ANNEX

to Decision of the
Eurasian Economic Commission's Council
No. _____ dated __ _____ 20___

AMENDMENTS

made to the Rules for Conducting Bioequivalence Studies of Medicinal Products within the Eurasian Economic Union

1. Paragraph 18 shall read as follows:

18. When choosing a reference medicinal product, proceed from the following sequence:

a) an original medicinal product, the safety, efficacy and quality of which have been established at market authorisation in the Union, and which is authorised in accordance with the legislation of the Member State (the original medicinal product authorised in the Union);

b) an original medicinal product authorised in the ICH countries or, when it is justified by its non-availability in the pharmaceutical market or impossibility to purchase it, a generic (or hybrid) medicinal product authorised at least in one Member State and having confirmed bioequivalence to the original medicinal product (if the generic medicinal product choice is approved by the Expert Committee on Medicines), if item a) of this paragraph is impractical;

c) medicinal products approved by the Expert Committee on Medicines (list of decisions can be found at the Union's web-site), if items a) and b) of this paragraph are impractical;

d) a medicinal product with at least 20 years of experience of use in the territory of one of the Member States (subject to approval by the Expert Committee on Medicines), if items a) through c) of this paragraph are impractical;

e) a combined medicinal product reviewed and approved by the Expert Committee on Medicines, if items a) through c) of this paragraph are impractical.

The Expert Committee in Medicines may choose a combined medicinal product containing known active substances and authorised at least in one of the Member States as a reference product in case of its restricted study programme, which does not allow to acknowledge its originality, considering assessment of this medicinal product use experience and reasonability for creating a fixed combination, if items a) through d) of this paragraph are impractical.

When it is impossible to recognize a medicinal product as a reference one due to the combination irrationality, unproved efficacy and safety, it is proposed to conduct a programme of preclinical and clinical studies for development of a new combined medicinal product complying with the Recommendation of the Eurasian Economic Commission Board No. 25 dated September 2, 2019 ‘On Guidelines for Preclinical and Clinical Development of Combined Medicinal Products’.

2. Annex No. 4 shall be read as follows:

‘Annex No. 4

to the Rules for
Conducting Bioequivalence Studies
of Medicinal Products within the Eurasian
Economic Union

REQUIREMENTS
for Biowaver Based on Biopharmaceutics Classification System

I. General provisions

1. Two medicinal products containing identical active substance(s) are considered bioequivalent, if their bioavailbility, i.e. the active substance absorption rate and degree, falls within the predefined acceptance limits after their administration at the same molar dose. These limits are to be defined to ensure similar *in vivo* activity of the investigational medicinal product (similarity in its safety and efficacy to the reference/original medicinal product). To estimate the absorption rate and degree of the active substance, the basic pharmacokinetics parameters such as AUC and C_{max} shall be used in the *in vivo* bioequivalence studies.

2. The approach based on the Biopharmaceutics Classification System (BCS) at biowaiver is aimed at reducing the necessity to conduct *in vivo* bioequivalence studies (that is, it may serve as a surrogate study for assessing *in vivo* bioequivalence). It is acceptable to skip the *in vivo* bioequivalence studies, if the suggestion on the activity *in vivo* bioequivalence is justified through the satisfactory *in vitro* data. The BCS categorises active substances in the following four classes:

Class I: high solubility, high permeability;

Class II: low solubility, high permeability;

Class III: high solubility, low permeability;

Class IV: low solubility, low permeability.

3. This Annex contains instructions for defining the BCS class for an active substance and using a BCS-based biowaiver for *in vivo* bioequivalence

studies of medicinal products. The BCS-based biowaiver principles are also applicable for the objectives of the medicinal product dosage form bioequivalence studies not covered by this Annex provided that the indicated principles are scientifically established.

II. BCS-Based Biowaiver Application Criteria

4. The BCS-based biowaiver is used to justify the *in vivo* bioequivalence. This procedure is applied to compare a medicinal product with the pharmaceutical products used during the period from the medicinal product clinical development until its industrial batches release, to assess the post-marketing changes and for application for authorisation as a generic medicinal product.

5. The BCS-based biowaiver is applicable only to oral solids or immediate release suspensions intended for the active substance delivery into systemic blood flow. As for the narrow therapeutic index medicinal products, the BCS-based biowaiver may not be applied for substitution of the *in vivo* bioequivalence studies. For a combination of fixed doses of medicinal products, the BCS-based biowaiver is applied when all active substances contained in the combined medicinal product meet the criteria stated in sections 2 and 3 of this Annex.

6. The BCS-based biowaiver is applicable to the medicinal products, if the active substance(s) has high solubility and high permeability (BCS Class I) or high solubility and low permeability (BCS Class III).

7. The BCS-based biowaiver is applicable, if the active substance(s) of the investigational medicinal product and the reference medicinal product is identical. The BCS-based biowaiver is also applied when the investigational medicinal product and the reference medicinal product contain different salts provided that both the salts belong to the BCS Class I (high solubility and

high permeability). The BCS-based biowaiver is not applicable, if the investigational medicinal product contains an active substance in the form of another ether, ester, isomer, isomer mixture, complex or another derivative differing from the active substance of the reference medicinal product, since such differences may cause differences in bioavailabilities, which can not be obtained in an experiment based on application of the BCS concept for the biowaiver. The BCS-based biowaiver may be applied to pro-drugs, if they are absorbed in the form of the pro-drug.

III. Active substance Biopharmaceutics Classification System

1. Solubility

8. An active substance is classified as highly soluble, if its maximum single therapeutic dose (as per the summary of the reference product characteristics) is completely soluble in 250 mL or less of aqueous medium within the pH range of 1.2 to 6.8 at temperature of (37±1)°C. In the cases when the maximum single therapeutic dose does not meet this criterion, but weight amount of the active substance corresponding to the maximum dose of the reference medicinal product is completely soluble under the above-mentioned conditions, additional data shall be provided for justification of the BCS-based biowaiver procedure in the marketing authorisation application of the medicinal product.

9. The active substance solubility within the pH range of 1.2 to 6.8 at a temperature of 37±1 °C shall be established experimentally. At least three pH levels shall be studied in this range including buffer media with pH of 1.2, 4.5 and 6.8. Moreover, the active substance solubility shall be studied at the pH with its lowest solubility provided that the pH value is within the specified range. The studies indicated shall confirm that the active substance

solubility is maintained for the time intervals corresponding to the expected duration of the active substance absorption.

10. The solubility should be assessed using the method selected basing on the active substance properties.

11. The equilibrium solubility studies using the flask agitation procedure or an alternative procedure are acceptable, if it is justified in the marketing authorisation application of the medicinal product. Small medium volumes may be used for the solubility assessment, if the equipment available for studies allows that. To ensure measurement of solubility at the stated pH value, the pH value for each test solution shall be measured after adding active substance and at the end of the equilibrium solubility study. If required, the pH value should be adjusted. The experiment shall be conducted for the time period sufficient to reach the equilibrium.

12. As alternative option of the studies, the solubility study, in which the maximum single therapeutic dose of the active substance is to be studied in 250 mL of buffer medium or the less dose of the active substance in the proportionally less volume of the buffer medium, may be conducted.

13. To determine the active substance class according to the BCS, the least measured solubility within the pH range of 1.2 to 6.8 shall be used.

14. To confirm the obtained solubility value, at least 3 repeated solubility determinations at each condition or pH with use of the corresponding compendial buffer media and validated active substance quantification method are required.

15. The sufficient active substance stability in the solubility assessment buffer medium shall be confirmed. When the active substance is instable (i.e. its degradation is over 10% during the solubility assessment), the active substance solubility and the BCS Class can not be determined accurately. In addition to the experimental data, it is acceptable to provide research

chemical-engineering and pharmaceutical data in the marketing authorisation application of the medicinal product to prove and justify the solubility values. Herewith, it shall be considered that not all articles of the scientific peer-reviewed journals/publications contain study data necessary to assess the quality of such studies.

2. Permeability

16. The permeability assessment shall be preferably based on determination of the active substance absorption degree obtained from the pharmacokinetcs studies in human (e.g. at absolute bioavailability or mass balance studies).

