Annex 4

Protocol to conduct equilibrium solubility experiments for the purpose of Biopharmaceutics Classification System-based classification of active pharmaceutical ingredients for biowaiver

1. Scope

The objective of this document is to provide guidance on the design and conduct of equilibrium solubility studies undertaken for the purpose of active pharmaceutical ingredient (API) classification within the Biopharmaceutics Classification System (BCS) (*1*, *2*).

Notably, the definition and guidance given in this document for performing solubility studies apply to APIs and there might be differences in requirement with respect to the conditions for dissolution studies that are applicable to solid finished pharmaceutical products (FPPs).

A study protocol has been developed to provide a harmonized approach when performing solubility studies; however, alternative approaches to determining the solubility of an API, such as phase solubility analysis (*3*), can also be valid if the appropriate test conditions are employed.

The aim of the WHO biowaiver guidance is to reduce the risk of *bioinequivalence* to an acceptable level when granting biowaivers supporting pharmaceutical development. In this context, the solubility, the release from the drug product, and the subsequent absorption phase are considered critical processes underlying the equivalence of the test and reference product.

Equilibrium solubility profiles of APIs contained in medicines in the *WHO Model List of Essential Medicines* (EML) (*4*) can be used in conjunction with absorption/permeability data, FPP dissolution studies, and comparative consideration of FPP excipient content, to enable an informed decision on whether a biowaiver could be granted safely.

2. Experimental considerations

Overall, the API sample should be dissolved/suspended in buffer, then separated by appropriate methods, and the solubilized API concentration measured using a suitable analytical method.

According to the World Health Organization (WHO) definition given in the guidance document *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability* (*5*), an API is considered highly soluble when the highest single therapeutic dose (e.g. the total amount of API administered orally at once), as determined by the relevant regulatory authority, typically defined by the labelling for the innovator product, is soluble in 250 mL or less of aqueous media over the pH range of 1.2–6.8. The pH solubility profile of the API should be determined at 37 ± 1 °C in aqueous media. A minimum of three replicate determinations of solubility at each pH condition is recommended (*5*).

In general, equilibrium solubility experiments should be employed. However, in exceptional cases, for example, where the API is not available in

sufficient quantities; it is prohibitively expensive; or when it is not possible to maintain the pH of the medium with pharmacopoeial buffers, experiments where the highest therapeutic single dose as recommended by the approved label/summary of product characteristics (SmPC) is examined in a 250 mL volume, or a proportionally smaller amount examined in a proportionally smaller volume of buffer, can be considered. As these are equilibrium solubility experiments, small volumes of solubility media may be employed without issue if the available experimental apparatus will permit it.

The source and purity of the API should be reported according to the *Report template for equilibrium solubility experiments for Biopharmaceutics Classification System-based classification of active pharmaceutical ingredients for biowaiver* (Appendix 1). Additional characterization of the solid API used in the solubility experiments may be necessary; for example, the solid form of the drug should be confirmed (the crystallinity and polymorphic form). The depth of the characterization will depend on 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 necessary.

The "shake flask" method for solubility determination is recommended; a mechanical agitation device should be used (e.g. orbital shaker) and an appropriately validated analytical assay method should also be employed. The device used should be capable of maintaining a temperature of 37 ± 1 °C and an appropriate agitation speed that ensures particle contact with the buffer solution. The agitation speed should be optimized based on the shape of the flask or tube and volume of the liquid, in order to prevent particle agglomeration and ensure particle contact with the diluent. Vortex formation should be avoided. With an optimized agitation rate, it is expected that samples will generally reach equilibrium within 24 hours (*6*). Samples should be collected and analysed at several time points, until equilibrium has been reached.

To address issues such as poor wettability and the tendency of the API to float on the surface of the solubility medium, it may be necessary to include tools such as glass microspheres to aid de-aggregation of the particles with agitation or sonication (*6*). Surfactant should be avoided in equilibrium solubility studies because they would produce biased results. Once wetting is successfully achieved, that is, there are no visible particles floating on the surface or sticking to the container, the solubility experiment should proceed toward equilibrium.

