PROTOCOL TO CONDUCT EQUILIBRIUM SOLUBILITY EXPERIMENTS FOR THE PURPOSE OF BIOPHARMACEUTICS CLASSIFICATION SYSTEM-BASED CLASSIFICATION OF ACTIVE PHARMACEUTICAL INGREDIENTS FOR BIOWAIVER

(July 2018)

TAKEN FROM DRAFT NOTES ON THE CONDUCT OF SOLUBILITY STUDIES (AUGUST 2017)

REVISED DRAFT FOR DISCUSSION

Should you have any comments on the attached text, please send these to Dr Valeria Gigante, Technical Officer, Medicines Quality Assurance, Technologies Standards and Norms (gigantev@who.int) with a copy to Mrs Xenia Finnerty (finnertyk@who.int) by 30 September 2018.

Working documents are sent out electronically and they will also be placed on the Medicines website (http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en/) for comments under the “Current projects” link. If you have not already received our draft guidelines, please send your e-mail address to jonessi@who.int and we will add you to our electronic mailing list.
**SCHEDULE FOR THE PROPOSED USE OF DOCUMENT QAS/17.699/Rev.2:**

**PROTOCOL TO CONDUCT EQUILIBRIUM SOLUBILITY EXPERIMENTS FOR THE PURPOSE OF BIOPHARMACEUTICS CLASSIFICATION SYSTEM-BASED CLASSIFICATION OF ACTIVE PHARMACEUTICAL INGREDIENTS FOR BIOWAIVER**

<table>
<thead>
<tr>
<th>Event Description</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation to the forty-seventh meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP). ECSPP recommended to update the WHO Biowaiver List.</td>
<td>October 2012</td>
</tr>
<tr>
<td>Presentation to the forty-eighth meeting of the WHO ECSPP. ECSPP recommended to continue revisions and to align the WHO Biowaiver List with the 18th Model List of Essential Medicines (EML).</td>
<td>October 2013</td>
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<td>Discussion during an informal consultation held together with regulatory experts from national regulatory authorities, the WHO Prequalification Team: medicines (PQTm) and the WHO Collaborating Centre for Research on Bioequivalence Testing of Medicines, Frankfurt/Main, Germany.</td>
<td>5–6 July 2014</td>
</tr>
<tr>
<td>Presentation to the forty-ninth meeting of the ECSPP. ECSPP recommended to continue revisions of the WHO Biowaiver List in a format that facilitates updating in line with the EML.</td>
<td>October 2014</td>
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<tr>
<td>Presentation to the fiftieth meeting of the ECSPP and discussion of the remaining revisions before public consultation. Adoption of the revised <em>Multisource (Generic) Pharmaceutical Products: Guidelines on Registration Requirements to Establish Interchangeability</em> (WHO Technical Report Series, No. 992, 2015) which includes a chapter on <em>in vitro</em> equivalence testing in the context of the Biopharmaceutics Classification System (BCS) and qualification for a biowaiver based on the BCS.</td>
<td>October 2015</td>
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<tr>
<td>Visit of the WHO Collaborating Centre for Research on Bioequivalence</td>
<td>February 2016</td>
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Testing of Medicines, from Frankfurt/Main, Germany, to review and discuss the progress made.

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<th>Event</th>
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<tr>
<td>Presentation of the WHO Biowaiver List and revisions at the Biowaiver Working Group of the International Generic Drug Regulators Programme (IGDRP).</td>
<td>May 2016</td>
</tr>
<tr>
<td>Discussion during an informal consultation held together with regulatory experts from national regulatory authorities, PQTm and the WHO Regulatory Systems Strengthening team (RSS).</td>
<td>8–9 July 2016</td>
</tr>
<tr>
<td>Presentation to the 51st meeting of the ECSPP.</td>
<td>October 2016</td>
</tr>
<tr>
<td>Proposal discussed with experts from national quality control laboratories and specialists in assessing bioequivalence data.</td>
<td>April–June 2017</td>
</tr>
<tr>
<td>Proposal discussed during the Joint Meeting on Regulatory Guidance for Multisource Products with Regulators with the WHO Medicines Quality Assurance group, the Prequalification of Medicines team (PQTm), and the Regulatory Systems Strengthening team held in Copenhagen, Denmark.</td>
<td>7–8 July 2017</td>
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<tr>
<td>Revision of text including feedback received.</td>
<td>August 2017</td>
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<tr>
<td>Mailing and posting of the working document on the WHO website for public consultation.</td>
<td>September 2017</td>
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<tr>
<td>Compilation of comments received.</td>
<td>October 2017</td>
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<td>Presentation to the 52nd ECSPP .*</td>
<td>16–20 October 2017</td>
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<tr>
<td>Preparation of draft Protocol to conduct equilibrium solubility experiments for the purpose of BCS-based classification of APIs for</td>
<td>November 2017</td>
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</table>
biowaiver and the Model Certificate of equilibrium solubility experiments for BCS-based classification of APIs for biowaiver (Annex 1) by Dr Valeria Gigante, WHO Medicine Quality Assurance group (MQA), Dr John Gordon from PQTm, Professor Giovanni Pauletti, Professor Marival Bermejo, Professor Virginia Merino Sanjuán, Professor Carla M. Caramella, Mr Xu Mingzhe and Professor Jin Shaohong.