17. The conclusion on high permeability may be made, if absolute bioavailability of the active substance is $\geq 85\%$. The conclusion on high permeability of the active substance can be made, if $\geq 85\%$ of its administered dose is excreted in urine unchanged (in the form of the parent compound) or as a sum of the parent compound and its oxidized (Phase I) and conjugated (Phase II) metabolites. As for the metabolites detected in feces, considering only oxidized and conjugated metabolites is acceptable as well. The metabolites formed as a result of reduction or hydrolysis shall not be included into the general bioavailability assessment, unless there is a way to demonstrate that they are not formed before absorption (e.g. through the influence of the gastrointestinal microorganisms). The unchanged active substance in feces shall not be included into the absorption assessment, unless the corresponding data justify that the amount of the parent compound in feces counted as the absorbed active substance is caused by its biliary excretion, intestinal secretion or it is formed from instable metabolite such as glucuronide, sulphate, N-oxide, which is subject to inverse transformation to the parent compound as a result microorganism activity.

18. It is acceptable to provide *in vivo* data obtained in human, taken from a scientific medical literature (e.g. knowledge of the medicinal product properties and bioavailability studies). Herewith, it shall be considered that not all scientific peer-reviewed journals/publications contain the required data on the studies that allow assessing the quality of such studies.

19. The active substance permeability shall be also assessed using validated and standardised methods of *in vitro* studies using the Caco-2 cells (in accordance with Annex No. 1 to these Requirements). The results of the active substance permeability assay in Caco-2 cell line shall be analysed considering the available data on the active substance pharmacokinetics in human. If the conclusion on high permeability of the active substance is made basing on the *in vitro*-cell system study, it is required to prove the active substance permeability not dependent on the active transport, in accordance with Annex No. 1 to the Requirements.

20. If high permeability is not established, the active substance is considered as having low permeability for its classification in accordance with the Biopharmaceutics Classification System.

3. Gastrointestinal Stability of the Active Substance

21. When mass balance studies are used to confirm high permeability of the active substance, additional data on the gastrointestinal stability of the active substance shall be provided, except for the cases when ≥ 85% of the active substance dose is excreted with urine in the form of unchanged active substance. Confirmation of the active substance gastrointestinal stability is required, if *in vitro* studies in Caco-2 cell line is used to justify its high permeability. The active substance gastrointestinal stability shall be documented using compendial media or simulated gastric and intestinal fluids. Other relevant methods for assessing the active substance stability

may be used in the marketing authorisation application of the medicinal product subject to proper justification. The active pharmaceutical substance solutions shall be incubated at 37.0± 0.5 °C for the period corresponding to the *in vivo* contact time of the active substance with these fluids, namely: 1 hour in gastric fluid and 3 hours in intestinal fluid. After that, the active substance concentrations in the solution shall be determined using validated method. The significant (> 10%) degradation of the active substance does not allow classifying it as highly permeable substance according to the Biopharmaceutics Classification System.

IV. Medicinal Product Eligibility for the BCS-Based Biowaiver

22. A medicinal product is suitable for the BCS-based biowaiver when all the following conditions are fulfilled:

the active substance(s) of the investigational medicinal product corresponds to the BCS Class I and Class III according to the solubility and permeability criteria;

the investigational medicinal product and the reference medicinal product are the oral dosage forms with immediate release and systemic effect;

the investigational medicinal product has the same dosage form and strength as the reference medicinal product.

23. In the cases when the maximum single therapeutic dose of the medicinal product does not meet the high solubility criterion, but the highest dose of the reference medicinal product is soluble under the required conditions, the BCS-based biowaiver application shall be justified through demonstrating the dose-proportional pharmacokinetics of the investigational medicinal product (AUC and C_{max}) within the dose range which includes the maximum single therapeutic dose.

24. Medicinal products with buccal or sublingual absorption are not eligible for the BCS-based biowaiver. Approach based on application of the Biopharmacueticals Classification System in biowaiver is applicable only when the medicinal product is taken with water. If use without water is also acceptable for the medicinal product (e.g. orodispersible medicinal products), an *in vivo* bioequivalence study in which the medicinal product is to be used without water shall be conducted.

25. To ensure that medicinal product may be claimed for application of the BCS-based biowaiver, the criteria set for the composition (excipients) and *in vitro* solubility characteristics of the medicinal product specified in subsections 1 and 2 of this section shall be met.

1. Excipients

26. It is necessary to aim for the composition of the investigational medicinal product to fully mimic the composition of the reference medicinal product. If there are any differences between the excipients of the investigational medicinal product and the reference medicinal product, their potential impact on the *in vivo* active substance absorption shall be assessed. Such assessment includes analysis of the active substance properties and effect of excipients on its absorption. To apply the BCS-based biowaiver, the Sponsor must justify why the differences proposed in the medicinal product excipients will not affect the absorption profile of the active substance in question (i.e. its absorption rate and degree) using physico-chemical studies and calculations, as well as the risk-based approach.

The decision tree for such assessment is presented in figures 1 and 2 of Annex No. 2 to the Requirements.

27. The Applicant shall analyse the possible effect of excipients on the following active substance absorption aspects *in vivo*:

solubility of the active substance;

effect of the excipients on the gastrointestinal motility;

the active substance transit time and its intestinal permeability including transfer mechanisms.

28. Excipients that may affect absorption include: aldols (sugar alcohols, e.g. mannitol, sorbitol) and surfactants (e.g. sodium dodecyl sulfate).

29. The risk that an excipient will affect the active substance absorption shall be assessed by physico-chemical methods and calculations taking into consideration the following:

a) amount of excipient used in the dosage form unit;

b) mechanism through which an excipient may affect the active ingredient absorption;

c) absorption characteristics (absorption rate, degree and mechanism) of the active substance.

30. During the pharmaceutical development of a medicinal product, it is necessary to take into consideration the excipients amount in the composition of the investigational medicinal product and the reference medicinal product that may affect the active substance absorption in order to ensure minimal changes in the composition and excipients content in the investigational medicinal product. It is acceptable not to take into consideration a small amount of excipients included in the tablet coat, or the amount of excipients below their established/proven limit of influence.

31. Since the BCS Class I active substances are well absorbed and have absorption that is neither limited by solubility nor permeability, they are considered to be a low-risk compound group in terms of the excipients potential to affect the absorption compared to other BCS classes of substances.

32. The assessment of the excipient effect on the medicinal products containing BCS Class I active substances shall include analysis of potential changes in the absorption rate or degree. For example, if the active substance is known to have high permeability due to active uptake, the BCS-based biowaver will not be applicable due to the presence of excipients capable of inhibiting uptake transporters in the medicinal product composition. As for the BCS Class I active substances having slow absorption, it is also required to consider the capability of the excipients included in the composition of the medicinal product to increase its absorption.

33. For medicinal products containing the BCS Class I active substances, qualitative and quantitative differences in the excipients of the investigational medicinal product and the reference medicinal product are acceptable, except for the excipients may affect the active substance absorption, which shall match in quality and be similar in quantity (i.e. differ within the range ±10% from the excipient quantitative content in the reference medicinal product). Moreover, the total difference in the content of all excipients with the potential to affect absorption shall be within ±10%.

34. The behavior in the body of the BCS Class III active substances largely depends on the excipients effect. These active substances have low permeability and may be absorbed in a specific portion of the gastrointestinal tract, therefore, there are many mechanisms through which excipients may affect their absorption compared to the BCS Class I active substances. With regard to the BCS Class III active substances, all excipients shall be of the same qualitative composition and similar in quantitative composition (except for the excipients in the film coat or capsule shell) to the composition of the reference medicinal product. Excipients with the potential to affect the active substance absorption shall be identical in qualitative composition and similar in quantitative composition to the composition of the reference medicinal

product (i.e. differ within the range of ±10% from the excipient amount in the reference medicinal product, while the total difference in the content of all excipients with the potential to affect the absorption shall be within ±10%). The limits of differences in the excipients composition indicated in Table 1 shall be observed. Examples of acceptable differences in excipients are given in Addendum II. Differences in dyes and flavours are acceptable if their content in the medicinal product composition is insignificant.

35. In some cases (e.g. when determining the weight of the reference product film coat is problematic), the use of the indicators specified in the Table is difficult. The Table provides indicative values to be considered in the pharmaceutical development process. Deviations from specified indicator values require appropriate justification in the marketing authorisation application of the medicinal product based on the principles set forth in paragraphs 26 through 34 of the Requirements.