In some circumstances, it can be difficult to obtain true equilibrium solubility, for example, due to excessive material requirements (i.e. very high-solubility, micelle-forming API etc.), long equilibrium times (e.g. slow conversion to a salt form), or poor chemical stability. In these cases, particularly when the compound solubility is very high, it may be appropriate to report a lower solubility limit; that is, the actual solubility is greater than the reported value,

which is still higher than the solubility required to dissolve the highest single therapeutic dose in 250 mL of medium.

3. Buffers for determination of equilibrium solubility

The pH–solubility profile of the API should be determined over the pH range of 1.2–6.8, with the API's solubility classification being based on the lowest solubility measured over this pH range. Measurements should be made in triplicate or more, according to the observed variability, under at least three pH conditions, pH 1.2, 4.5 and 6.8, using for example, 0.1 N HCl test solution or simulated gastric fluid without enzymes – pH 1.2; acetate buffer – pH 4.5; and phosphate buffer – pH 6.8 solution. If there are any known solubility minima for the API in aqueous media within that pH range (for example the pK_a of the API is within the tested pH range of 1.2–6.8), data should also be collected at that pH. Pharmacopoeial buffer solutions are recommended for use in solubility experiments, as reported in Section 3.1. The pH of the buffers should be adjusted at the same temperature as that at which the equilibrium solubility experiments are performed, that is, at 37 ± 1 °C. The pH should be verified after addition of the API and at the end of the experiment, with a calibrated pH meter. If the pH of the buffer changes upon combination with the solute, adjustment of the pH with an appropriate acid or base solution may be sufficient to address the issue, or a buffer with a stronger buffering capacity may be employed. After adjustment of the pH, the solution should be allowed to re-equilibrate for at least one hour before a sample is taken.

3.1 Composition of buffers

Solution pH 1.2, TS (test solution)

Dissolve 2.52 g of sodium chloride R (reagent) in 900 mL of water R, adjust the pH to 1.2 with hydrochloric acid (~70 g/L) TS and dilute to 1000 mL with water R.

Buffer pH 4.5, TS

Dissolve 2.99 g of sodium acetate R in 900 mL of water R, adjust the pH to 4.5 by adding about 14 mL of acetic acid (~120 g/L) TS and dilute to 1000 mL with water R.

Buffer pH 6.8, TS

Dissolve 6.9 g of sodium dihydrogen phosphate R and 0.9 g of sodium hydroxide R in 800 mL of water R, adjust the pH to 6.8 with sodium hydroxide (~80 g/L) TS and dilute to 1000 mL with water R.

Information on the reagents to be used can be found by consulting *The International Pharmacopoeia section on Reagents, test solutions and volumetric solutions* (*7*).

4. Experimental design

The details of the solubility experiment's design should be based on the characteristics of the API under investigation. It is recommended that preliminary testing be conducted to assess the amount of API required and the length of time required for the pivotal solubility experiment.

5. Preliminary assessment of the time to equilibrium and expected solubility

Preliminary estimation of solubility from chemical structure may be used as a starting point, using an open-source tool (e.g. ChemSpider (*8*); Virtual Computational Chemistry Laboratory (*9*); Swiss ADME (*10*)) or by estimating the data.

From this calculation, the amount of solid needed to have approximately 30–40% excess of undissolved solid in 5 mL (or the selected working volume) of buffer solutions at pH 1.2, 4.5 and 6.8 can be determined. This amount of solid should be weighed in a glass or non-leaching vial of suitable material and of appropriate volume, for instance a 10 mL tube, if 5 mL of the buffer will be used (corresponding to the expected minimum solubility condition).

If the solubility is greater than expected, the working volume should be reduced to 3 mL, while if the expected solubility is low and the API is available in sufficient quantity, higher volumes should be used.

Alternatively, the volume can be kept at 5 mL and the mass can be increased or reduced as appropriate.

The presence of undissolved solid should be checked; if the entire solid dissolves when adding the buffer, additional solid should be added until such time that some solid remains undissolved in the tube. To solve any potential issues related to solid wettability or agglomeration, the tubes should be put in the agitation system, such as shaker or magnetic stirrer, adding glass beads.