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<th>Event</th>
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<tr>
<td>Circulation for comments and compilation of comments received for the Protocol and the Model Certificate of equilibrium solubility experiments for BCS-based classification of APIs for biowaiver.</td>
<td>December 2017</td>
</tr>
<tr>
<td>Start of APIs equilibrium solubility studies for BCS-based classification by Professor Giovanni Pauletti, Professor Marival Bermejo, Professor Virginia Merino Sanjuán, Mr Xu Mingzhe and Professor Jin Shaohong. Collation of regulatory information from the European Medicines Agency (EMA), Agência Nacional de Vigilância Sanitária (ANVISA) and PQTm.</td>
<td>February 2018</td>
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<tr>
<td>Protocol and Model Certificate (Annex 1) optimization during the WHO pilot project for BCS-based classification of API for biowaiver.</td>
<td>March–April 2018</td>
</tr>
<tr>
<td>Presentation of regulatory documents and tests results during the Joint Meeting on Regulatory Guidance for Multisource Products with MQA and PQTm in Copenhagen, Denmark.</td>
<td>18–19 May 2018</td>
</tr>
<tr>
<td>Updated Protocol and Model Certificate of equilibrium solubility studies for BCS-based classification of APIs mailed and posted for public consultation.</td>
<td>June–September 2018</td>
</tr>
<tr>
<td>Consolidation of comments received.</td>
<td>September 2018</td>
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<tr>
<td>Presentation of updated Protocol and Model Certificate of equilibrium solubility experiments for BCS-based classification of APIs for biowaiver to the 53rd meeting of the ECSPP.</td>
<td>22–26 October 2018</td>
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<tr>
<td>Any other follow-up action as required.</td>
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8.2.1. Revision of the biowaiver list

As part of its 2006 guidance on waiving of bioequivalence requirements for immediate-release oral solid dosage forms on the WHO EML, WHO had provided a list of APIs that are eligible for biowaivers. The intention is for this list to be updated and maintained as a living document on the WHO website. In 2016 it was agreed that the list should be based on verified laboratory data instead of a literature-based approach. The WHO Secretariat intends to coordinate a new multicentre project to determine the solubility profiles of APIs contained in medicines on the WHO EML to enable an informed decision on whether a biowaiver could safely be granted. The WHO Secretariat proposed that the Expert Committee contribute to updating the biowaiver list by proposing appropriate laboratories to perform the tests, review experimental protocol templates, review laboratory results, determine the APIs’ Biopharmaceutics Classification System (BCS) class, and/or participate in the publication of results. A number of members responded positively to the WHO Secretariat’s call for support for the envisaged studies. The Committee noted the report on the update of the WHO biowaiver list. WHO gratefully acknowledged the support offered by the members of the Expert Committee.

8.2.2 Conduct of solubility studies

During the design of studies to support the revision of the WHO biowaiver list, it became apparent that more guidance was needed on how to design and conduct solubility studies for the purpose of classifying APIs within the BCS. A guidance text was drafted in March 2017, building on recently adopted WHO guidance on equilibrium solubility experiments and on the general chapter on solubility measurements included in the Brazilian Pharmacopoeia in 2016. The proposed guidance was discussed with relevant specialists and at a joint meeting on regulatory guidance held in July 2017. The proposal was further revised and circulated for public consultation in September 2017. It was presented to the Committee at its fifty-second meeting together with comments received.