Table

Parameters confirming the similarity in the quantitative composition of excipients in the investigational medicinal product and the reference medicinal product containing BCS Class III active substances

Excipient type	Weight deviation, NMT
Excipients affecting absorption:	
individual substance	10%[1]
total substances	10%[1]
Other excipients:	
filler	10%[2]
Disintegrants:	
starch	6%[2]
other	2%[2]
Binder	1%[2]

Excipient type	Weight deviation, NMT
Lubricants:	
stearates	0.5%[2]
other	2%[2]
Glidants:	
talc	2%[2]
other	0.2%[2]
Changes in all excipients in total including those affecting absorption	10%[2]

Note:
[1] Deviation from the excipient weight in the reference medicinal product;
[2] Deviation from the dosage form core weight of the reference medicinal product. The core does not include the tablet film coat or capsule shell.

36. The BCS-based biowaiver is applicable to fixed dose combinations in the same dosage form and strength in the investigational medicinal product and the reference medicinal product. The fixed-dose combination medicinal products containing the BCS Class I active substances shall meet the excipient criteria for the BCS Class I active substance. The fixed-dose combination medicinal products containing only the BCS Class III active substances or BCS Class I and Class III active substances shall meet the excipient criteria for the BCS class III active substances.

2. *In Vitro* Comparative Dissolution Kinetics Test for the Medicinal Product

37. When applying the BCS-based biowaiver, the *in vitro* comparative dissolution kinetics tests using one industrial or pilot batch of medicinal product manufactured as a result of the process submitted for authorisation shall be performed in comparison with a reference medicinal product.

38. The investigational medicinal product shall be sampled from a batch with a size of at least of the industrial batch size or 100,000 units

(whichever is greater), unless otherwise justified by the Applicant/Manufacturer. When performing the *in vitro* comparative dissolution kinetics test to select a medicinal product for clinical development, it is acceptable to use a smaller pilot-scale batch of the medicinal product with proper justification. The *in vitro* comparative dissolution kinetics tests shall be performed with an apparatus complying with the Pharmacopoeia of the Eurasian Economic Union and a properly validated analytical method(s).

39. To characterise the dissolution profile of the medicinal products, the following conditions shall be met in the comparative dissolution kinetics test:

apparatus: paddle mixer or rotating basket;

dissolution medium volume: 900 mL or less (it is preferable to use the volume selected for the quality control medium);

dissolution medium temperature: 37.0 ± 1.0 °C;

mixing speed: 50 rpm for paddle mixer, 100 rpm for rotating basket;

for each experiment of the dissolution profile, at least 12 dose units of the reference medicinal product and the investigational medicinal product shall be used;

three buffer media: pH 1.2; pH 4.5 and pH 6.8. Compendial buffers shall be used. For the pH level, at which the minimum solubility of the medicinal product (if this medium differs from the buffers above) is observed, further study shall be considered;

use of organic solvents and the addition of surfactants to the dissolution medium are not acceptable;

during sampling, it is necessary to ensure their filtration, except when the *in situ* detection methods for the active substance are used;

for gelatin capsules or tablets with gelatin coat, if presence of gelatin molecule cross-links is found in their composition, use of enzymes is allowed under proper justification.

40. If the paddle mixer at 50 rpm exhibits high variability in active substance release or coning for both the reference medicinal product and the investigational medicinal product, the rotating basket at 100 rpm may be used. Moreover, alternative methods (e.g. use of sinkers or other properly justified approaches) may be used to overcome problems such as coning. In the study report, it is necessary to present all the experiments results.

41. To compare dissolution profiles, where applicable, it is necessary to evaluate the similarity factor f_2 using the following equation:

$$f_2 = 50 \times lg\left\{\left[1 + \frac{1}{n} \times \sum_{t=1}^{n}(\bar{R}_t - \bar{T}_t)^2\right]^{-0,5} \times 100\right\},$$

where:

f_2 is the similarity factor;

n is the number of time points;

$R_{(t)}$ is the average proportion (in percent) of the dissolved active substance of the reference medicinal product at time t after the study start;

$T_{(t)}$ is the average proportion (in percent) of the dissolved active substance of the investigational medicinal product at time t after the study start.

42. The similarity factor calculation is used when all of the following conditions are met:

dissolution has been assessed at least at three time points (excluding the zero sampling point);

selected sampling time points for the two medicinal products being compared shall be the same;

for each time point, the average value of the individual dissolution indicators for active substance of each of the compared medicinal products has been calculated;

availability for any of the drug products of at most one time point with the average active substance dissolution value of ≥ 85% of its label claim in the medicinal product;

coefficient of variation for the average dissolution values of the medicinal product active substance shall not exceed 20% at early time points (up to 10 minutes) and 10% at other time points.

43. Two dissolution profiles of the compared medicinal products active substances are considered similar if the f_2 value is ≥50. If both the investigational medicinal product and the reference medicinal product demonstrate that ≥85% of the label claim of the active substance is dissolved within 15 minutes, comparison by similarity factor f_2 assessment is not required, and the dissolution profiles are considered similar. If the coefficient of variation for the average dissolution values of the active substance exceeds the values specified in paragraph 42, the similarity factor f_2 calculation is considered incorrect, and it is impossible to conclude that the active substance dissolution profiles of the compared medicinal products are similar.

44. To apply the BCS-based biowaiver for the BCS Class I active substances, both the investigational medicinal product and the reference medicinal product shall demonstrate:

either very rapid (≥ 85% for average amount of substance in solution in ≤ 15 minutes) characteristics of the *in vitro* dissolution profile;

or rapid (≥ 85% for average amount of substance in solution in ≤ 30 minutes) and similar *in vitro* dissolution profile characteristics (e.g. based on comparison by similarity factor f_2) under all dissolution conditions.

When one of the medicinal products (investigational or reference one) has rapid dissolution and the other product shows very rapid dissolution, the similarity of the profiles shall be demonstrated based on comparison by the f_2 factor.

45. To apply the BCS-based biowaiver for medicinal products containing the BCS Class III active substances, both the investigational medicinal product and the reference medicinal product shall exhibit very rapid ($\geq$ 85% for average amount of substance in solution in $\leq$ 15 minutes) and similar *in vitro* dissolution profile characteristics under all dissolution conditions.

46. To apply the BCS-based biowaiver for the medicinal products with fixed dose combination, the dissolution profiles of all active substances in the composition of the considered medicinal products with fixed dose combination shall be similar. The assessment of the dissolution profile similarity of the medicinal products with fixed dose combination depends on their composition:

drug products containing only the BCS Class I active substances shall meet the similarity criteria for dissolution profiles for each BCS Class I active substance;

drug products containing only the BCS Class III active substances shall meet the similarity criteria for dissolution profiles for each BCS Class III active substance;

drug products containing a combination of active substances of both BCS Class I and Class III shall meet the criteria for the dissolution profile similarity corresponding to the BCS Class for each component in the combination.

47. For medicinal products with more than one strength, the BCS-based biowaiver approach shall be applied to each strength of the medicinal

product, i.e. the dissolution profiles of the investigational medicinal product and the reference medicinal product shall be compared for each strength.

IV. Documentation to be Submitted as a Part of the Marketing Authorisation Application with the BCS-Based Biowaiver

48. The Sponsor must provide complete information on the critical quality attributes of the investigational active substance(s) and medicinal product, as well as detailed information on the reference product including also the polymorphic form type, enantiomeric purity of the active pharmaceutical substance and any information on bioavailability or bioequivalence issues for the active substance(s) or medicinal product including medical scientific data and data from own research. All protocols and study reports shall be submitted. Information on analytical method validation shall meet the requirements:

for bioanalytical procedures, of Annex No.6 to the Rules for Conducting Bioequivalence Studies of Medicinal Products within the Eurasian Economic Union;

for analytical procedures, of the Guidelines for the Validation of Analytical Procedures for Testing Medicines, approved by the Decision of the Board of the Eurasian Economic Commission No. 113 dated July 17, 2018.

49. The report shall include description of all excipients contained in the investigational medicinal product and the reference medicinal product, indicating their qualitative and, if applicable, quantitative differences.