When the amount of solute and volume of buffer has been determined to obtain a saturated solution, a minimum of three replicate samples for each pH should be prepared to allow measurements at multiple time points for identification of the equilibration time. The pH of the solution should be measured at the time intervals. pH adjustments may be made, if necessary and scientifically justified.

Filtration is normally recommended to remove undissolved API from collected samples, although centrifugation is a valid alternative method, particularly if the medium volume is small.

Either approach is acceptable, and should be scientifically justified.

The samples should be filtered (using, for example, a filter pore size of 0.45 μm) during or immediately after withdrawal, or dissolved API separated from undissolved API by centrifugation as appropriate.

Solubility experiments are performed at 37 ± 1 °C; therefore, if samples are to be left at room temperature until analysis, they should be diluted immediately after centrifugation or filtration, in order to avoid precipitation of the solute. This should be taken into account for back calculations.

To determine equilibrium solubility, the concentration of the solution should be measured at different time points, for example 2 h, 4 h, 8 h, 24 h, 48 h and 72 h, until it does not deviate significantly (e.g. 10%) between sequential measurement. The shortest time needed for reaching the plateau of drug concentration against time could be considered a suitable equilibration time.

Samples should be collected over time, to establish a plateau for the amount of solute dissolved and also to monitor the stability of the API at each pH (see Section 6).

The pH value of the buffer solutions should be measured after establishing the time to obtain equilibrium.

6. Stability

The API's stability across the pH range should be monitored, in order to measure true solubility (*11*, *12*),

To distinguish the drug substance from its degradation products, a validated, stability-indicating analytical method should be employed for solubility determination of APIs such as those indicated in *The International Pharmacopoeia* (*13*) or other pharmacopoeias adapted as appropriate, if available, for example, high-performance liquid chromatographic (HPLC) analysis (see chapter *1.14.4 High-performance liquid chromatography* in *The International Pharmacopoeia* (*14*)) or an alternative, validated stability-indicating assay. An advantage of an HPLC method over a spectrophotometric one is that the HPLC method can also be employed to detect impurities and instability (*11*, *12*). If degradation of the drug substance is observed as a function of buffer composition and/or pH, it should be reported. If a stability-indicating analytical method is not available, separate stability experiments will be necessary to demonstrate that the API is stable in the buffer medium employed.

7. Recommendations for the analytical method

Calibration curves should be constructed, ideally with 5–6 standards for regression and estimation of intercept, slope, and correlation coefficient, and three additional control standards independently prepared for estimation of precision and accuracy.

If necessary, samples should be diluted to be on the range of the calibration curve, recording the dilution factor for back calculations.

Each sample should be run in duplicate and a calibration curve established. It is anticipated that at least 3–4 analytical runs are expected (e.g. the first for the samples of 2 h, 4 h, 8 h and then possibly another three for 24 h, 48 h and 72 h), depending on the stability of the samples. In the end, data for intra- and inter-day accuracy and precision estimation should be available. In general, the control standards are intercalated with the samples.

To check filter influence, control standards could be injected without and after filtration. Recovery should be between 98% and 102% (if less than this value, there is some adsorption happening; if more, some filter component is affecting the analysis). If necessary, a change of filter type or switch to centrifugation is recommended.

The specificity, linearity, range, accuracy, repeatability and intermediate precision should be determined (*12*), which should meet the minimum acceptance limits.

8. Pivotal experiment

Pivotal experiments should be designed considering the results of the preliminary experiments. The following steps are presented as a general example of a pivotal solubility experiment:

1. In triplicate, for each pH condition to be evaluated, weigh approximately a 10% excess amount of API (as determined during the preliminary test) and combine with an appropriate volume of the necessary buffer solutions (at least pH 1.2, 4.5 and 6.8 buffers) in a flask. Sufficient API and volume should be used to allow for collection of residual API following the experiment.
2. Mix and measure the pH value.
3. Stop and secure the flask to the orbital shaker, with controlled temperature and shaker speed.
4. Collect an aliquot of the supernatant solution at the time equilibrium was established in the preliminary experiment.

5. Immediately separate dissolved from undissolved API by filtration or centrifugation.
6. Record the pH value of the solution at the end of the experiment.
7. Dilute the sample to avoid precipitation before quantifying.
8. Determine the concentration of the API.