The Committee endorsed the proposed approach to conducting solubility studies for the purpose of revising the WHO biowaiver list.”
PROTOCOL TO CONDUCT EQUILIBRIUM SOLUBILITY EXPERIMENTS FOR
THE PURPOSE OF BIOPHARMACEUTICS CLASSIFICATION SYSTEM-BASED
CLASSIFICATION OF ACTIVE PHARMACEUTICAL INGREDIENTS FOR
BIOWAIVER

1. OBJECTIVE OF THE DOCUMENT

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 to perform solubility studies apply to APIs and
there might be differences in requirement from the conditions for dissolution studies applicable
to finished solid pharmaceutical products (FPP).

A study protocol has been developed to provide a harmonized approach when performing
solubility studies.

2. EXPERIMENTAL CONSIDERATIONS

Overall, the API sample should be dissolved/suspended in the buffer, then separated by
appropriate methods and, in the end, its concentration should be determined in the liquid
phase/supernatant.

According to the World Health Organization (WHO) definition given in the guidance document
*Multisource (Generic) Pharmaceutical Products: Guidelines on Registration Requirements to
Establish Interchangeability* (3), an API is considered highly soluble when the highest single
therapeutic dose 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 (3).
In general, equilibrium solubility experiments should be employed. However, in exceptional cases, where the API is not available in sufficient quantities, and 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 (e.g. 50 mL) 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 Model Certificate of equilibrium solubility experiments for BCS-based classification of APIs for biowaiver (Annex 1). Additional characterization of the solid API used in the solubility experiments may be necessary. The depth of the characterization will depend on the existing knowledge of the solid-state properties of the API in question. For example, if it has been established that the API exists as a single polymorphic form, then less solid-state characterization is necessary.

The “shake flask” method for solubility determination is recommended; a mechanical agitation device should be used (e.g. orbital shaker) and an appropriate validation 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 diluent. 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 (4). Samples should be taken at several time points to verify that 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 in the de-aggregation of the particles with agitation or sonication (4). Once wetting is successfully achieved, the solubility experiment should proceed toward equilibrium.
3. BUFFERS FOR EQUILIBRIUM SOLUBILITY DETERMINATION

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 study variability, under at least three pH conditions, pH 1.2, 4.5 and 6.8 using, for example, 0.1 M HCl 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 pKa of the API when within this range), data should also be collected at that pH. Pharmacopoeial buffer solutions are recommended for use in solubility experiments as reported below. Adjust the pH of the buffers at the same temperature as the equilibrium solubility experiments are performed, i.e. at 37 °C ±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 an hour before a sample is taken.

3.1 Buffers composition

- **Buffer 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 (~80g/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, Reagents, Test Solutions and Volumetric Solutions Section*, [http://apps.who.int/phint](http://apps.who.int/phint) (5).

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 TIME TO EQUILIBRIUM AND EXPECTED SOLUBILITY**

Preliminary estimation of solubility from chemical structure is suggested as a starting point, using an open source tool (e.g. ChemSpider [www.chemspider.com](http://www.chemspider.com); Virtual Computational Chemistry Laboratory [http://www.vcclab.org](http://www.vcclab.org); Swiss ADME [http://www.swissadme.ch](http://www.swissadme.ch)) or by estimating this data.

From this calculation, determine 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. Weigh this amount of solid and put in, 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, reduce volume to 3 mL while if the expected solubility is low and the API is available in sufficient quantity, use higher volumes.

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

Check for the presence of undissolved solid; 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, put the tubes 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, minimum three replicates samples for each pH should be prepared to allow measurements at 2, 4, 8, 24, 48, and 72 hours to identify the equilibration time.

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. Filter the samples (using, for example, a filter pore size of 0.45 μm) immediately after withdrawal or separate dissolved 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, samples 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 (approximately six; i.e. 2, 4, 8, 24, 48, and 72 hours) until it does not deviate significantly between each measurement. The shortest time needed for reaching the plateau of drug concentration against time could be considered a suitable equilibration time. Collect samples over time to establish a plateau for the amount of solute dissolved and also to monitor stability of the API at each pH (see below).

Measure the pH value of the buffer solutions 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 (6,7).

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 Ph. Int. or other Pharmacopoeias adapted as appropriate, if available, e.g. high-performance liquid chromatographic (HPLC) analysis (see chapter 1.14.4 High-performance liquid chromatography in The International Pharmacopoeia (5)) or an alternative, validated stability-indicating assay. An advantage of an HPLC method over a spectrophotometric method
is that the HPLC method can also be employed to detect impurities and instability (6,7). 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 media employed.