50. The report shall provide full description of the analytical procedures used, including their validation and analytical equipment qualification. It is also necessary to provide full description of all medicinal product dissolution media, as well as information on the investigational medicinal product and

the reference medicinal product batches (single dose/strength used in the comparative dissolution kinetics test, batch analysis protocols), batch number, manufacturing date and batch size (if known), expiration date/shelf life). The report on the determination of the active pharmaceutical substance solubility and comparative dissolution kinetics test of the medicinal product shall include detailed description of the experimental conditions and analytical procedures, including information on dissolution conditions such as the compendial apparatus used, deaeration procedures, filtration during sampling, sample volume and other parameters allowing describing the process of obtaining experimental data.

51. Moreover, the report shall provide information with full description of the methods used to determine the active substance permeability in Caco-2 cell line, if it has been used (in accordance with the instructions in Annex No. 1 to the Requirements).

52. The report shall include tabulated and graphic data corresponding to individual and average results, as well as generalising statistical data.

Annex No. 1

to the Requirements for Biowaver Based on Biopharmaceutics Classification System

PROCEDURE

for the Active Substance Permeability Assay in Caco-2 Cell Line

1. General instructions

Intestinal absorption of active substance in humans can be assessed through the active substance permeability assay using cultured Caco-2 epithelial cell monolayers derived from human colon adenocarcinoma cell line. The Caco-2 cells are subject to spontaneous morphological and biochemical differentiation into enterocytes and exhibit cellular polarity by having apical brush border, tight intercellular junctions and several active transporters identical to the active transporters in human small intestine cells. Due to the low or zero expression potential of efflux transporters (e.g. P-gp, PCRP, MRP2) and uptake transporters (e.g. PepT1, OATP2B1, MCT1), the results of the Caco-2 assay as the only evidence of high permeability of active substance for its classification according to the Biopharmaceutics Classification System (BCS) may only be used for active substances transported by passive diffusion.

2. Validation of the Permeability Assay Procedure

The suitability of Caco-2 permeability assays for the active substance according to BCS shall be confirmed by establishing a rank/order relationship between the experimental permeability values and the active substance

absorption degree in humans using model active substances with zero, low (< 50%), moderate (50–84%) and high (≥ 85%) permeability. It is necessary to use a sufficient number of model active substances to validate the analytical procedure to characterise high, moderate and low permeability (at least 5 active substances for each permeability degree), as well as a marker characterising the zero permeability. Possible examples of model active ingredients are shown in Table 2. To obtain a reliable assessment of the active substance permeability, it is required to use a sufficient number (at least 3) of replicates in the Caco-2 assay. The established rank/order relationship shall allow differentiating active substances with low, moderate and high permeability.

Pre- and post-experimental integrity of the Caco-2 cell line monolayer shall be confirmed by comparison of transepithelial electrical resistance and/or other appropriate parameters.

In addition, the Caco-2 cell monolayer integrity shall be demonstrated using the proven zero permeable active ingredients listed in the Table.

The description of the analytical procedure validation for the assay shall include:

list of model active substances along with data on their absorption degree in humans (average, standard deviation, coefficient of variation), used to establish the method suitability;

permeability values of each model active substance (average, standard deviation, coefficient of variation);

the permeability class of each model active substance;

graph of active substance absorption degree vs. its permeability (with representation of the values in graph as average ± standard deviation or 95% confidence interval);

indication of high permeability limit and the selected model active substance to determine the high permeability used in determining the BCS class of the investigational active substance.

In addition, the report shall include:

description of the assay procedure;

the active substance concentrations used in the donor fluid;

description of the analytical procedure;

equation used to calculate the permeability;

information on the efflux potential of the Caco-2 cell line (e.g. bidirectional transport data for a known active substance).

3. Procedure for Permeability Assay

The passive diffusion of the investigational active substance shall be established. The active substance passive diffusion is to be confirmed with use of a suitable test system that expresses known efflux transporters, for example through establishing the independence of the measured *in vitro* permeability of the active substance on its initial concentration (e.g. for strengths of 0.01; 0,10 and 1.00 of the highest strength of active substance dissolved in 250 mL of medium), or on the active substance transfer direction (efflux ratio, i.e. the ratio of the apparent permeability of the active substance [P_{app}] between the basolateral-to-apical and apical-to-basolateral directions, which shall be < 2 for the selected concentrations of the active substance). The efflux ratio is calculated using the following equation:

$$\text{Эффлюкс отношение} = \frac{P_{\text{каж, БЛ→АП}}}{P_{\text{каж, АП→БЛ}}},$$

where:

$P_{app,\ BL\rightarrow AP}$ is the apparent permeability of the active substance in the basolateral-to-apical direction;

$P_{app, AP \rightarrow BL}$ is the apparent permeability of the active substance in the apical-to-basolateral direction.

Functional expression of efflux transporters shall be verified using bidirectional transport assays confirming asymmetric permeability of the selected efflux transporter substrates, e.g. digoxin, vinblastine and rhodamine 123 used at transporter non-saturating concentrations.

The investigational active substance concentrations used in the permeability studies shall be justified by the Applicant. In the validated analytical procedure in the Caco-2 cell line used for the active substance permeability assay, it is necessary to use the conditions established during the procedure validation and include model active substances with moderate and high permeability in the donor fluid with the investigational active substance as in-house standards to confirm the robustness of the analytical procedure. The choice of in-house standards shall be based on their compatibility with the investigational active substance, i.e. they shall not demonstrate any significant physical, chemical or transfer-mediated interactions with the investigational active substance. Permeability of the in-house standards can be determined after assessment of the investigational active substance in the same Caco-2 cell monolayers or cell monolayers in the same plate, if it is impossible to include in-house standards in the same cell culture in which the permeability of the investigational active substance has been assessed. The permeability values of in-house standards obtained in a series of assays, as well as during the validation of the analytical procedure shall correlate (be similar). The acceptance criteria for in-house standards and the model substrate for the efflux transporter shall be indicated in the report. The average release of the active substance and in-house standards at the test end shall be stated. If the release is < 80%, a mass balance assessment shall be

performed, including measurement of the active substance residual amount in the Caco-2 cell monolayer and in the test device environment.

The assessment of the investigational active substance permeability for its BCS classification can be simplified by choosing an in-house standard with high permeability, the value of which is close to the established border between moderate and high permeabilities. In this case, the investigational active substance is considered as highly permeable, if its permeability value is equal to or greater than the permeability value of the selected in-house standard with high permeability.

Information to justify the high permeability of the investigational active substance (average permeability, its standard deviation, coefficient of variation) shall include:

data on the investigational active substance permeability;

data on permeability of the used in-house standards;

data on the active substance gastrointestinal stability *in vitro*;

data justifying the mechanism of passive diffusion of the active substance.

Table

Examples of model active substances for the permeability assay procedure validation

Group	Active substance
High permeability ($f_a \geq 85\%$)	Antipyrin Caffeine Ketoprofen Naproxen Theophylline Metoprolol Propranalol Carbamazepine Phenytoin

Group	Active substance
	Disopyramide Minoxidil
Moderate permeability (f_a = 50% to 84%)	Chloramphenicol 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
Zero permeability	FITC-dextran Polyethylene glycol 4000 Lucifer yellow Inulin Lactulose
Model substrates for efflux transporters	Digoxin Paclitaxel Quinidine Vinblastine

Annex No. 2

to the Requirements for Biowaver Based on Biopharmaceutics Classification System

ALGORITHMS AND EXAMPLES for Assessing Differences in the Excipients Composition in the Investigational Medicinal Product and the Reference Medicinal Product

1. Algorithms for assessing differences in the excipients composition in the investigational medicinal product and the reference medicinal product

