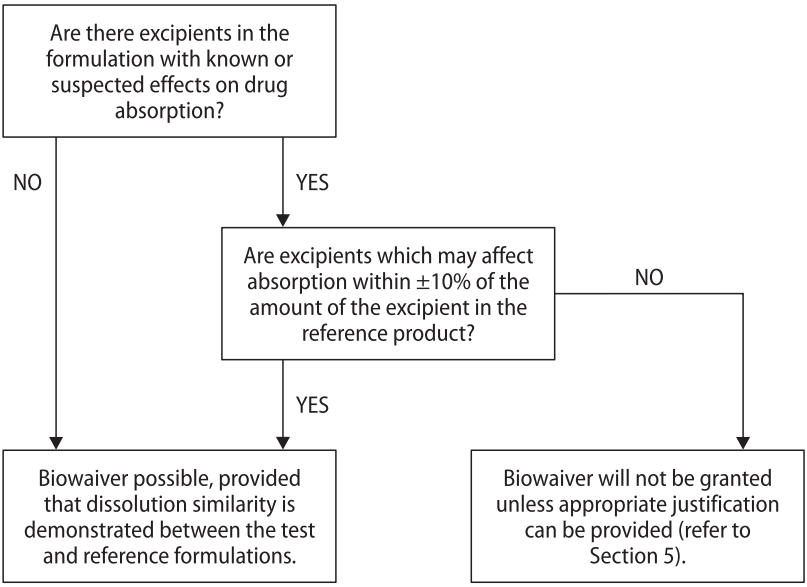
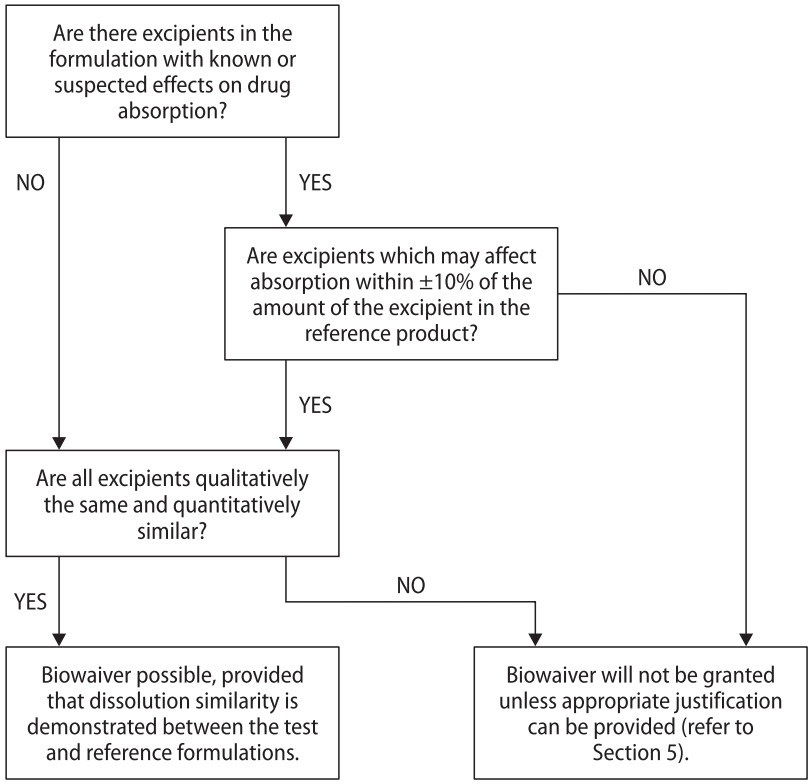


Fig. 2
Biopharmaceuticals Classification System class III active pharmaceutical ingredients



Examples of differences in excipients

Example 1. BCS class I biowaiver

The formulation of the test product is qualitatively the same as that of the comparator product. Additionally, it contains sorbitol, an excipient with known or suspected effects on API absorption. The amount of sorbitol in the test formulation is within the permitted range of 45 milligrams (mg) to 55 mg based on the amount of sorbitol in the comparator formulation (that is, 50 mg ± 10%).

Component	Amount (mg) comparator	Amount (mg) test
API	100	100
Microcrystalline cellulose (filler)	100	95

Table *continued*

Component	Amount (mg) comparator	Amount (mg) test
Sorbitol (filler)	50	55
HPMC (binder)	10	10
Talc (glidant)	5	5
Total	265	265

Example 2. BCS class III biowaiver

The test formulation is qualitatively the same as the comparator formulation. Additionally, it contains sorbitol, an excipient with known or suspected effects on API absorption. The amount of sorbitol in the test formulation is within the permitted range of 9 mg to 11 mg based on the amount of sorbitol in the comparator formulation (that is, 10 mg ± 10%). Any differences in the amount of other excipients are within the criteria outlined in Table A7.1, subsection 5.1.

Component	Comparator product		Test product		Absolute % difference relative to core weights
	Composition (mg)	Proportion relative to core weight (%w/w)	Composition (mg)	Proportion relative to core weight (%w/w)	
API	100	49.3%	100	46.5%	–
Lactose monohydrate (filler)	85	41.9%	97	45.1%	3.2%
Sorbitol (filler)	10	4.9%	9	4.2%	0.7%
Croscarmellose sodium (disintegrant)	6	3.0%	7	3.3%	0.3%
Magnesium stearate (lubricant)	2	1.0%	2	0.9%	0.1%
Total	**203**	**100%**	**215**	**100%**	
				Total change	4.3%

Example 3. Ineligible BCS class III biowaiver

The formulation of the test product is qualitatively the same as that of the comparator product. Further, the quantitative differences in excipient content between the products, based on percentage of core weight, satisfy the limits expressed in Table A7.1, subsection 5.1. However, the total core weight of the proposed product deviates by more than 20% from the total core weight of the comparator product, making the product ineligible for a biowaiver.