7. RECOMMENDATIONS FOR THE ANALYTICAL METHOD

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

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

Run each sample in duplicate and establish a calibration curve. It is anticipated that at least 3 to 4 analytical runs are expected (e.g., the first for the samples of 2, 4, 8 hours and then possibly another three for 24, 48 and 72 hours samples), depending on the stability of the samples. In the end, data for intra, 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 98–102% (if less than this value there is some adsorption happening; if more some filter component is affecting the analysis). If necessary, change the filter type.

Estimate specificity, linearity, range, accuracy, repeatability, and intermediate precision (7), which should meet the minimum acceptance limits.

8. PIVOTAL EXPERIMENT

Pivotal experiments should be designed considering the results of 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;  
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; and  
8. determine the concentration of the API.

9. REPORTING OF RESULTS

Test results should be reported in the Model Certificate of Equilibrium Solubility Experiments for Biopharmaceutics Classification System-based classification of Active Pharmaceutical Ingredients for Biowaiver appended to this Protocol as Annex 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 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 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 (RSD) between 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 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 milliliter) obtained in the study. An API is considered highly soluble when the DSV is less than 250 mL over the entire pH range 1.2-6.8.

REFERENCES


FURTHER READING


ANNEX 1

MODEL CERTIFICATE OF EQUILIBRIUM SOLUBILITY
EXPERIMENTS FOR BIOPHARMCEUTICS CLASSIFICATION
SYSTEM-BASED CLASSIFICATION OF ACTIVE
PHARMACEUTICAL INGREDIENTS FOR BIOWAIVER

Name and address of the laboratory issuing the study report: ____________________________
________________________________________________________________________________

Name of the 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: __________________________
Information about the API

Chemical structure (please report here):

Nature of the drug (i.e. acid, basic, neutral, amphoteric):

Dissociation constants [i.e. pKa(s)]:

Molecular weight (g/mol):

Equilibrium solubility experiment

Apparatus:

Highest therapeutic dose:

Recorded temperature (target 37 °C ± 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 where used):_____________________________________________________

Separations of samples (please indicate how and when samples were filtered, filter type and pores 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

<table>
<thead>
<tr>
<th>Theoretical pH</th>
<th>Individual pH measurement</th>
<th>Final pH before correction</th>
<th>Adjusted with (ml of 0.1 N HCl or NaOH)</th>
<th>Final pH corrected</th>
<th>API equilibrium concentration (mg/ml)</th>
<th>Concentration mean (mg/ml)</th>
<th>CV %</th>
<th>Conc. (mg/ml) 2 h</th>
<th>Conc. (mg/ml) 4 h</th>
<th>Conc. (mg/ml) 6 h</th>
<th>Conc. (mg/ml) 12 h</th>
<th>Conc. (mg/ml) 24 h</th>
<th>Conc. (mg/ml) 48 h</th>
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\(^a\) 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.

\(^d\) Coefficient of variation.
Result of the **pivotal** solubility experiment

<table>
<thead>
<tr>
<th>Theoretical pH</th>
<th>Individual pH measurement</th>
<th>Final pH before correction</th>
<th>Adjusted with (ml of 0.1 N HCl or NaOH)(^{a})</th>
<th>Final pH corrected</th>
<th>Time to equilibrium</th>
<th>APIs Weight</th>
<th>Buffer volume</th>
<th>API equilibrium concentration (mg/ml)(^{b})</th>
<th>API equilibrium concentration mean (mg/ml)(^{c})</th>
<th>CV % (^{d})</th>
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<td>pH 1.2</td>
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<td>pH 6.8</td>
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</table>

\(^{a}\) 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.

\(^{d}\) Coefficient of variation.
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 mean.

Example chart

<table>
<thead>
<tr>
<th>pH</th>
<th>Highest therapeutic dose (mg)/Solubility (mg/ml)</th>
<th>Concentration mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>highest therapeutic dose (mg)/Solubility (mg/ml)</td>
<td>Concentration mean</td>
</tr>
<tr>
<td>4.5</td>
<td>highest therapeutic dose (mg)/Solubility (mg/ml)</td>
<td>Concentration mean</td>
</tr>
<tr>
<td>6.8</td>
<td>highest therapeutic dose (mg)/Solubility (mg/ml)</td>
<td>Concentration mean</td>
</tr>
</tbody>
</table>

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>
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<High> / <Low>

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

________________________________________________________________________________

Telephone: __________________ E-mail: _________________________________

Signature: _______________________________ Date: _______________________

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