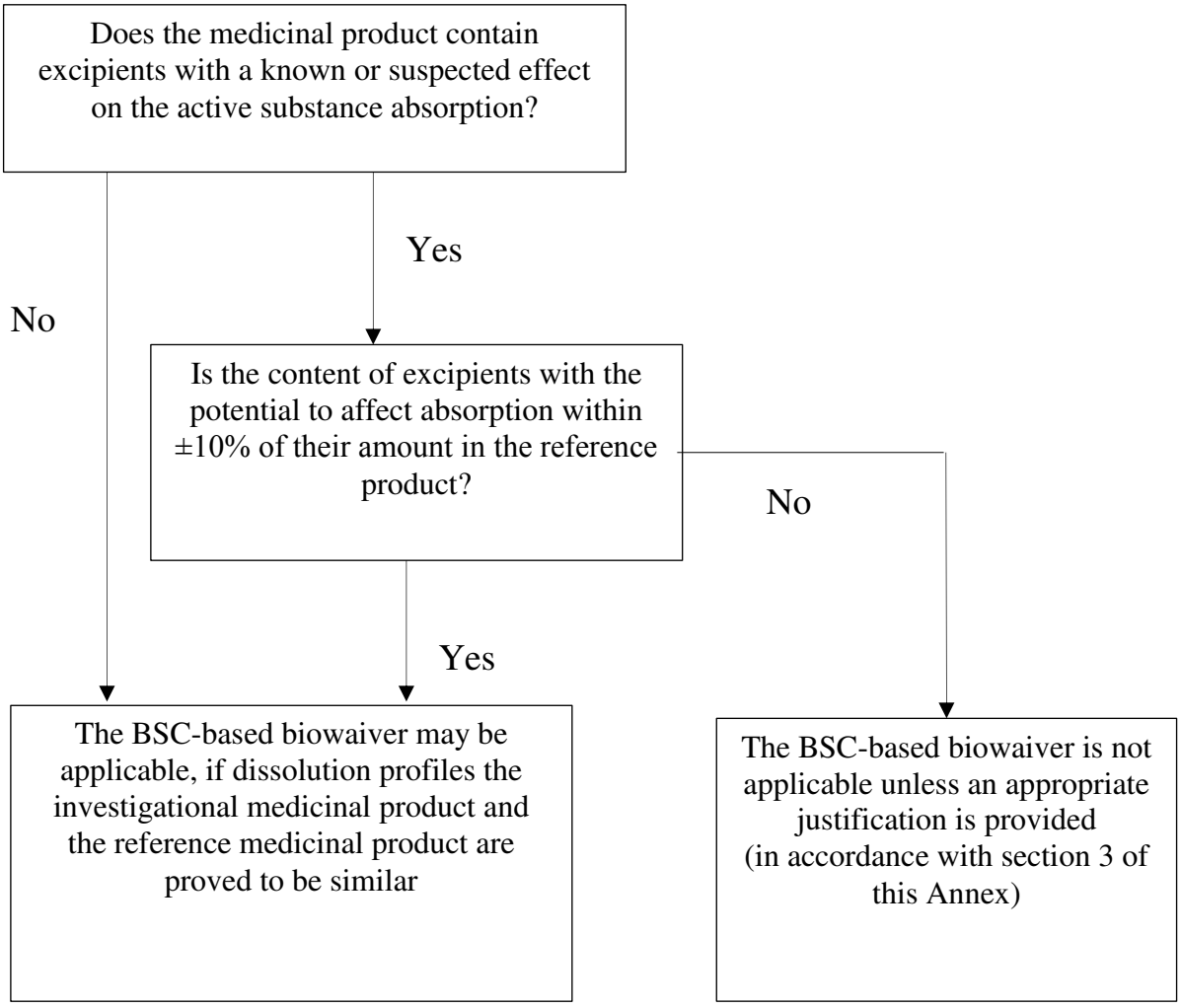

Figure 1. Algorithm for the assessment of medicinal product containing Class I active substances

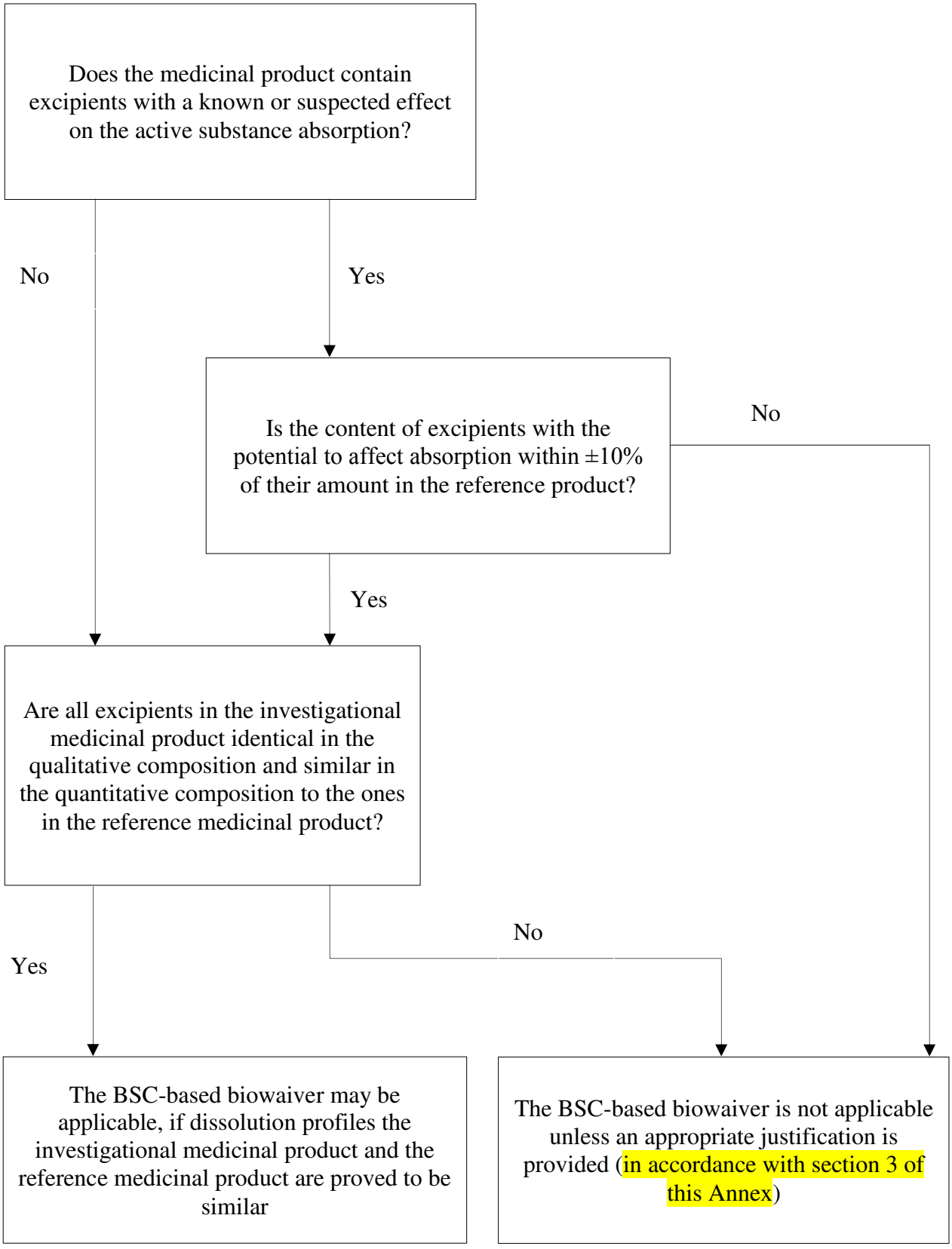

Figure 2. Algorithm for assessing medicinal product containing BCS Class III active substances

2. Examples of acceptable differences in the excipients composition in the investigational medicinal product and the reference medicinal product

Example 1. The BCS-based biowaiver for a medicinal product containing a BCS Class I active substance

The qualitative composition of the investigational medicinal product coincides with the qualitative composition of the reference medicinal product. The quantitative content of sorbitol, an excipient with known or suspected effect on the active substance absorption, differs from its content in the reference medicinal product. At that, the sorbitol quantitative content in the investigational drug product is within the acceptable range of 45 mg to 55 mg, calculated basing on the sorbitol content in the reference drug product (50 mg ±10%).

Component	Functional purpose	Content in reference product (mg)	Content in investigational medicinal product (mg)
Active substance		100	100
Microcrystalline cellulose	filler	100	95
Sorbitol	filler	50	55
Hydroxypropyl methylcellulose	binder	10	10
Talc (glidant)	glidant	5	5
Total		265	265

Example 2. Biowaiver for medicinal product containing the BCS Class III active substance

The qualitative composition of the investigational medicinal product coincides with the qualitative composition of the reference medicinal product. The quantitative content of sorbitol, an excipient with known or suspected effect on the active substance absorption, differs from its content in

the reference medicinal product. At that, the sorbitol quantitative content in the investigational drug product is within the acceptable range of 9 mg to 11 mg, calculated basing on the sorbitol quantitative content in the reference medicinal product (10 mg ±10%). Differences in the quantitative content of other excipients are within the limits specified in the Table of paragraph 35 of the Requirements.