9. Reporting of results

Test results should be reported in the *Report template for equilibrium solubility experiments for Biopharmaceutics Classification System-based classification of active pharmaceutical ingredients for biowaiver* appended to this protocol as Appendix 1. The report should include information on the API (chemical structure, molecular weight, known dissociation constants, etc.), the actual experimental conditions, including information on buffer composition and the analytical method, results (raw data plus mean values with standard deviations), and any observations such as, for example, the degradation of an API due to pH or buffer composition. The section describing the experimental conditions should include the initial and equilibrium pH of solutions and de facto buffer concentrations. If samples are filtered, the type and pore size of the filter should be recorded, along with data from filter adsorption studies. If samples are centrifuged, the conditions of centrifugation should be recorded. A graphic representation of the mean pH–solubility profile should be provided.

Any deviations from the protocol should be noted and duly justified.

The solubility should be reported in mg/mL. The relative standard deviation of the obtained solubility results should not be more than 10% between the replicates of each test condition.

The dose:solubility volume (DSV) represents the volume of liquid necessary to completely dissolve the highest single therapeutic dose of the API (as recommended by the approved label/SmPC) at the pH where the lowest solubility was observed. Based on the lowest solubility calculated in mg/mL, the DSV can be calculated by dividing the highest therapeutic dose (in milligrams) by the solubility (in milligrams per millilitre) obtained in the study. An API is considered highly soluble when the DSV is ≤ 250 mL over the entire pH range 1.2–6.8.

References

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5. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations, fifty-first report. Geneva: World Health Organization; 2017: Annex 6 (WHO Technical Report Series, No. 1003; http://apps.who.int/medicinedocs/documents/s23245en/s23245en.pdf, accessed 14 February 2019).

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12. Supplementary guidelines on good manufacturing practices: validation. Appendix 4: Analytical method validation. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations, fortieth report. Geneva: World Health Organization; 2006: Annex 4 (WHO Technical Report Series, No. 937; http://apps.who.int/medicinedocs/documents/s20108en/s20108en.pdf, accessed 9 February 2019).

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Further reading

- Guidance for organizations performing in vivo bioequivalence studies. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations, fiftieth report. Geneva: World Health Organization; 2016: Annex 9 (WHO Technical Report Series, No. 996; https://www.who.int/medicines/publications/pharmprep/WHO_TRS_996_annex09.pdf?ua=1, accessed 14 February 2019).
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Appendix 1

Report template for equilibrium solubility experiments for Biopharmaceutics Classification System-based classification of active pharmaceutical ingredients for biowaiver

Header:
Logo of the laboratory/company issuing the certificate (if applicable)
Identification No. of the study: *page X of Y*

Name and address of the laboratory issuing the study report: _____

Name of the active pharmaceutical ingredient (API) (international nonproprietary name [INN], brand name, etc.): _____

Certificates of analysis (CoAs) from manufactures provided: assay within specifications <*Yes/No*> _____

Batch number: _____
Date received: _____ Quantity received: _____
Date of manufacture (if available): _____
Expiry date/retest date: _____

Details of original manufacturer

Name and address: _____

Telephone: _____
Email: _____

Information about the API

Chemical structure (please report here): _____

Nature of the drug (i.e. acid, basic, neutral, amphoteric): _____
Dissociation constants [i.e. pK_a(s)]: _____
Molecular weight (g/mol): _____

Equilibrium solubility experiment

Apparatus: _____

Highest therapeutic dose: _____

Recorded temperature (target 37 ± 1 °C): _____

Volume of the buffer: _____

Sampling times: _____

Time to equilibrium: _____

Buffer composition (*please indicate if different buffers from those recommended in the* Protocol to conduct equilibrium solubility experiments for the purpose of BCS-based classification of APIs for biowaiver *were used*): _____

Separations of samples (*please indicate how and when samples were filtered, filter type and pore size. If samples are centrifuged, the conditions of centrifugation should be recorded. If separated through different methods, this should be justified*): _____

Stability (*report and discuss any problems with pH-related stability of samples*): _____