Component	Comparator product		Test product		Absolute % difference relative to core weights
	Composition (mg)	Proportion relative to core weight (%w/w)	Composition (mg)	Proportion relative to core weight (%w/w)	
API	8	8.0%	8	0.8%	–
Lactose monohydrate (filler)	75	75.0%	802	80.2%	5.2%
Silicon dioxide (glidant)	2	2.0%	20	2.0%	0.0%
Croscarmellose sodium (disintegrant)	13	13.0%	150	15.0%	2.0%
Magnesium stearate (lubricant)	2	2.0%	20	2.0%	0.0%
Total	**100**	**100%**	**1000**	**100%**	
				Total change	**7.2%**

Appendix 3

Equilibrium solubility experiments for the purpose of classification of active pharmaceutical ingredients according to the Biopharmaceutics Classification System

Introduction

The Biopharmaceutics Classification System (BCS) was proposed in 1995 by Amidon et al. (*1*). It is a scientific framework that divides active pharmaceutical ingredients (APIs) into four groups according to their solubility and permeability. The recommended method for determination of the solubility is described below. of the condom. If a defect can be expected to affect the performance, safety or acceptability of the condom, it should be classified as a critical defect.

Recommendations for conducting experiments for assessing solubility of APIs

Prior to the experiment, a solubility study protocol should be prepared describing the equipment and procedures in detail. The protocol should include, for example, methods of sample preparation, experimental conditions such as temperature, method and rate of agitation, method of solid/solution separation of the API, and method of sample analysis. The source and purity of the API to be used in the study should also be recorded in the protocol, as well as the methods that will be used to characterize the material.

Characterization of the solid API should be completed prior to the investigation. The depth of the characterization will depend on the existing knowledge of the solid-state properties of the API in question. For example, if it has been established that the API exists as a single polymorphic form, then less solid-state characterization is needed. In some cases, it may be necessary to characterize the solid starting material as well as the solid residue remaining after equilibrium has been reached and sampling has been completed. For a discussion of the factors that should be considered when planning the solid-state characterization studies, see Avdeef et al. (*2*).

Solubility experiments should preferably be carried out with the shake flask method, which is used to determine equilibrium solubility, although other methods are possible if justified. A discussion of the factors that should be considered when designing the study can be found in Avdeef et al. (*2*). The conditions employed should be fully described in the study protocol.

The pH solubility profile of the API should be determined over the pH range of 1.2–6.8 at 37 (± 1) °C. Measurements should be made in triplicate under

at least three pH conditions, pH 1.2, 4.5 and 6.8, as well as at the pH of any known solubility minima in aqueous media within that pH range. Pharmacopoeial buffer solutions are recommended for use in solubility experiments – see, for example, Chapter 5.5 "Dissolution test for solid oral dosage forms" in *The International Pharmacopoeia* (*3*). Factors such as common ion effects and ionic strength should be considered when selecting buffers for the study. The pH should be verified after addition of the API and at the end of the experiment with a calibrated pH meter. Samples should be taken at several time points to ensure that the equilibrium solubility has been reached. Strong agitation followed by a period of sedimentation is suggested, to achieve solubility equilibrium.

A description of the methods of solid/solution separation employed, including details such as filter type and pore size or centrifugation speed, should be provided in the study protocol. Sedimentation, centrifugation and filtration are the standard methods of separation. The factors described by Avdeef et al. (*2*) should be considered when selecting the most appropriate approach for the API under study.

A validated, stability-indicating analytical method should be employed for determination of the solubility of APIs, for example, chromatography– see Chapter 1.14.1 "Chromatography" in *The International Pharmacopoeia* (*3*) – or an alternative, validated stability-indicating assay.

A study report should be created after the experiment detailing the actual experimental conditions, results (raw data plus mean values with standard deviations), and any observations, for example, the degradation of an API as a result of pH or buffer composition. The section describing the experimental conditions should include initial and equilibrium pH of solutions and de facto buffer concentrations. If applicable, filter adsorption studies should be documented. Any deviations from the protocol should be noted and justified.

The dose–solubility ratio is calculated as follows: highest single therapeutic dose (mg) divided by solubility (mg/mL). An API is considered highly soluble when the highest single therapeutic dose is soluble in 250 mL or less of aqueous media over the pH range 1.2–6.8, that is, the dose–solubility ratio is ≤ 250.

References

1. Amidon GL, Lennemas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 1995;12:413–20.
2. Avdeef A, Fuguet E, Llinàs A, Ràfols C, Bosch E, Völgyi G et al. Equilibrium solubility measurement of ionizable drugs: consensus recommendations for improving data quality. ADMET & DMPK. 2016;4:117–78.

3. Health products policy and standards. In: The International Pharmacopoeia, 11th edition. Geneva: World Health Organization; 2023 (https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/norms-and-standards-for-pharmaceuticals/international-pharmacopoeia accessed 5 February 2024).