Component	Functional purpose	Reference product		investigational product		Absolute difference with reference to the tablet core weight
		Composition (mg)	Tablet core weight fraction (% w/w)	Composition (mg)	Tablet core weight fraction (% w/w)	
Active substance		100.0	49.3%	100.0	46.5%	–
Lactose monohydrate	filler	85.0	41.9%	97.0	45.1%	3.2%
Sorbitol	filler	10.0	4.9%	9.0	4.2%	0.7%
Sodium croscarmellose	disintegrant	6.0	3.0%	7.0	3.3%	0.3%
Magnesium stearate	lubricant	2.0	1.0%	2.0	0.9%	0.1%
Total		203.0	100.0%	215.0	100.0%	
Total difference:						4.3%

Annex No. 3

to the Requirements for Biowaver Based on Biopharmaceutics Classification System

ADDENDA
to the Application Conditions of the Requirements for Biowaver Based on Biopharmaceutics Classification System

Requirements Provision	Application Conditions
General provisions	
Applicability of the biowaiver procedure based on the Biopharmaceutics Classification System to active substances with non-linear pharmacokinetics	The BCS-based biowaiver may be applied to active substances with non-linear pharmacokinetics if they meet the solubility and permeability criteria for Class I or Class III substances according to the Biopharmaceutics Classification System (BCS).
Acceptability of applying the BCS-based biowaiver to the combined medicinal products containing only one of the active substances meeting the criteria for the BCS-based biowaiver and the rest of the substances not meeting the said criteria	The BCS-based biowaiver may be applied if all active substances of the combined medicinal product meet the criteria for the BCS Class I or Class III. If at least one of the active substances is not the BCS Class I or Class III active substance, the probability that the dosage form and composition of the combined medicinal product will affect the behavior of this active substance *in vivo* cannot be ruled out.
Reason for ineligibility of a medicinal product with a narrow therapeutic index for the BCS-based biowaiver procedure, although the absorption rate and degree of such BCS Class I or Class III active substances depend only on their solubility and permeability and do not	The medicinal products with narrow therapeutic index are the drug products for which minor differences in dose or blood concentration can lead to dose- or concentration-dependent serious issues (therapeutic failures or adverse drug reactions). These medicinal products exhibit a steep dose-response pattern over the usual dose range or a narrow range between effective drug concentrations and concentrations associated with serious toxicity. Therefore, doses of such medicinal products shall be carefully titrated and monitored.

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depend on the narrow therapeutic index	Notwithstanding the absence of medicinal products with narrow therapeutic index, there are special requirements for confirming the *in vivo* bioequivalence of such medicinal products (e.g. more stringent acceptance criteria [90% confidence interval for C_{max} and/or AUC shall be within the range of 90.00% to 111.00%] and certain special aspects of the study design). The BCS-based biowaiver principles do not allow for more stringent biowaiver criteria. Due to that, the BCS-based biowaiver is not suitable as a substitute for an *in vitro* study to establish the bioequivalence of the medicinal products with narrow therapeutic index.
BCS-Based Biowaiver Eligibility	
Acceptability of the BCS-based biowaiver, if the investigational medicinal product and reference medicinal product contain different forms of salts of the same active substance	The BCS-based biowaiver may be applied if the investigational medicinal product and the reference medicinal product contain different (single) salts of the active substance, provided that both salts belong to the BCS Class I (high solubility and high permeability). The BCS-based biowaiver is not applicable if the investigational medicinal product contains active substance differing in its structure (ether, ester, isomer, isomer mixture, complex compound or other chemical derivative) from the structure of the reference product active substance, since such differences may cause differences in bioavailability that cannot be determined through the experiments used to justify the BCS-based biowaiver. In addition to the scientific aspects of justification of the possibility to apply the BCS-based biowaiver, the requirements of these Rules shall be considered.
The necessity to consider changes in the active pharmaceutical substance weight associated with different active substance salt when assessing solubility	In the Biopharmaceutics Classification System, the active substance itself (the base) is subject to classification. The dose of a particular active substance in the experiment shall be identical regardless of the type of its salt. Therefore, the concept of difference in the active substance weight in this situation is not applicable.
Justification for the BCS-based biowaiver	The Biopharmaceutics Classification System is based on solubility and permeability criteria for a

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application to prodrugs that are only absorbed as a prodrug	specific active substance. The classification group cannot be extrapolated to other compounds, such as the parent compound or metabolite. Moreover, the solubility criterion is assessed basing on the assumption that the medicinal product is taken orally with a certain amount of water. This condition does not apply to metabolite, unless it is formed immediately after ingestion and before absorption. The Biopharmaceutics Classification System is applied to an active substance contained in a medicinal product, since *in vitro* dissolution of this active substance is used to confirm the similarity of drugs.
Solubility	
Maintaining consistent pH during the solubility assessment experiment	Various methods of maintaining the solution pH are acceptable. If pH consistency is required, the method chosen shall be justified. The pH deviation within ±0.1 is considered acceptable.
Determination of the solubility determination duration	In the case of equilibrium solubility assessment, duration of the solubility determination shall be scientifically justified basing on the time required to reach the equilibrium state. When the equilibrium solubility cannot be determined, the duration of the solubility experiment shall be scientifically justified considering the expected *in vivo* absorption time.
Accounting for the buffer solution ionic strength effect on solubility testing	The ionic strength of buffer solutions does not affect the active substance solubility.
Acceptability of using average solubility values of the assessment replicates or the lowest of the obtained solubility values at significant variability between individual solubility results at a given pH	As for the highly soluble active substances, no significant variability between the values of the individual solubility results shall occur. However, if there is variability, the solubility determination shall be based on the average solubility value of the assessment replicates.
Acceptability of using scientific literature data or other scientific data to justify solubility as	To establish the active substance solubility, experimental solubility data shall be provided. Scientific chemical process and pharmaceutical data are to be provided only to further support the

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reference data to classify the active substance in accordance with the Biopharmaceutics Classification System	solubility conclusions.
Acceptability of using a different limit for the active substance degradation when assessing the solubility compared to the limit established by the Requirements of not more than 10%?	The active substance degradation limit of not more than 10% is set to prevent upward bias of the solubility index associated with the substance transition into solution, but not with degradation of this active substance. This limit can be ensured under experimental conditions.
Permeability	
Acceptability of using other fully validated cell lines (e.g. MDCK-II, LLC-PK1) instead of the Caco-2 cell line to ensure the BCS permeability assessment	The active substance permeability can essentially be assessed using other *in vitro* (other cell lines, e.g. MDCK-II), *in situ* (Loc-I-Gut) or *ex vivo* (everted rat intestinal sac model) methods. Since there is little experience in assessing the active substance permeability using *in vitro* approaches, it is necessary to apply the method for which the greatest experience has been accumulated, that is using the Caco-2 cell line. As experimental data accumulates in relation to other *ex vivo* and *in situ* methods based on cell lines or animals, these data can be used in the future with strict validation and standardisation in accordance with the principles set forth in Annex No. 1 to the Requirements.
Reasons to classify active substances demonstrating moderate permeability (50% to 84%) in validated studies in the Caco-2 cell line and being unstable in the gastrointestinal tract into the group of lowly permeable active substances	Only the active substances with high permeability are assigned to the BCS Class I (that provides additional flexibility for the manufacturer to change the excipients composition and allows for broader dissolution criteria (i.e. ≥ 85% in 30 minutes)). Assignment to this class of active substances with permeability other than the high one (i.e. substances with moderate or low permeability) is not acceptable in the context of the BCS-based biowaiver. As for the active substances unstable in the gastrointestinal tract, it is impossible to

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	demonstrate high permeability *in vivo*. When the high permeability cannot be conclusively demonstrated using one of the methods described in the Requirements, the applying the BCS-based biowaiver is possible if the active substance meets the BCS Class III criteria (the medicinal product has restrictions regarding changes in the composition and excipients content and is very rapidly soluble (releasing ≥ 85% of the active substance within 15 minutes)).
Ensuring the sampling size necessary to obtain reliable assessment of the medicinal product permeability.	The planned number of replicates required to determine the permeability class accurately is difficult to determine conclusively since it depends on the variability of each individual assay. This group of assays is characterised by high between-laboratory variability, potential variability sources for which have been identified in the scientific medical and chemical-pharmaceutical literature (J Pharm Sci (97), 2008; Eur J Pharm&Biopharm (114), 2017)). The between-laboratory variability is significantly lower for Class I than for Class III. As for the active substances with $P_{app} > 10 \times 10^{-6}$ cm/s, there is evidence of moderate variability (5% to 20%) presented in the scientific medical and chemical-pharmaceutical literature (Eur J Pharm Sci (56), 2014; J Pharmcol & Toxicol Methods (70), 2014). Due to that, it is unlikely that high variability will result in inaccurate assessment of high permeability. For assays based on the Caco-2 epithelial cell monolayers, the minimum justification is to perform the assay in 3 replicates.
Approaches to statistical differentiation of the obtained P_{app} values for medicinal products with low, moderate and high permeability in case they overlap when comparing individual values for the medicinal products from	The purpose of the statistical differentiation of the experimental values is to obtain a dichotomous result (active substance demonstrates or does not demonstrate high permeability). The *in vivo* permeability of the reference active substances listed in Annex No. 1 to these Requirements has been confirmed in human studies, which demonstrate that average values clearly allow classifying them as substances with low, moderate

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each group	and high permeability. The Caco-2 cell line systems are successfully validated by laboratories for the purposes of the Biopharmaceutics Classification System using these reference active substances, which makes necessary to obtain differentiated results for active substances with high, moderate and low *in vitro* permeability. If the average values for active substances with low, moderate and high permeability values are overlapped in the experimental determination, this indicates the probable issues in setting up the study procedure in the Caco-2 cell line in the organization or its implementation. In order to confirm the permeability class of the investigational active substance, the assay is standardised against the indicated reference active substances. The investigational active substance shall demonstrate apparent permeability (P_{app}) equal to or greater than that of the reference active substance with high permeability in order to be classified as a highly permeable active substance; in that case no further statistical differentiation of the results is required.
The Medicinal Product Eligibility for the BCS-Based Biowaiver	
Restrictions in use of different dosage forms of the investigational medicinal product and the reference medicinal product for the BCS-based biowaiver	Differences in dosage forms of the same active substance can affect its *in vivo* behavior. Specific instructions for different dosage forms and excipients are covered in the Requirements, including taking into consideration the impact of differences introduced by the dosage form on the active substance *in vivo* behavior and to reduce the risk associated with the possibility of making incorrect conclusion on the bioequivalence. The provisions of the Requirements may be used to replace one dosage form with another during the development of a medicinal product, if it is justified, for example, using previous *in vivo* data.
Restrictions in applying the BCS-based biowaiver for orodispersible tablets	Since the residual stomach volume is significantly less than 250 mL, assessment of the active substance solubility in 250 mL of dissolution

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provided that they are used without water	medium is not applicable to medicinal products taken without water. Determining the dissolution medium volume required to establish the solubility class will be difficult for the orodispersible tablets provided they are used without water. The current dissolution test procedure is of limited use for orodispersible medicinal products (not required to be washed down with water). For such medicinal products, a bioequivalence study shall be carried out with orodispersible tablets used without water.
Excipients	
The acceptability of using the *in silico* physiologically based pharmacokinetic absorption modelling procedure to assess the risk of changes in the dosage form behavior associated with the potential effect (inclusion or exclusion) of changing the excipient beyond the recommended ranges.	The *in silico* physiologically based pharmacokinetic absorption modelling is used to assess risk in the drug product behavior due to changes in dosage form. However, at present, such models cannot exhaustively predict all potential differences in absorption mediated by critical excipients. Validation of *in silico* models for such purposes is limited due to the lack of understanding of the mechanisms behind some of the observed effects of excipients and the lack of good quality *in vivo* data for some classes of excipients. In this regard, the risk of incorrect assessment based on the effects predicted by the model does not justify changing the excipient beyond the recommended range. In some cases, the *in silico* physiologically based pharmacokinetic modeling provides necessary justification within a wider excipients risk assessment, such as sensitivity analysis using properly validated physiologically based pharmacokinetic absorption modeling for excipients, when the mechanism of effect is well established.
The effect of excipients listed in Table of paragraph 35 of the Requirements as ‘Other Excipients’ on absorption	The Table of paragraph 35 of the Requirements contains criteria for confirming the quantitative similarity of medicinal products containing the BCS Class III active substances. The classes of excipients listed in the Table are functional classes, however, within each class, an excipient may be able to affect absorption. In this case, the percentage difference in the amount of such excipient compared to its content in the

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	reference medicinal product shall be within 10% deviation from this substance weight.
Data considered as appropriate justification for deviation from the acceptable difference in excipients specified in the Table of paragraph 35 of these Requirements	Such data are the results regarding the *in vivo* behavior of the dosage form obtained during the medicinal product development program. Such data (e.g. for dosage forms with different content ranges of excipients not affecting the active substance absorption including detailed assessment of their effect mechanisms), can be used as a justification for changing the excipients content beyond the limits indicated in the Table of paragraph 35 of the Requirements.
Acceptability of using excipients in the medicinal product composition of the same type as the substances in the composition of the reference medicinal product, but of different functional class, as to ensure the ‘qualitatively similar composition‘ requirement in relation to the BCS Class III active substances	If it is justified, the difference in the excipient functional class shall be assessed in relation to its functional properties directly in the industrial formulation and dosage form of a particular medicinal product. For some types of excipients, there is no evidence of the effect of changing the excipient functional class on the medicinal product behavior in the human body. For other excipients, class modification can potentially affect the medicinal product dissolution (e.g. changes in particle size distribution, viscosity and degree of substitution of hydroxypropyl methylcellulose; changes in specific surface area of stearate lubricants). To evaluate the excipient comparability, it is required to make decisions whether such comparability is acceptable on an individual basis in order to conclusively confirm the preservation of the composition qualitative similarity.
Acceptable limits of differences in the content of excipients from the polyhydric alcohol group	There are currently insufficient data to qualify the limits at which the effect of these excipients on the medicinal product behavior becomes significant. Moreover, the impact of changes caused by such excipients will depend on the active substance properties (sensitivity of the active substance pharmacokinetic profile to changes in intestinal transit). Changes in the content of this group excipients shall comply with the same restrictions that apply to other excipients that can affect the active substance absorption, that is, it shall be within ±10% of the excipient amount in the

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	reference medicinal product.
Examples of medicinal products compositions containing the BCS Class III active substances, for which the excipients meet and do not meet the criterion of the qualitative composition match and the quantitative composition similarity	Examples of such medicinal products with similar quantitative excipients compositions are given in Annex No. 2 to the Requirements. Additional instructions on allowable differences in the excipients composition are presented in the table of example 2 of Annex No. 2 to the Requirements in the form of percentage difference relative to the weight of the tablet core (mass fraction). If the investigational medicinal product complies with these instructions, but has greater differences in the absolute amounts of excipients (e.g. if the core weight of the investigational medicinal product is not similar to the one of reference medicinal products), the authorised authority (expert organisation) has the right to request additional justification.
Comparable *In Vitro* Dissolution Kinetics Test for the Medicinal Product	
The Acceptability of using sinkers in the comparative dissolution kinetics test not only when coning occurs, but also in other cases (e.g. when particles of the dosage form stick to the paddle mixer, their flotation, etc.)	The use of sinkers to eliminate the problems identified during dissolution experiments is allowed if justified. In this case, the same experimental conditions shall be applied to the investigational and the reference dosage forms.
Approach to comparing the dissolution profiles for BCS Class I medicinal products when one dissolution profile meets the criteria for very rapid dissolution (≥ 85% of average amount of substance transfers in solution in ≤ 15 minutes) and the other dissolution profile meets the criteria for rapid dissolution	If one drug product demonstrates over 85% dissolution in 15 minutes and the other does not, enough sampling points shall be provided to calculate the f_2 parameter to confirm the similarity of the dissolution profiles.

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(≥85% of average amount of substance transfers in solution in ≤ 30 minutes), the *in vitro* dissolution profile characteristics	
The necessity to calculate f_2 from sampling time points to compare dissolution profiles if they differ between the investigational medicinal product and the reference medicinal product (rapid and very rapid dissolution)	In these cases, f_2 shall be calculated from the sampling time points. If one drug product demonstrates over 85% dissolution in 15 minutes and the other does not, enough sampling points shall be provided to calculate the f_2 parameter to confirm the similarity of the dissolution profiles.
Acceptability of using insufficient number of sampling points to calculate the f_2 parameter in dissolution profile comparisons if this insufficient number is associated with exclusion of early time points due to high variability	For the BCS Class I active substances, high variability in dissolution is not expected, therefore alternative statistical methodologies (e.g. bootstrap analysis) to confirm the similarity of dissolution profiles are considered inapplicable. Where high variability is caused by coning, alternative methods for performing the dissolution study (e.g. using sinkers or other reasonable approaches) are considered to address such issues, if scientifically justified.
Approach to comparing dissolution profiles when different sampling time points lead to different f_2 values requiring conflicting conclusions (e.g. time points of 10, 20, 30 minutes lead to $f_2 < 50$, while time points of 8, 20, 30 minutes lead to $f_2 > 50$)	Such a situation shall only arise in exceptional cases. The time points for calculating the f_2 parameter shall be preset. When calculating the parameter, all eligible preset sampling time points shall be used and the choice of these time points shall be justified.
Acceptability of extrapolation of the BCS-based biowaiver for one strength of the medicinal product to other available strengths of the medicinal	Such conclusion is unacceptable. The BCS-based biowaiver requires submission of supporting data for each individual strength from the strength line of medicinal products. Comparison of *in vitro* strength of the investigational medicinal product with the corresponding strengths of the reference medicinal

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product	product eliminates possible drift of similarity results that may occur if release of additional amount of the active substance at a strength is assessed without comparison with the corresponding strength of the reference medicinal product.
Acceptability of comparison between the following dosage forms for applying the BCS-based biowaiver: a) uncoated tablets versus film-coated tablets; b) tablets versus capsules.	a) non-coated tablets and film-coated tablets that do not fulfill a specific functional purpose are considered as the same dosage form when compared according to the biowaiver procedure. Comparison between the indicated dosage forms is allowed for the BCS-based biowaiver; b) tablets and capsules are not considered as the same dosage form of comparison for the biowaiver. Comparison between the indicated dosage forms is not allowed for the BCS-based biowaiver.
Required indicators of the medium mixing speed for comparative dissolution kinetics assessment in the case of suspension dosage forms.	In the case of suspensions, the recommended paddle mixer speed is 50 rpm (instrument 2). It is acceptable (but not mandatory) to use a lower rotation speed of the paddles.
Procedure for the active substance permeability assay in Caco-2 cells	
Acceptability of using 12 model active substances not subject to active transport (4 of 12 model active substances are subject to active efflux [digoxin, paclitaxel, quinidine and vinblastine], the remaining 8 substances are subject to active transport [furosemide by OAT3, metformin by OCT1 and OCT2, amirolide by OCT2, famotidine by the OCT2, acyclovir by OAT1 and OCT1, theophylline by OAT2, and enalapril by PepT1 and PepT2])	The results of a comparison of the permeability of 24 active substances presented in the scientific medical and chemical-pharmaceutical literature (Pharm Res (19) 2002 и Drug Discover Today (17) 2012) and obtained *in vivo* and *in vitro*, in the human jejunum and in the Caco-2 cell line had a good correlation for the substances absorbed by passive diffusion and somewhat worse for active transport. Therefore, the Caco-2 cell monolayers can be used to predict passive diffusion of active substances in humans, while prediction of active transport by transporter systems may be less accurate given the altered expression of transporters by this cell line. Thus, the model medicinal products for determining high permeability include the rapid (passively) permeable medicinal products such as naproxen, antipyrine and metoprolol with comparable permeability coefficients in Caco-2 cells and in the human jejunum. Although some

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specified in the table of Annex No. 1 to the Requirements to validate the Caco-2-based method for BCS classification of an active substance through establishing *in vitro* permeability by means of passive diffusion.	model medicinal products partially undergo active transport, the permeability of their active substances in Caco-2 cell monolayers has strong correlation with *in vivo* permeability. Since expression of transporters by cell lines may differ from *in vivo* conditions, this correlation is not universal for all active substances transported by the active transport. Due to that, without providing evidence-based *in vivo* data, *in vitro* data are not the only way to determine the permeability class of active substances carried by the active transport. The final conclusion on the active substance classified as a highly permeable based on the Caco-2 cell monolayer assay will only be valid for active substances not transported by any active transport.
Applying the BCS-based biowaiver for active substances with low K_m values is acceptable if all of the following conditions are met: a) *in vitro* data for the active substance subject to efflux in Caco-2 cells evidence that the apparent K_m value is significantly lower than the corresponding concentrations of this active substance in the intestine; b) the efflux process activity reaches saturation at all concentrations of the active substance, and the active substance permeability is determined only by its passive diffusion; c) the clinical pharmacokinetics of the active substance in human is linear;	The absence of efflux or of efflux transporter saturation cannot be experimentally distinguished at physiologically reasonable concentrations (e.g. in accordance with Annex No 1 to the Requirements at 0.01, 0.10 and 1.00 of the highest strength of the medicinal product dissolved in 250 mL of medium) exceed the K_m value of the active substance. In this case, the active substance is considered to be highly permeable if the apparent permeability (P_{app}) is greater than or equal to the P_{app} of the highly permeable model reference medicinal product. The assay using Caco-2 cells shall be validated, confirming the bidirectional transport nature of known model medicinal products (table of Annex No. 2 to these Requirements) and proving the functional activity of the efflux transporter(s). If it is also possible to provide *in vivo* data demonstrating high permeability of the active substance in accordance with the Requirements (i.e. pharmacokinetic data (absorption, distribution, metabolism, elimination) in human or absolute bioavailability), the high permeability class for the active substance can also be assigned. It should be noted that the BCS-based biowaiver option is also available for the BCS Class III active substances that do not meet the high permeability class criteria if all the conditions in accordance with the Requirements are met.

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d) auxiliary data are presented, for example, data on pharmacokinetics (absorption distribution, metabolism, elimination) in humans.	
Acceptability of excluding actively transported active substances the permeability of which may be accurately predicted using Caco-2 cells from consideration in the BCS-based biowaiver procedure	For actively transported active substances, the conditions described above for active substances with low K_m values apply. Actively transported active substances shall not be excluded from consideration for the BCS-based biowaiver if *in vivo* data obtained in human allow justifying their classification as highly permeable substances. For this case, it is not acceptable to use only the permeability assay in Caco-2 cells (since expression of transporters by Caco-2 systems may differ from such expression *in vivo*).
Acceptability of justifying the efflux ratio limit higher than 2 using model compounds (data sets from validation results) in the case of individual validated models of Caco-2 cell monolayers for which the efflux ratio higher than 2 is more acceptable.	In the absence of any active transport (absorption or efflux), the ratio between absorptive P_{app} (in the apical (AP) to basolateral (BL) or AP–BL direction) and $P_{app\ BL–AP}$ shall be equal or close to 1. Any deviation from 1 will indicate some contribution of active transport to the active substance transfer process. The efflux ratio higher than 2 is taken as limit indicating that the active substance is the efflux transporter substrate.
Publications providing data on selection of model medicinal products for validation of the active substances permeability assessment method	The relevant data are provided in the following publications: Volpe DA. Application of Method Suitability for Drug Permeability Classification. AAPS J. 2010; 12(4):670-8; Li C. et al. Development of In Vitro Pharmacokinetic Screens Using Caco-2, Human Hepatocyte, and Caco-2/Human Hepatocyte Hybrid Systems for the Prediction of Oral Bioavailability in Humans. Journal of Biomolecular Screening 2007; 12(8):1084-1091; Peng Y. et al. Applications of a 7-day Caco- 2 cell model in drug discovery and development. European Journal of Pharmaceutical Sciences 2014;

Requirements Provision	Application Conditions
	56: 120-130; Kasim NA et al. Molecular Properties of WHO Essential Drugs and Provisional Biopharmaceutics Classification. Molecular Pharmaceutics 2004; 1(1): 85-96; Lennernäs, H. Intestinal permeability and its relevance for absorption and elimination, Xenobiotica 2007; 37(10): 1015 – 1051; Thiel-Demby VE. Biopharmaceutics Classification System: Validation and Learnings of an In Vitro Permeability Assay. Molecular Pharmaceutics 2009; 6(1): 11-18; Giacomini, et al. Nat Rev Drug Discov. 2010; 9:215-236; FDA, United States In Vitro Metabolism-and Transporter-Mediated Drug-Drug Interaction Studies Guidance for Industry (October 2017)