Solubility method and conditions: _____

Brief summary of analytical methods, including validation: _____

Result of the *preliminary* solubility experiment

Theoretical pH	Individual pH measure-ment	Final pH before correction	Adjusted with (mL of 0.1 M HCL or NaOH)[a]	Final pH corrected	API equilibrium concentra-tion (mg/mL)[b]	Concen-tration mean (mg/mL)[c]	CV %	Concentration (mg/mL)						
								2 h	4 h	6 h	12 h	24 h	48 h	72 h
pH 1.2	1 2 3													
pH 4.5	1 2 3													
pH 6.8	1 2 3													
Potential additional pH	1 2 3													

CV: coefficient of variation; HCl: hydrochloric acid; NaOH: sodium hydroxide.

[a] Amount of acid or base needed to adjust the measured pH. The measurement should be conducted in triplicate and, per each measurement, the corresponding pH values should be reported.

[b] Report here the three measurements registered at each pH.

[c] Report here only the mean of the individual values reported in the previous column.

Result of the *pivotal* solubility experiment

Theoretical pH	Individual pH measure-ment	Final pH before correction	Adjusted with (ml of 0.1 M HCL or NaOH)[a]	Final pH corrected	Time to equilibrium	APIs weight	Buffer volum e	API equilibrium concentra-tion (mg/mL)[b]	API equilibrium concentra-tion mean (mg/mL)[c]	CV %
pH 1.2	1 2 3									
pH 4.5	1 2 3									
pH 6.8	1 2 3									
Potential additional pH	1 2 3									

CV: coefficient of variation; HCl: hydrochloric acid; NaOH: sodium hydroxide.

[a] Amount of acid or base needed to adjust the measured pH. The measurement should be conducted in triplicates and, per each measurement, the corresponding pH values should be reported.

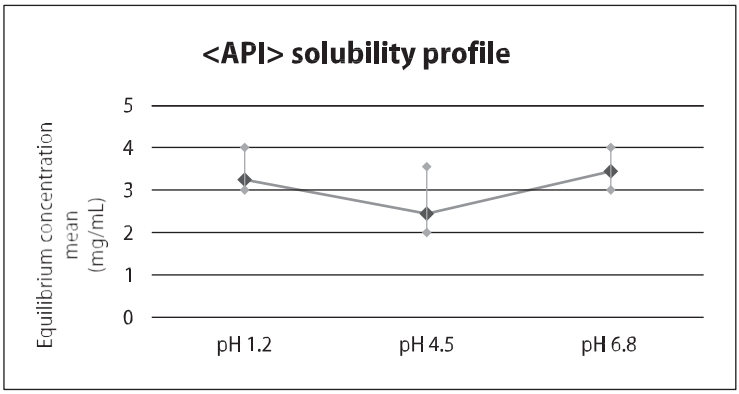
[b] Report here the three measurements registered at each pH.

[c] Report here only the mean of the individual values reported in the previous column. It is the mean solubility value for each pH.

Plot of solubility

To identify the pH of minimum solubility: plot concentration at saturation versus pH and provide a graphical representation of the results. Include error bars on the mean.

Example chart



Please report here the intermediate calculation for the dose:solubility colume (DSV) at (add additional pHs tested as appropriate):

- pH 1.2 Highest therapeutic dose (mg)/Solubility (mg/mL)] [concentration mean] =
- pH 4.5 Highest therapeutic dose(mg)/Solubility (mg/mL) [concentration mean] =
- pH 6.8 Highest therapeutic dose(mg)/Solubility (mg/mL)] [concentration mean] =

Conclusion: is the highest single therapeutic dose (according to the approved originator product labelling) soluble in 250 mL of buffer over the pH range of 1.2–6.8 at 37 ± 1 °C, i.e. in all buffers tested including buffers at pH 1.2, 4.5 and 6.8?

<Yes>/<No>

Solubility classification (*please refer to Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability*:[1]

<High>/<Low>

[1] Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations, fifty-first report. Geneva: World Health Organization; 2017: Annex 6 (WHO Technical Report Series, No. 1003; http://apps.who.int/medicinedocs/documents/s23245en/s23245en.pdf).

Name of the head of laboratory or person authorized to approve the certificate: _____

Telephone: _____

Email: _____

Signature:

Date: