English translation of **Attachment 1 of Clerical Notification** of Pharmaceutical and Food Safety Bureau, dated February 29, 2012

# Guideline for Bioequivalence Studies of Generic Products

## Q & A

### General Matters

Q-1 This Guideline differs from the corresponding WHO guideline* in terms of the extent of requirements, mainly in the following 3 points. Please explain the reason for the differences.

(1) A difference in the lot size of test products.

(2) The WHO guideline requires a minimum of 12 subjects, but this guideline accepts studies with 12 subjects or less.

(3) If the dissolution profile exhibits similarity or equivalence**, some products may be assessed as biologically equivalent even if the confidence intervals are wider than the acceptable range of bioequivalence.

* Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability (WHO Technical Report Series, No. 937, Annex 7, 2006).

** In the guideline, similarity of the dissolution profile is applied to immediate-release products and enteric-coated products. Equivalence in the dissolution profile is applied to extended-release products. Refer to Q35 in "Q&A on the Guideline for Bioequivalence Studies for Different Strengths of Oral Solid Dosage Forms and the Guideline for Bioequivalence Studies for Formulation Changes of Oral Solid Dosage Forms" for further information on equivalence and similarity of dissolution profiles.

(A) With regard to (1), the WHO guideline specifies that test products should ideally be taken from industrial scale batches, and when this is not feasible, from batches not smaller than 10% of the expected full production batches, or 100,000 units (hereafter referred to as tablets), whichever is higher. However, in Japan, full production of generic products is often approximately 100,000 tablets. If the lot scale is 1/10 or larger of the full production lot, and the same manufacturing method as the full production lots is used, drug product characteristics of the test products are considered to be equivalent to those of the full production lots. This can be confirmed in dissolution tests. Therefore, a lot size of the test product is stipulated as at least 1/10 of the full production size, and it does not necessarily require more than 100,000 tablets.

With regard to (2), it is possible to demonstrate bioequivalence in a study with 12 subjects or less, when intra-subject variability is small. This Guideline does not specify the number of subjects to avoid an unnecessary increase in the sample size.

With regard to (3), the assessment method was introduced for the following reasons:

The aim of bioequivalence studies is to prevent approval and marketing of generic products that have less than 80% or more than 125% of the bioavailability of the innovator product

(assessed using the population means of logarithmic AUC and $C_{max}$). The Guideline uses the 90% confidence interval for the assessment of bioequivalence, which is currently accepted in Europe and the US. In the assessment using the 90% confidence intervals, the probability (level of consumer risk) for a low-quality generic product that does not satisfy the above-mentioned bioavailability requirements to pass a bioequivalence study, does not exceed 5%. Risk to consumers must be kept below 5%, even when assessment methods other than the 90% confidence interval are used. The extent of intra-subject variability in clearance varies depending on the medicinal products to be tested in the bioequivalence study. The assessment method using the confidence intervals is suitable for a bioequivalence study because the level of consumer risk is to remain constant without being affected by residual variability of the study. However, when $\sigma/\sqrt{n}$ is large, the actual risk to consumers decreases with assessment methods using the confidence intervals. As a result, the actual risk to the manufacturer (probability of a good product rejected by the study) increases (D. J. Schuirmann, A comparison of the two one-sided tests procedure and power approach for assessing the equivalence of average bioavailability, J. Pharmacokinet. Biopharm., 15, 657 (1987)).

Therefore, in order to demonstrate bioequivalence of a medicinal product with a high intra-subject variability in clearance (usually, a medicinal product which has a residual sum of squares of over 25% to 30% of CV), using assessment methods that depend on the 90% confidence intervals, an unfeasibly large number of subjects could be required. The Guideline provides the other assessment method (geometric mean ratio) for drugs of which bioequivalence is statistically difficult to demonstrate; the difficulty arises because of wide confidence intervals caused by high intra-subject variability in clearance. These products should be assessed to be bioequivalent when the geometric mean ratios of bioavailability (AUC and $C_{max}$) of the test

Table Relationship of the geometric mean ratio of bioavailability of test product to reference product ($\mu_t/\mu_r$), and the acceptable rates in human studies (number of total subjects is 20)

| | Acceptable rate | | | | | |
|---|---|---|---|---|---|---|
| $\mu_t / \mu_r$ | 1 | | 0.9 | | 0.8 | |
| Residual variation in logarithmic data[*1] | 90% confidence interval[*2] | Geometric mean ratio[*3] | 90% confidence interval[*2] | Geometric mean ratio[*3] | 90% confidence interval[*2] | Geometric mean ratio[*3] |
| 0.100(0.100) | 1.00 | 1.00 | 0.98 | 0.50 | 0.05 | 0.00 |
| 0.149(0.150) | 1.00 | 0.96 | 0.78 | 0.50 | 0.05 | 0.01 |
| 0.198(0.200) | 0.93 | 0.89 | 0.56 | 0.50 | 0.05 | 0.04 |
| 0.246(0.250) | 0.73 | 0.81 | 0.42 | 0.49 | 0.05 | 0.07 |
| 0.294(0.300) | | 0.73 | | 0.48 | < 0.05 | 0.11 |
| 0.385(0.400) | | 0.60 | | 0.45 | < 0.05 | 0.17 |
| 0.472(0.500) | | 0.51 | | 0.41 | < 0.05 | 0.20 |

[*1] Numbers in parentheses show coefficients of variation in data before logarithmic transformation. The relationship between variables for logarithmic normal distribution (x) and coefficients of variation (CV) is $CV^2 = \exp(\sigma r^2)-1$.

[*2] Assessment method using the 90% confidence intervals.

[*3] The assessment method used in the Guideline using geometric mean ratios of bioavailability.

product to the reference product are within the range of log 0.90 to log 1.11 on the condition that dissolution test results should indicates very low possibility of bio-inequivalence. The level of consumer risk depends on the variability when the sample size in human studies is constant, and when this assessment method is used (see Table above; at $\mu_t/\mu_r = 0.80$ in the table, the acceptable rates show the level of consumer risk). Therefore, this assessment method is not suitable for a bioequivalence study in which a high variability is anticipated. In this guideline, the paddle method at 50 and 75 rpm, and the basket method at 100 rpm are used for the comparison of dissolution characteristics between test and reference products. These methods are mildly destructive to formulations, and are highly capable of discriminating the differences in dissolution characteristics. With these methods, dissolution tests are carried out using 3 or more test fluids for immediate-release products, enteric-coated products, and 5 or more test fluids for extended-release products. Furthermore, tests with different stirring speeds are also carried out. Drug products showing similar or equivalent dissolution characteristics under all of these conditions may have very low possibility to be bio-inequivalent. Thus, it is expected that the actual level of consumer risk remains at 5% or less in the assessment method using the combined result of dissolution test and the human study. When the results of the dissolution test are used as supportive data for cases in which bioequivalence is difficult to demonstrate with human studies alone, similarity in the dissolution profile is required for immediate-release products and for enteric-coated products. For extended-release products, equivalence in the dissolution profile is required because content of active ingredient is expected to be larger than that of immediate-release products (refer to Q-63).

Q-2 Can data obtained in human bioequivalence studies that are carried out abroad be accepted?

(A) Accepting data from overseas clinical trials requires submission of references to evaluate the possibility for the extrapolation of such data to the Japanese population, as shown in “Handling of Data from Clinical Studies for Pharmaceuticals Conducted in Overseas,” Bureau-Notification No. 739 of the Pharmaceutical and Medical Safety Bureau, dated August 11, 1998, and “Ethnic Factors Considered to Accept Data from Overseas Clinical Studies,” Division-Notification No. 672 of the Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, dated August 11, 1998. Because factors such as ethnic differences in physiological features associated with the digestive tract, including the level of gastric acidity, may affect bioequivalence evaluation, confirmation that the ethnic differences do not affect bioequivalence evaluation is required. The results of human bioequivalence studies are used to evaluate the therapeutic effectiveness and safety of the medicinal products, and approval of a generic product is based on pharmacokinetic equivalence to the innovator product. Therefore, when the results of human bioequivalence studies conducted overseas are included as supporting data, the information related to bioavailability of such generic products must be sufficient to evaluate the possibility for the extrapolation of the study data to the Japanese population. It is preferable to include the results of studies conducted in Japan as supporting data.

Q-3 The scope of the Guideline according to Notification No. 487 of Pharmaceutical and Medical Safety Bureau, dated December 22, 1997 is “medicinal products as stipulated in (8) of Attached Table 2-(1) in Director-Notification No. 698 of the Pharmaceutical Affairs Bureau, dated May 30, 1980. Are medicinal products for dentistry and radiopharmaceuticals within the scope?

(A) The Guideline can be applied to all medicinal products that are categorized as generic drugs and that are required to undergo a bioequivalence study.

**For Each Section**

Section 3. Tests

A. Immediate-release products and enteric-coated products

I. Reference and test products

Q-4 According to the Guideline, the reference product should be selected from 3 lots of the innovator product. In exceptional cases, such as when it is difficult to obtain 3 lots of the innovator product, is it acceptable to select a reference product from 2 lots or less?

(A) It is acceptable to select reference product from less than 3 lots only if acceptable reasons are submitted for the difficulty in obtaining 3 lots of innovator product for the selection of reference product.

Q-5 Since “at least 1/10 of full production” is far larger than the quantity required for a bioequivalence study, a large amount of product will be discarded after the study. Can an applicant choose any lot size for a bioequivalence study, if the dissolution profile of the lot used for the bioequivalence study is confirmed to be equivalent to that of 1/10 or greater of full production?

(A) There is a risk of bioavailability changing in association with scale-up of drug product manufacturing. Scale-up of more than 10 times is undesirable in order to have equivalent quality to the product used in the bioequivalence study. The WHO* and EMA** stipulate that “The test product should usually originate from a batch of at least 1/10 of production scale or 100,000 units, whichever is greater” and their conditions are more strict than the conditions outlined in this Guideline. Therefore, “at least 1/10 of full production” should be ensured. This requirement is also for the purpose of international harmonization.

* See Q-1 for reference.

** European Medicines Agency, Guideline on the investigation of bioequivalence, 2010.

Q-6 When a bioequivalence study uses a lot that is not manufactured at the same scale as full production lots, is it acceptable to confirm equivalence of bioavailability between the full production lots and the lots used in the study using a dissolution test?

(A) The Guideline is intended to ensure that the bioavailability of the full production lots of the

test product is equivalent to that of the reference product. If a bioequivalence study is not performed using lots manufactured at the same scale as the production lots, equivalence of the quality and bioavailability between the production lots and the lots used in the bioequivalence study must be demonstrated. No extra test is required if the similarity or equivalence of the dissolution profile between the full production lots and the lots used in bioequivalence studies are confirmed using dissolution tests conducted with the appropriate procedures. In some cases, however, bioequivalence should be determined by a human study.

Q-7 Is it acceptable to select a test product to be used in the bioequivalence study by conducting a preliminary study (in humans) with several lots of the test product?

(A) The applicant may use any appropriate method for the selection of a test product.

II. Bioequivalence studies

1. Test methods

Q-8 What are “medicinal products with an extremely long elimination half-life”?

(A) Medicinal products with an extremely long elimination half-life are those products for which the value of $t_{max}$ plus 3 times the elimination half-life is 72 hours or more.

Q-9 If the number of subjects is too large and the study is difficult to conduct all at once, is it acceptable to divide the subjects into 2 groups, or to carry out the study in 2 separate facilities, and then to analyze the combined data?

(A) It is acceptable to regard a study in 2 separate groups as a single study, and to analyze the combined data from 2 separate groups, if the following conditions are fulfilled: (1) the study is planned to be conducted in 2 separate groups *a priori*. (2) the tests are conducted in both groups during the same time period; (3) the same protocol and analytical methods are used in both groups; and (4) the number of subjects is similar between the groups.

Q-10 If bioequivalence is demonstrated in the preliminary human study, are further human studies still necessary?

(A) As long as a study meets the requirements stipulated in the guideline, it is acceptable to use the preliminary study data as bioequivalence study data for assessment.

Q-11 Under which conditions can the data obtained in the add-on subject studies be analyzed with those of the initial study? Is it acceptable to use the data of the preliminary study as add-on subjects for the bioequivalence analysis?

(A) Unlike a clinical study for comparing the efficacy of different drugs, in a bioequivalence study, significant differences between the results from the initial study and the add-on subjects study is rarely observed when the 2 studies share a common protocol and similar sample sizes. Thus, it is

acceptable under this Guideline to conduct an add-on study. This is an exceptional measure for avoiding unnecessary human studies; therefore, it is not acceptable to conduct bioequivalence studies as sequential tests. Subjects should be added-on to the study only once. A study should be started with a sufficient number of subjects with the purpose to reach a conclusion from the single study. When the initial and add-on subject studies show no substantial differences in the test results (ratio of bioavailability between the 2 medicinal products) and in the distribution of residual errors, the results obtained from the combined data of the 2 studies can be used for analysis.

If an add-on study is planned for the situation where no conclusion is reached in the initial study because of an insufficient number of subjects, the plan should be defined in the protocol before starting the initial study. The use of data from a preliminary study as an add-on study must be defined in the protocol before conducting the initial study.

In a general add-on subject study, there may be an increase in the probability (α) of a type I error caused by multiple testing. However, this potential increase in the type I error may not be considered to be a significant issue for the following reasons: the probability of products that are bio-inequivalent showing a mean ratio of bioavailability within the margin of bioequivalence at the first stage, and entering the second stage, i.e. an add-on study, is unlikely to be in excess of 50%. The maximum contribution of an add-on study to α is 2.5%. Because α□ for highly variable drugs may be 5% or less, an increase in α□ in the add-on subjects study is of less concern (K.F. Karpinski; Ed. by I.J. McGilveray, et al., Proceedings Bio International '89, Issues in the evaluation of bioavailability data, October 1-4, 1989, Pharma Medica Research Inc., Toronto, Canada, p. 138 (1990)).

Q-12 The Guideline states that subjects should be healthy adult volunteers. What are the criteria for determining the subjects (for example age, sex, bodyweight, and gastric acidity)?

(A) As long as they are assessed to be healthy, any specific rules on age, sex, bodyweight, and the gastric acidity level of the subject are not stipulated.

Q-13 Please explain how to select subjects with low gastric acidity and the criteria for low gastric acidity.

(A) Gastric acidity can be estimated by several methods including measurements using a fiber pH meter inserted directly into the stomach and a pH measurement of gastric juice collected through an inserted gastric tube. In those measurements, lower and normal gastric acidity should be distinguished by following the rules for each test method or by using pH 5.5 as an identification index. Past investigations showed that the ratio of subjects with low gastric acidity increases with older subjects. People in their 20s have a low gastric acidity population rate of 10% or less. The studies also showed that subjects who have low gastric acidity have a high probability of maintaining the low gastric acidity.

The first priority is to conduct a bioequivalence study enrolling subjects with low gastric

acidity. Also, it is considered appropriate to conduct the study with healthy adult volunteers by co-administration of gastric acid reducers.

Q-14 Why are bioequivalence studies with subjects from a specific population or with subjects with low gastric acidity necessary? Please explain what is meant by "application of the medicinal product is limited to a specific population," and please provide some examples.

(A) The phrase "the application of the medicinal products is limited to a special population" means that the drug is frequently administered to populations of a certain age and sex, and that the special population includes both healthy subjects and patients. Possible differences in the factors affecting bioavailability between healthy volunteers recruited from a non-specific population and the subjects in the specific populations lead to different bioavailability of the drug products between the 2 populations. Therefore, if a significant difference between drug products is observed under more than 1 dissolution testing condition, the possibility of the difference in bioavailability in the specific population cannot be ruled out. Thus, a bioequivalence study must be conducted with the specific population receiving the medicinal products. However, it is not statistically correct to use data collected in a non-specific population to extract data on the specific population.

The studies in a population with low gastric acidity indicated that percentage of Japanese people with low gastric acidity is larger than that in people from Western countries. Therefore, if there is a significant difference in mean dissolution at pH 6.8, a bioequivalence study in subjects with low gastric acidity must be conducted. The following references present cases where the degree of differences in bioavailability of formulations differ between a group of subjects with normal gastric acidity and a group of subjects with low gastric acidity:

H. Ogata, et al., The bioavailability of diazepam uncoated tablets in humans. Part 2: Effect of gastric fluid acidity. Int. J. Clin. Pharmacol. Ther. Toxicol., 20, 166 (1982).

N. Aoyagi, et al., Bioavailability of sugar-coated tablets of thiamine disulfide in humans. I. Effect of gastric acidity and in vitro correlation. Chem. Pharm. Bull., 34, 281 (1986).

H. Ogata, et al., Bioavailability of metronidazole from sugar-coated tablets in humans. I. Effects of gastric acidity and correlation with in vitro dissolution rate. Int. J. Pharm., 23, 277 (1985).

Q-15 The older guideline stated that bioequivalence should be demonstrated in animal studies "when it is not recommended to use healthy volunteers because of strong pharmacological action or adverse effects." However, this Guideline specifies that, "a study should be conducted with a population receiving the medicinal products." Enrolling patients as subjects for such studies may raise an ethical issue. Please explain the reason for the change. Higher variability is expected in studies using patients (not healthy volunteers). Are there specific assessment criteria for such studies?

(A) Current research indicates that the results from bioequivalence studies using animals, such as

beagles, do not necessarily correlate with those from human bioequivalence studies. Some medicinal products with potent pharmacological actions or adverse reactions require especially strict assessment of equivalence. Therefore, bioequivalence has to be ensured by conducting such a study in humans, not in animals. The change was made based on the idea that medicinal products without demonstrated therapeutic equivalence should not be supplied for use in clinical practice. It is unlikely that an ethical issue should arise when a bioequivalence study with patients is conducted according to Good Clinical Practice. If participation in a bioequivalence study requires anything that could be detrimental to the patient, such as the discontinuation of a patient's regular treatment, the study should be conducted while continuing with the medicinal product or with their regular treatment in conjunction with the products investigated. Study of the administrating products could also be conducted at steady-state. The assessment criteria for studies enrolling patients are the same as those for the studies enrolling healthy volunteers.

Q-16 The Guideline states "If the clearance of drug differs to a large extent among subjects due to genetic polymorphism, subjects with higher clearance should be employed," in a bioequivalence study. What is the reason for the addition of this information? How is the extent of clearance evaluated? Which option should be selected from the following alternatives, when the data from the subjects with high clearance is used in the analysis: (1) conduct screening in advance on the subjects to determine the clearance; or (2) use data of the subjects with high clearance only?

(A) A study in subjects with high clearance can be conducted only when the presence of genetic polymorphism in the medicinal product is known either from published data or from accumulated data. The reason for recommending the removal of subjects with low clearance from studies is to ensure the safety of the subjects, and additionally because subjects with high clearance have higher sensitivity for detecting differences of bioavailability. The extent of clearance can be assessed with statistical outliers, and an estimation based on genetic information is not necessary. It is preferable to select subjects as stated in (1), but (2) is also acceptable. However, the protocol should state that, "Due to its genetic polymorphism, data from subjects with low clearance may be excluded" prior to initiating the study (2). The number of subjects may be insufficient for statistical analysis if data from subjects with especially low clearance are excluded as described in (2). In that case, an add-on study may be necessary.

Q-17 In the cases of "postprandial administration," is it acceptable to concurrently administer the products to all subjects, 50 minutes after starting the meal, on condition that the meal is finished within 20 minutes?

(A) It is important to administer the products 30 minutes after finishing a meal. Therefore, it is not acceptable to concurrently administer the products to all subjects 50 minutes after starting the meal.

Q-18 The Guideline states that studies of drugs with anticipated poor bioavailability in the fasting

state or with a high incidence of serious adverse events should be performed in the fed state. It is also indicated that if the dissolution rate of the test product is significantly different from that of the reference product, the study should be conducted in subjects with low gastric acidity or in a specific population receiving the medicinal product. In that case, is it acceptable to conduct a bioequivalence study in the fed state?

(A) The differences in bioavailability in immediate-release products tend to be smaller when administered postprandially, compared to the fasted state. Therefore, bioequivalence of medicinal products with dissolution rates significantly different from those of the reference product cannot be properly assessed if they are administered postprandially to subjects with lower gastric acidity or to the specific population.

Q-19 Which dosing conditions (single high dose or multiple doses) should be preferred when analytical issues occur, such as high detection limit?

(A) It is preferable to chose single high-dose administration rather than multiple-dose administration, in conditions where higher detection of differences in $C_{max}$ occur.

Q-20 In multiple-dose studies of a product in which administration is required 3 times daily, it is practically impossible to administer at constant intervals (for example, at 10:00 am, 6:00 pm, and 2:00 am) for a long period of time. What action should be taken in such cases?

(A) In principal, the test products should be administered at constant intervals. However, if it is impossible to follow the instruction, reference and test products may be administered at intervals in accordance with the Dosage and Dose regimen, from the initial administration to 2 days before the sampling of biological fluid. On the day before the sampling, the subjects should receive administration of products at the same hour in accordance with the Dosage and Dose regimen.

Q-21 In what cases could urine be used as a biological fluid for sampling?

(A) Urine can be used as a biological fluid when the unchanged drug or active metabolites are excreted in the urine, and these substances are measurable. If it is impossible to assess the maximum urine concentration ($U_{max}$) because of the problems associated with sampling intervals, assessment using urine samples is not appropriate.

Q-22 Why are inactive metabolites not appropriate as substances to be measured?

(A) The aim of a bioequivalence study is to guarantee therapeutic equivalence. Therefore, it is not appropriate to assess bioavailability with an inactive metabolite that is not involved in the therapeutic effect.

Q-23 The Guideline states that, in principle, the unchanged drug should be measured. Is it acceptable to measure the prodrug for assessment?

(A) If bioavailability of a prodrug in 2 formulations is equivalent, these 2 formulations are

bioequivalent. It is recommended to use a prodrug for assessment if the analysis of the prodrug is available. This is because a difference in bioavailability is generally easier to detect in prodrugs than in active metabolites. However, if the active metabolite is measured and used for the assessment, analysis of the prodrug data is unnecessary.

Q-24 Which is the more appropriate way to measure antibiotics: bioassays or instrumental analyses?

(A) In a bioequivalence study, in principle, the method for specific analysis of active chemical species should be used. Measuring the sum of multiple chemical species is not appropriate for evaluating bioequivalence. Antibiotics should preferably also be analyzed using a method with high specificity, such as instrumental analyses. However, the use of a bioassay will be accepted, if unavoidable.

Q-25 If an active metabolite exists in the both non-conjugate and conjugate forms, should equivalence assessment be conducted for the non-conjugate alone or for both?

(A) If the conjugate is inactive, bioequivalence should be assessed with the non-conjugate only. When both are active, either non-conjugates or conjugates that are scientifically adequate should be selected for the assessment. It is not appropriate to assess equivalence from combined measurements of the conjugate and non-conjugate.

Q-26 The Guideline states that, “for drugs consisting of a mixture of stereoisomers, an isomer largely contributing to the main pharmacological effect should be a substance to be measured.” What is the reason for this?

(A) In the development and approval of medicinal products, each isomer in the mixture is usually considered as a separate chemical compound. Therefore, in principle, the isomers should be measured separately, and the isomer with the greatest contribution to the main pharmacological effect should be regarded as a substance to be measured. Separate measurements are particularly essential in medicinal products that contain isomers and for which there is a possibility of substantial differences in the results of bioequivalence assessment between the isomers; these differences may be because of significant differences in pharmacokinetics between the isomers, such as the first pass effect and clearance. If no pharmacokinetic differences between the isomers have been reported for the active ingredient, it is acceptable to measure isomers together as the unchanged drug because a possibility that there is a difference in bioequivalence conclusion between the isomers is very low.

Q-27 Please describe the analytical validation in detail.

(A) In the analytical validation using biological samples, the following items should be primarily validated, and a summary of the following findings should be included in the results section of the bioequivalence study:

- Stability of substances to be measured in samples under the storage conditions (including freeze and thaw stability)
- Accuracy
- Precision (repeatability and intermediate precision)
- Specificity (to be investigated with samples collected from several individuals considering intra-subject variations)
- Study results related to the calibration curve
- Quantitation limit

The following criteria should be established before conducting a study, and routine analyses should be maintained. The results of routine analytical validation are not required to be included in the bioequivalence study report.

- Criteria for accepting analytical results
- Criteria for requiring reanalysis

Refer to the following literature for information on analytical validation:

V.P. Shah, et al.,Bioavailability, bioequivalence and pharmacokinetic studies. J. Pharm. Sci., 81, 309 (1992).

Naoko Kaniwa, Analytical validation of drugs. Hayashi Pure Chemicals Industry Co. Ltd. Osaka, 2003.

ISO 5725-6 Accuracy (trueness and precision) of measurement methods and results - part 6: Use in practice of accuracy values.

JIS Z 8402-6: Accuracy (trueness and precision) of measurement methods and results - part 6: Use in practice of accuracy values., Japanese Standards Association, Tokyo, 1999.

## 2. Assessment methods

Q-28 Which methods should be used for the calculation of the AUC?

(A) AUC is calculated by a trapezoidal rule as the area under the straight lines connecting the measured points.

Q-29 Please stipulate references to be used when determining relative absorption F using a deconvolution method.

(A) The following are the examples of relevant references:

D. P. Vaughan, and M. Dennis, Mathematical basis of point-area deconvolution method for determining in vivo input functions. J. Pharm. Sci., 67, 663 (1978).

K. Iga, et al., Estimation of drug absorption rates using a deconvolution method with nonequal sampling times. J. Pharmacokinet. Biopharm., 14, 213 (1986).

D. Verotta, An Inequality-constrained least-squares deconvolution method. J. Pharmacokinet. Biopharm., 17, 269 (1989).

Q-30 What is the significance of submitting reference parameters?

(A) AUC and $C_{max}$ may not always be sufficient to assess bioequivalence. In the Guideline, $t_{max}$ is considered a reference parameter, not an assessment parameter, because of its small statistical power. However, when significant differences appear in the reference parameter, the medicinal product cannot be unconditionally regarded as bioequivalent even if the AUC and $C_{max}$ are equivalent. If a significant difference in the elimination rate constant is detected during hypothesis testing, it is implied that the measured slope in the elimination phase indicates the absorption rate constant, not the elimination rate constant, suggesting the difference in the absorption rates between the formulations. Thus, in this regard, submission of reference parameters is required. In the case that a statistically significant difference is detected, submission of an acceptable rationale is required to explain whether or not the difference is therapeutically negligible. Reference parameters used for analysis should be selected depending on the characteristics of the medicinal product. For example, for extended-release products, the use of parameters with which variance residence time (VRT) or fluctuation range of blood concentrations can be assessed is considered. Also, if there is the potential that the variability in the action onset time could affect the clinical effectiveness of medicinal product, $t_{max}$ can be adopted as a reference parameter for equivalence assessment.

Q-31 Is mean residence time (MRT) necessary as a reference parameter?

(A) For pharmaceutical substances with a low elimination rate constant, MRT has little ability to detect differences in the rates of bioavailability between formulations. For pharmaceutical substances with a high elimination rate constant, in contrast, MRT is a good parameter to identify the differences in the rates between formulations, and it also has strong statistical power. $T_{max}$ is a highly sensitive parameter to detect the variability in rates of bioavailability between formulations, but it has been pointed out that this parameter has low statistical power. Therefore, MRT is useful as a reference parameter to compliment $t_{max}$.

Q-32 Is logarithmic transformation always necessary? Is it acceptable to carry out logarithmic transformation only if necessary?

(A) Based on the principle of international harmonization, assessment should be made using logarithmically transformed values. However, when it is not appropriate to conduct an analysis with transformed data, assessment can be made with untransformed data. For example, the assessment can be made using untransformed data if the parameters are normally distributed or transformed data can be used with non-normally distributed data.

Q-33 How can logarithmic transformation be applied if the value 0 is included in the parameter of a subject?

(A) For logarithmic transformation of data, excluding the subjects with a value of 0 for the parameter is not preferred because some of the information is excluded. Therefore,

untransformed data that includes the subjects with the value 0 for the parameter should also be analyzed.

Q-34 Please explain how to assess bioequivalence using untransformed data.

(A) The acceptable range of bioequivalence using untransformed data is -0.20 to +0.20 as the ratios of the population means of the parameters for the test and reference products. Therefore, if the 90% confidence interval of the difference in the means of the parameters to be assessed between the test and reference products is within the acceptable range of -0.20m to +0.20m (m is the mean of the reference product), the test product is assessed to be bioequivalent. When the outcomes of dissolution tests exhibit similarity or equivalence between the reference and test products, they are assessed as bioequivalent if the differences in the mean values of the parameters are between -0.10m and +0.10m.

Q-35 Please provide references on sample size design, multiple-dose studies, and studies wherein stable isotopes are administered simultaneously. The Guideline states that a statistical analysis may be conducted using methods, other than those described in the Guideline, if an acceptable rationale is presented. Please show examples of acceptable analytical methods.

(A) (1) Sample size

Determining the sample size can be accomplished by estimating the extent of individual variation for a medicinal product using published data and the results of preliminary studies, or by determining the subject number by using the method described below. The references listed below are related to the determination of the sample size from an assessment applying 2 one-sided tests with untransformed data, which gives exactly the same results as those using the 90% confidence intervals. The references regarding the application of 2 one-sided tests to a bioequivalent study are also listed below.

[On logarithmically transformed data] E. Diletti, et al., Sample size determination for bioequivalence assessment by means of confidence intervals, Int. J. Clin. Pharmacol. Ther. Toxicol., 30 Suppl. 1,S51 - 58 (1992).

[On untransformed data] K.F. Phillips, Power of the two one-sided tests procedure in bioequivalence. J. Pharmacokinet. Biopharm., 18, 137 (1990).

[On the application of two one-sided tests to a bioequivalence study] D.J. Schuirmann, A comparison of the two one-sided tests procedure and power approach for assessing the equivalence of average bioavailability, J. Pharmacokinet. Biopharm., 15, 657 (1987).

(2) The following report outlines the benefits of multiple dose studies:

el-Tahtawy AA, Jackson AJ, Ludden TM, Comparison of single and multiple dose pharmacokinetics using clinical bioequivalence data and Monte Carlo simulations, Pharm. Res., 11, 1330-1336 (1994).

(3) The following report outlines the benefits of studies wherein stable isotopes are administered simultaneously:

Heck HA, Buttrill SE Jr, Flynn NW, Dyer RL, Anbar M, Cairns T, Dighe S, Cabana BE, Bioavailability of imipramine tablets relative to a stable isotope-labeled internal standard: increasing the power or bioavailability tests, J. Pharmacokinet. Biopharm., 7, 233-248 (1979).

(4) Analytical methods other than those shown in this Guideline: Nonparametric procedure: If a parameter is not normally distributed, the 90% confidence interval obtained from the non-parametric procedure may be used for the assessment. Reference material is cited below.

V. W. Steinijans and E. Diletti, Statistical Analysis of Bioavailability Studies: Parametric and Nonparametric Confidence Intervals, Eur. J. Clin. Pharmacol., 24, 127 (1983).

Parallel group comparative studies: For medicinal products with an extremely long elimination half-life, parallel-group comparative studies may be carried out rather than cross-over studies. Analytical methods should follow the conventional one-way layout study design.

Q-36 Please indicate the acceptance criteria for bioequivalence when analysis is implemented using a nonparametric procedure and 2 one-sided tests.

(A) In assessing bioequivalence using the confidence intervals determined from a non-parametric procedure, the same criteria as those given in the Guideline for a parametric procedure should be applied when logarithmically transformed data are used. When using untransformed data, the acceptance criteria shown in Q-34 should be followed.

The null hypothesis and alternative hypothesis of 2 one-sided tests are as follows:

$H_0$: $\mu \leq \theta_1$, $\mu \geq \theta_2$

$H_1$: $\theta_1 < \mu < \theta_2$

In the logarithmically transformed data, $\mu$ is log ($\mu_t/\mu_r$), $\theta_1 = \log 0.80$, and $\theta_2 = \log 1.25$. In the untransformed data, $\mu$ is $(\mu_t-\mu_r)/\mu r$, $\theta_1 = -0.20$, and $\theta_2 = +0.20$. $\mu_t$ and $\mu_r$ represent the population mean of the parameter used for assessment of bioequivalence of test and reference products, respectively. When the 2 null hypotheses above are rejected at the 5% significance level, the 2 formulations can be assessed as bioequivalent.

Q-37 If there is a significant carry-over effect, is it necessary to redo the study?

(A) In general, the group effect and the carry-over effect are indistinguishable in a 2-period, 2-sequence, cross-over study. If the carry-over effect is significant, it is not possible to interpret the results. However, if the group effect is significant, it is possible to interpret the results. The data obtained in 2-period, 2-sequence, cross-over studies were previously accepted if the applicant concluded that the significant difference was caused by the group effect and not by the carry-over effect, because that indicated a bias in group allocation. However, the presence of allocation bias is generally difficult to prove, and in a bioequivalence study comparing bioavailability of the same drugs, the incidence of carry-over effects is rarely expected as long as the protocol is followed. Therefore, the Guideline does not require a discussion of carry-over

effects.

Q-38 Is it acceptable to assess bioequivalence using symmetrical confidence intervals?

(A) The Guideline's criteria for assessing bioequivalence by using the 90% (shortest and unsymmetrical) confidence intervals or 2 one-sided tests ($\alpha = 0.05$) are designed so that medicinal products with 80% or 120% bioavailability of the reference product will have a 95% probability of rejection. This means that the consumer risk is 5%. Consumer risk should also be 5% or less when methods other than those in the Guideline are used for assessment. Therefore, in order to satisfy the requirement, the confidence coefficient must be 95% when symmetrical confidence intervals are applied. It is acceptable to use this procedure, because the risk to the manufacturer increases when symmetrical confidence intervals are used as compared to the use of the shortest non-symmetrical confidence intervals. However, the use of symmetrical confidence intervals will offer little advantage (V.W. Steinijans and D. Hauschke, Update on the statistical analysis of bioequiavelence studies, Int. J. Clin. Pharamcol. Ther. Toxicol., 30, 543 (1992).

Q-39 Please provide examples of "drugs with mild actions."

(A) For each example, the applicant is to provide scientific grounds, based on its characteristics, as to why the medicinal product was determined to have mild pharmacological actions. The acceptable criteria should be established before commencing the study.

Q-40 Is it acceptable to use the non-parametric test method for $t_{max}$?

(A) Yes, it is acceptable. When $t_{max}$ is a reference parameter, it is acceptable to show no significant difference in $t_{max}$ between the reference and test products by using nonparametric tests. When the $t_{max}$ is a parameter to be assessed, if there is a possibility that the difference in $t_{max}$ between the reference and test products affects the clinical effectiveness, there should be preliminary establishment of appropriate bioequivalence criteria and the 90% confidence interval for the difference in $t_{max}$ should be calculated and assessed (see the reference in Q-35 4)).

III. Pharmacodynamic studies

Q-41 Is it acceptable to demonstrate bioequivalence in pharmacodynamic studies in animals for drugs that have a direct action on the gastrointestinal tract or that have effects and functions inside the tract, such as laxatives, anti-diarrheal agents, contrasts, adsorbents, mucous membrane-protecting agents, and anti-flatulents?

(A) Animal studies may be accepted on a case-by-case basis for the above-mentioned medicinal products that satisfy when the active ingredient does not reach the site of action via the systemic circulation and when the pharmacological action is mild; animal studies in these products are considered to be scientifically appropriate based on published research and other

information. However, this exception is limited to cases in which similarity or equivalence in dissolution between the test and reference products is demonstrated, or if a dissolution test is impossible, to the cases in which a physicochemical test demonstrates similarity or equivalence between the properties of both products. In the latter case, the acceptable range of similarity or equivalence should be established appropriately based on the test characteristics. When bioequivalence is demonstrated in animal studies, the relationship between dose and pharmacological effects should be studied in order to set the dose, and bioequivalence should be assessed using methods similar to those in the human studies.

V. Dissolution test

Q-42 If bioequivalence of the medicinal products can be demonstrated in human studies but the results from a dissolution test do not meet the Guideline's requirement, can those products be considered "bioequivalent"? Or should results of dissolution tests be considered as supportive data when it is difficult to demonstrate bioequivalence by human studies alone?

(A) For immediate-release products, dissolution tests provide (1) information on subject selection and (2) supportive data when bioequivalence in medicinal products with high variability in pharmacokinetic parameters is difficult to prove in human studies alone. Therefore, if bioequivalence of immediate-release products is demonstrated in a human study, the products are assessed to be bioequivalent irrespective of the dissolution test results.

In contrast, for extended-release products, a generic product would not be approved without proving a similar release mechanism as for the innovator product by the demonstration of a similar dissolution profile. .

The "dissolution profile," as discussed above, is the dissolution time course of the measured ingredient. When the apparent decrease in dissolved measured ingredients occurs by degradation, reaction, or precipitation, the dissolution profiles should be compared during the time course up to the maximum solubility. The Guideline stresses the results of a dissolution test because they have the following advantages:

(1) Some relationship may be observed between physiological factors affecting patients' gastrointestinal tracts and the bioavailability of the medicinal products. The relationship between gastric acidity of a patient and bioavailability is a well-known example. The detection of relationships in a bioequivalence study depends on the ratio of the subjects that exhibit relationships to the particular formulation. By using dissolution tests, it is possible to detect the relationships that are observed in particular combinations of patients and formulations by selecting appropriate test solutions.

(2) Even when bioequivalence between reference and test products is demonstrated in a bioequivalence study, it is important to explore the reason if similarity or equivalence in dissolution is not shown in dissolution tests. For example, the justification for the

dissolution conditions used in the tests should be reviewed. For dosage forms for which official dissolution specifications are published with the progression of the quality re-evaluation program, approval reviews will be implemented taking into consideration the setting of the dissolution tests, after evaluating data on suitability of the official dissolution specifications (Bureau-Notification No. 634 of the Pharmaceutical and Medical Safety Bureau, dated July 15, 1998).

Q-43 Please indicate analytical validation for dissolution testing and for assays used in dissolution tests.

(A) The purpose of analytical validation is to scientifically assure the validity of the test method and the repeatability of data. The dissolution tests should conform to the rules in the Japanese Pharmacopeia (JP), and the suitability of the testing apparatus should be confirmed regularly. It is also useful to apply the calibrators used in the United States Pharmacopeia (USP). The stability of the drugs in the test solution and the validity of the test method using automatic sampling should be confirmed.

The following items are usually examined in analytical validation (Preliminary dissolution tests done to select conditions for dissolution test, such as pH of dissolution fluids or surfactant concentration, are out of the scope):

Accuracy (recovery rate is acceptable)
Precision (repeatability and intermediate precision)
Specificity
Linearity
Range

References for analytical validation:

Division-Notification No. 338 of the Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, dated October 28, 1997.

Division-Notification No. 775 of the Pharmaceutical and Medical Safety Bureau, dated July 20, 1995.

Naoko Kaniwa, Analytical validation of drugs, Hayashi Pure Chemical IND. Ltd., Osaka, 2003.

Q-44 Is it acceptable to regard the medicinal products that are administered in a completely dissolved state as a medicinal product whose reference and test products dissolve not less than 85% within 15 minutes?

(A) When the reference and test products dissolve completely in a certain test solution, it will be regarded as a medicinal product whose reference and test products dissolve not less than 85% within 15 minutes under the test conditions.

Q-45 Is it necessary to conduct dissolution tests for oral dosage forms whose active ingredients do

not reach the active site via systemic circulation such as laxatives, anti-diarrheal agents, contrasts, adsorbents, mucous membrane-protecting agents, anti-flatulents, and digestive enzyme formulations?

(A) For selecting the subjects and the reference product when the active ingredient dissolves, dissolution tests need to be carried out as physicochemical studies. If the active ingredient does not dissolve, appropriate physicochemical tests such as disintegration tests should be performed.

Q-46 Please explain the rationale for setting the pH of the dissolution testing solutions.

(A) The pH levels were set based on the physiological pH range in the gastrointestinal tract to assist in discriminating differences in the dissolution profiles between the products.

Q-47 How should preliminary dissolution tests be conducted to set the test conditions, such as pH and surfactant concentrations, for dissolution test fluids? Does the study require tests using 12 vessels or more? The pH of the second fluid for the dissolution test in the Japanese Pharmacopeia (JP 16), prepared by diluting the "Phosphate buffer solution, pH 6.8 listed in the Section of Reagents and Solutions in the JP" twice with water, is approximately pH 6.9. Is there any problem with using this solution as it is?

(A) One option is to perform a dissolution test for 3 lots of the innovator product in several test solutions at intervals of pH 0.5–1.0 in the pH range near which the dissolution rate starts decreasing, under the condition that average dissolution reaches 85% within the specified time based on drug solubility. Then, determine the pH from the dissolution profiles of the reference product lots considering the dissolution profiles under other conditions for the dissolution test. The pH where drug solubility is the maximum can be used as a condition of a dissolution test if none of the average dissolutions of the 3 lots (or the lot in the case that it was selected as a reference product in the specification dissolution testing solution, the lot instead of the 3 lots in the case below) reached 85% within the specified time at the pH . For certain pharmaceutical products with high solubility if the average dissolution of the 3 lots reached 85% within 15 minutes in the designated pH range, the pH at which the drug is least soluble can be used as a condition of a dissolution test. The surfactant concentration should be selected based on the active ingredient solubility in a designated concentration of polysorbate 80 solution, and then set in a similar manner to the above pH settings.

The numbers of dissolution tests (12 vessels, and 6 or more vessels) specified in the Guideline are applied to the dissolution comparison tests and selection of reference products, respectively. Therefore, the number of tests (vessels) in the preliminary dissolution test to set the test conditions is not specified. A preliminary dissolution test to set the pH is not required when an appropriate pH can be determined based on pH-solubility profile. However, the pH-solubility profile of the active ingredient does not always have a relationship with the dissolution rate of the product. The results of the preliminary dissolution tests conducted to set the conditions may be included in the results of the dissolution comparison tests.

The dissolution testing method in the JP states that “If the dissolution medium is a buffer solution, adjust the solution so that its pH is within 0.05 units of the specified pH.” However, “Phosphate buffer solution, pH 6.8” in the JP is not a buffered solution with a specified pH*. Therefore, the 2nd fluid for dissolution test mentioned in the JP (prepared by diluting “Phosphate buffer solution, pH 6.8" twice with water) should be used as it is. The range of actual pH values of the second fluid is 6.92 ± 0.05, and the use of the solution in this range is recommended.

* “Phosphate buffer solution, pH 6.8” is the name of a solution; its pH is not adjusted at preparation and then is not specified.

Q-48 Is it acceptable to select a Diluted McIlvaine buffer (pH 4.0) as the dissolution test fluid, based on the solubility of the active ingredient and the properties of the excipients, by explaining that the dissolution profile does not change largely in the pH range of 3.0 to 5.0?

(A) In determining the pH of Diluted McIlvaine buffers, it is possible to determine the pH by appropriate scientific reasoning based on the solubility of the active ingredients and the properties of the excipients. For example, in the case where a drug substance has high solubility in buffers of all pHs, and similar dissolution of the products is observed at pH 1.2, at pH 6.8, and in water (immediate-release products of which dissolution is independent of pH), the pH of Diluted McIlvaine buffers where solubility of drug substance is the lowest can be selected. However, when the solubility of a drug substance is considered to have almost no effect on the dissolution in the pH range, the intermediate value of the range, for example pH 4.0, can be selected.

Q-49 Is it necessary to specify the types of buffer and the rotation speed in dissolution tests?

(A) For oral dosage forms where the active ingredients reach the active site via the systemic circulation, results of dissolution tests are used for (1) selection of reference products, (2) selection of subjects for bioequivalence studies, and (3) judgment of bioequivalence in the case that it is difficult to judge bioequivalence by human studies alone. In the Guideline, the conditions of dissolution tests are not established from the point of *in vitro/in vivo* correlations, but are established so that relative differences in dissolution profiles between formulations are shown clearly. The Guideline uses the idea that bioequivalence in humans is strongly supported if similarity or equivalence in the dissolution profile is assessed under these conditions. Therefore, the Guideline allows only specified conditions, for the assessment of similarity or equivalence in dissolution profiles, rather than options for applicants. However, it is reasonable that other buffers at the same pH can be used instead of the stipulated buffer, when it is scientifically confirmed that the dissolution profile of the reference or the test product is affected by the components of the stipulated buffers (for example, when interaction with components of McIlvaine buffers hinders dissolution of the active ingredient). For immediate-release and enteric-coated products, dissolution similarity should be assessed under the conditions in which 85% or more of the drug dissolves within 6 hours. Therefore, a greater number of conditions for

assessment results in stronger support for bioequivalence.

Q-50 Water has low ionic strength and weak buffer action. Therefore, the dissolution profile of the reference and the test products can differ largely only in water, and can be affected by properties of the drug substance or medicinal product. In such cases, is it acceptable to evaluate the dissolution profile with the dissolution test results, excluding the results in water?

(A) When a human bioequivalence study is performed according to the Guideline for Bioequivalence Studies of Generic Products, the dissolution profile can be evaluated with the results, except for the ones in water, by demonstrating scientific reasons (e.g. adsorption of drug substance and excipient) for the difference in the dissolution profile of the reference and test products only in water. Dissolution tests in water should be conducted.

Q-51 If the pharmaceutical substance is amphoteric, which buffer condition is appropriate for the dissolution test, for products containing acid drugs and for basic drugs?

(A) It is important to compare dissolution profiles under many conditions in which differences in the dissolution rate between formulations can be detected. Therefore, test conditions should be chosen to allow more dissolution tests at various pH values to be carried out (where more than 85% is dissolved within a specified testing time), as determined from the pH-solubility profile of the drug. First, perform a test under the conditions as described in (2) Products containing neutral or basic drugs and coated products. If no, or only one, implementable condition for a dissolution test is found, the test should be performed under the conditions for (1) Products containing acidic drugs. The appropriate dissolution testing conditions for the dosage form should then be selected.

Q-52 The Guideline requires the coated products containing acidic drugs to be tested using the conditions of the dissolution test for “Products containing neutral or basic drugs, and coated products.” Some coating membranes, however, can dissolve at a neutral pH range. Is it acceptable to conduct a dissolution test for coated products under the conditions for "Products containing acidic drugs”?

(A) Some film-coating membranes have characteristics that they are soluble in neutral pH but only slightly soluble at around pH 3 to 5. If tests are conducted under the conditions for products containing acidic drugs, these characteristics may not be investigated. Therefore, conducting the dissolution test under the conditions for coated products is preferable for these dosage forms. If conducting a dissolution test is difficult because of low solubility of acidic drugs at approximately pH 3 to 5, the test may be conducted under the conditions for acidic drugs.

Q-53 Why is the paddle method primarily used in the Guideline?

(A) The paddle method is used because of the simplicity in implementing this method, because its results are highly reproducible, and because there is a large amount of previously reported data using this method.

Q-54 Is there a referred alternate dissolution method to address the following: “when a phenomenon that disintegrants deposit in the bottom of vessel or paddle is observed, the paddle method at 75 rpm or the rotating basket method at 100 rpm can be used instead of the paddle method at 50 rpm”? Also, is it necessary to compare the dissolution profiles in the paddle method at 50 rpm?

(A) Either the paddle method at 75 rpm or the rotating basket method at 100 rpm can be selected arbitrarily. Comparison of dissolution using the paddle method at 50 rpm should be performed to show the dissolution profile in that condition. “…[A] phenomenon that disintegrants deposit in the bottom of vessel or paddle” may be objectively demonstrated, for example, with photographs.

Q-55 When the paddle method at 75 rpm or the rotating basket method at 100 rpm is used instead of the paddle method at 50 rpm, which “Significant difference in dissolution” should be evaluated?

(A) The “significant difference in dissolution” should be evaluated in the dissolution condition where the dissolution profile is evaluated.

Q-56 In the case that active ingredients adsorb to the vessel or paddle, is it acceptable to use the vessel or paddle to which the active ingredient adsorb the least?

(A) The Japanese Pharmacopoeia does not stipulate the materials used to construct the vessel and paddle, therefore vessels and paddles made of appropriate materials can be used.

Q-57 When formulations float on the dissolution testing solution, is it acceptable to use sinkers?

(A) When formulations float on the dissolution testing solution, sinkers can be used. In this case, the sinkers should be used for both reference and test products.

Q-58 What is the significance in adding surfactants in the dissolution test of low solubility drugs?

(A) Comparing dissolution rates of products containing low solubility drugs is difficult because those products reach their saturated solubility at a lower dissolution rate. Surfactants are added in the dissolution tests of those products in order to compare dissolution rates between the products by increasing the drug solubility. Polysorbate 80 is recommended as the first choice to examine the effect of surfactant.

Q-59 Please indicate the acceptable range of values when the average dissolutions are compared in the assessment of similarity and equivalence in the dissolution profiles. For example, the Guideline states that, “the average dissolution rate of the test drug product is within the range of the average dissolution rate of the reference drug product ± 15%” Does the “±15%” indicate the relative or absolute value of the difference in the dissolution rates?

(A) The acceptable criteria (±15%) indicates the absolute value of the difference in the average dissolution rate of the test and reference product. For example, the Guideline states, “the average dissolved amount of test products does not deviate by more than 15% from that of the reference

product at two time points when the average dissolved amount of the reference product is around 60% and 85%," in determining dissolution similarity in immediate-release products and enteric-coated products. For a reference product that has average dissolution rates of 63% and 87%, the acceptable range of the test product would be 48% to 78% and 72% to 102%, respectively. For determining dissolution equivalence between extended-release products, the Guideline also states, "When the average dissolution of the reference products reaches between 50% and does not reach 85% within the testing time specified: the average dissolution of the test product are within that of the reference product ±8% at the testing time specified and at an appropriate time point when the average dissolution of the reference product reaches about a half of the average dissolution at the testing time specified." For a reference product that has average dissolution rates of 73% at the specified testing time and 35% at the time specified for the half of the average dissolution, the acceptable range of the test product would be 65% to 81% and 27% to 43% respectively.

Q-60 Why are some of the dissolution rate sampling times for calculation of the similarity factor (f2) in this Guideline different from those in the US SUPAC (Scale-up and Post-Approval Changes) guidance?

(A) The value of the f2 function depends on the time point at which the dissolution rates are compared. For example, f2 values become larger if the number of comparison points increases at the point at which the difference in the dissolution rates is small in the dissolution curve. The time for comparison is specified in the Guideline in order to avoid such errors. It is acceptable to set the comparison time points that are appropriate to implement dissolution tests that satisfy the dissolution rates specified for reference products, rather than the exact time that exhibits the specified dissolution rates when either comparing the mean value or applying the f2 calculation.

Q-61 The Guideline states that, "If dissolution of the reference product or test product has a lag time, the dissolution curve can be adjusted with the dissolution lag time." Is it acceptable to compare dissolution profiles without the adjustment even when there are lag times? Please explain how to adjust dissolution curves in dissolution tests with lag times.

(A) Adjustment with lag times is not always needed for comparing dissolution rates. Refer to Appendix A for the methods to adjust dissolution profiles with lag times.

## VI. Reporting of bioequivalence study results

Q-62 Items (6) to (9) such as solubility, particle size, and crystal form, are generally published by the innovator product manufacturers. Is it necessary to submit these items?

(A) A formulation design needs to be conducted with full knowledge of those physicochemical characteristics. Therefore, these items for the generic products should be investigated and reported, as much as possible.

Q-63 Why is the narrower criterion, the dissolution equivalence, employed for extended-release products to compare dissolution similarity for immediate-release products and also applied when determining bioequivalence when it is difficult to judge bioequivalence by human studies alone?

(A) Extended-release products usually contain larger amounts of active ingredients compared to immediate-release products because they have a longer dose interval. They may also remain for a longer time in the gastrointestinal tract. In addition, extended-release products have functions that control the release of the active ingredients. In order to ensure safety and assess function, the similarity criterion for dissolution profiles of extended-release products is stricter than that for immediate-release products.

Q-64 Should physicochemical studies of the drug substance be used for a generic product, based on disclosed information on the drug substance used in the innovator product? For example, should the same measurement methods for items such as particle size be used? If information on the innovator product is not available, are those data required for the generic product?

(A) Any method for physicochemical measurement can be used as long as the method is regarded as scientifically appropriate. However, the methods and devices used in the measurement, and the measured values, must be reported. Regardless of the availability of information on the innovator product, the required information on the drug substance used in the generic product should be reported.

Q-65 How should the time points used to determine the elimination rate constant ($k_{el}$) be represented? Is it acceptable to calculate $k_{el}$ from mean blood concentrations?

(A) The data should be represented in a table, or the points can be marked on individual subject blood concentration–time profiles because the individual profile should be attached. It is important to know mean and standard deviation of $k_{el}$ and thus it is not acceptable to calculate $k_{el}$ from the mean blood concentration curve.

Q-66 Are the items in “VI. Reporting of test results” those to be reported in the application form (E-5-1)? Are these reporting items also required for the clinical study report? Please explain how to relate “the Guideline for Bioequivalence Studies for Generic Products” with “the Guideline of structure and Contents for Clinical Study Report” when the clinical study report is attached to the application documents.

(A) The items should be those included in the (E-5) “Bioequivalence” part of the documentation, and should be submitted when applying for manufacturing/marketing approval of medicinal product for ethical use. The report of the items listed in “the Guideline for Bioequivalence Studies of Generic Products” should be prepared and should refer to “Structure and Content of Clinical Study Reports,” Director-Notification No. 335 of the Pharmaceutical Affairs Bureau, dated May 1, 1996.

B. Oral extended-release products

I. Reference and test products

Q-67 The Guideline states that in oral extended-release products, the size, shape, specific gravity, and release mechanism of generic products should not differ markedly from those of the innovator products. What is the reason for imposing these conditions?

(A) Unlike immediate-release products, extended-release products often transit through the digestive tract retaining their original shape for relatively long periods of time. Bioavailability of formulations with different shapes, sizes, specific gravity, and release mechanisms tend to vary depending on the subject and administration conditions because the properties of these formulations are susceptible to different physiological factors in the digestive tract. Therefore, generic oral extended-release products are required to have the same release mechanisms as the innovator product. The similarity of release mechanisms should be explained by distinguishing the formulation characteristics: whether they use a matrix system or a controlling membrane, a single unit or multiple units, and disintegrating or non-disintegrating types.

Q-68 Unlike immediate-release products, it is a prerequisite for the initiation of a bioequivalence study that the dissolution profile of extended-release test products is similar to that of the reference products. What is the reason for this?

(A) It is possible, under the diverse physiological conditions of the digestive tract, that 2 formulations with different release mechanisms may have different movement and/or release within the digestive tract. In human studies, bioequivalence is assessed only under fasting and certain fixed conditions, which does not always ensure bioequivalence under other conditions. Formulations with similar release mechanisms are expected to show similarity in movement and in releasing performance in the gastrointestinal tract, even under diverse physiological conditions. Therefore, as a prerequisite for conducting bioequivalence studies using extended-release formulations, it must be demonstrated that the formulations have the same control release mechanism. This condition must be met as a proof that the release mechanism of a test product is not different from that of a reference product. If comparison of dissolution profiles is not possible because of limited active ingredient solubility in any of the specified dissolution test solutions, other information is required to explain that the release mechanism of the test product does not differ from that of the reference product.

II. Bioequivalence studies

1. Test methods

Q-69 Why should bioequivalence be assessed in fasted and fed states?

(A) Extended-release products usually contain higher doses compared to immediate-release

products, and their releasing performance is guaranteed by the special releasing control mechanisms. Therefore, it is important to confirm that the test and reference product mechanisms work equivalently in both the fasting state and in the fed state, which is the more severe condition. The tests should be performed with a high fat diet to mimic the most severe conditions.

Q-70 What is the reason for the product to be administered 10 minutes after a high-fat diet but 30 minutes after a low-fat diet?

(A) Administration in the fed state is conducted in order to confirm that bioavailability of the product does not relatively change between formulations because of a meal. To investigate the effect of a meal on bioavailability, a shorter interval between the meal and administration is an optimal condition. Therefore, for a high-fat diet, it has been decided that the products are to be administered 10 minutes after the meal. When studies in the fasted state are difficult to implement, the products are to be administrated 30 minutes after eating a low-fat meal to minimize the effect of the meal.

Q-71 The paddle method at 200 rpm or the method using the disintegration testing apparatus is quite severe. Why are these methods used?

(A) Dissolution tests are used to demonstrate that the release-controlling mechanisms between formulations are the same and to assess their bioequivalence as supportive data. Therefore, if the dissolution profiles of the products under certain severe conditions are the same, it is possible to infer, in some cases, that functions of the products would be similar under severe conditions within the body.

C. Non-oral dosage forms

Q-72 In non-oral dosage products, the Guideline states that a dissolution (release) test or alternative physicochemical tests should be performed. What sort of physicochemical tests are required?

(A) Possible examples of physicochemical tests include release tests for suppositories and dissolution tests for aqueous suspensions for injections.

D. Dosage forms for which bioequivalence studies are waived

Q-73 Should the bioequivalence studies in the Guideline be performed even for solutions for subcutaneous or intramuscular injection where special excipients are not used?

(A) Bioequivalence studies should be performed for such medicinal products according to the Guideline because sufficient information on the effect of excipients and on the absorption rates of subcutaneous or intramuscular injections are not available currently.

Q-74 Can bioequivalence studies of “Injections for arterial administration, administered as an

aqueous solution" and "Injections for intraspinal administration, administered as an aqueous solution" be waived?

(A) Bioequivalence studies of medicinal products such as arterial injections and intraspinal or epidural injections are not waived. These medicinal products, different from intravenous injections, are categorized as a product for topical use that are applied directly on, or near to, the targeted tissues. Bioequivalence of these medicinal products should be assessed on the basis of the clinical studies specified in the Guideline, Section C.III.

## Appendix A: Adjusting Dissolution Curves with Lag Times

The dissolution curve with a lag time is adjusted according to the steps below. If adjustment of the dissolution curve or calculation of dissolution rates by interpolation is anticipated before initiating the study, the frequency of measurement should be arranged such that the rates can be measured at intervals of about 5 minutes, or at intervals of about 10% in the dissolution rate to avoid increasing the errors caused by interpolation.

Lag times of the individual reference and test products are determined using the following steps:

1. Predict the time interval in which a lag time ($t_L$) appears by obtaining the entire profile of the dissolution rate–time curve in the preliminary test. Select measurement points at small intervals before and after the time interval, and obtain the curve by connecting the points with a line. Determine the time ($t_L$) at which the dissolution rate of 5% is obtained by reading the curve (or graph) or by interpolation. The time obtained in these methods is defined as "lag time."
2. Calculate adjusted measurement times by adjusting measurement times for lag times for each medicinal product to obtain a dissolution curve with adjusted measurement times.
3. Obtain the average dissolution curve of the reference and test products as follows:
4. Determine the time needed to obtain an average dissolution curve ($t_{si}$). The number of measurement points should be almost the same as the number of points after the lag time in the unadjusted dissolution curve. The dissolution rates at $t_{si}$ of the reference and test medicinal products are determined by interpolation or by reading the values on the curve (graph). Calculate average dissolution rates at each $t_{si}$ to obtain an average dissolution curve.
5. The average dissolution curve of the test product is determined according to steps (1) to (3) of Section A-1 and A-2, described below. The $t_{si}$, the time needed to calculate average dissolution rates, should be the same as that for the reference product.
6. According to the Guideline, determine comparison times ($t_{ci}$) at which the dissolution rates of the reference and test products are compared. Determine an average dissolution rate of the reference product at $t_{ci}$ by interpolation or by reading the curve.

Examples for adjusting the dissolution curves are shown below when the average dissolution rates of the reference product reach 85% within the specified time and for cases in which they do not.

A-1 An example when the average dissolution rate of the reference product reaches 85% within the specified time:

Assume that a dissolution test is performed using 12 units of the reference product and the results in Table 1 are obtained.

Step 1. Calculating a lag time.

In each dissolution curve $t_A$ where a dissolution rate reaches $d_A$% is calculated according to the

formula below:

$$t_A = t_1 + (d_A - d_1) \times (t_2 - t_1) / (d_2 - d_1) \quad (1)$$

Here, $t_1$: measurement time just before a dissolution rate reaches $d_A$%.

$t_2$: measurement time just after a dissolution rate exceeds $d_A$%.

$d_1$: dissolution rate at $t_1$.

$d_2$: dissolution rate at $t_2$.

Table 1: Dissolution rate (%) of each reference product

| Products | Time (minutes) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 52.5 | 60 | 67.5 | 75 | 90 |
| ① | 0.0 | 1.3 | 8.1 | 17.8 | 29.3 | 41.6 | 51.6 | 60.1 | 68.3 | 75.2 | 81.8 | 84.1 | 91.2 | 97.2 | 100.0 |
| ② | 0.0 | 0.8 | 8.9 | 20.9 | 31.8 | 42.2 | 52.0 | 59.1 | 66.3 | 72.9 | 81.3 | 88.9 | 93.7 | 96.7 | 98.5 |
| ③ | 0.0 | 1.8 | 11.3 | 23.7 | 35.0 | 45.8 | 55.7 | 62.2 | 70.3 | 77.3 | 82.8 | 88.1 | 91.0 | 94.1 | 97.2 |
| ④ | 0.0 | 1.6 | 7.4 | 16.1 | 26.4 | 36.5 | 44.9 | 55.5 | 65.5 | 75.1 | 82.9 | 86.7 | 92.3 | 96.5 | 98.9 |
| ⑤ | 0.0 | 1.1 | 7.1 | 15.6 | 25.5 | 35.0 | 44.3 | 52.6 | 61.3 | 69.3 | 78.4 | 86.7 | 94.2 | 97.5 | 99.1 |
| ⑥ | 0.0 | 0.5 | 6.6 | 16.0 | 26.0 | 36.8 | 44.7 | 54.1 | 61.4 | 70.4 | 77.5 | 88.0 | 90.5 | 97.8 | 100.0 |
| ⑦ | 0.0 | 1.4 | 9.5 | 22.7 | 35.1 | 43.3 | 55.8 | 63.8 | 75.0 | 79.3 | 83.3 | 85.3 | 90.2 | 95.8 | 97.7 |
| ⑧ | 0.0 | 0.5 | 8.1 | 18.6 | 31.0 | 42.0 | 53.7 | 62.1 | 67.1 | 72.9 | 78.4 | 81.2 | 85.0 | 86.5 | 91.7 |
| ⑨ | 0.0 | 0.3 | 6.6 | 13.8 | 21.5 | 30.4 | 42.3 | 50.8 | 65.4 | 73.0 | 80.1 | 84.9 | 89.4 | 93.6 | 95.2 |
| ⑩ | 0.0 | 0.0 | 5.3 | 10.5 | 17.5 | 30.2 | 35.6 | 43.6 | 52.0 | 59.6 | 67.8 | 80.9 | 88.2 | 94.6 | 98.1 |
| ⑪ | 0.0 | 0.8 | 6.3 | 18.2 | 27.3 | 42.5 | 50.5 | 58.4 | 70.3 | 76.4 | 84.1 | 89.9 | 93.3 | 94.9 | 96.5 |
| ⑫ | 0.0 | 1.8 | 13.6 | 27.5 | 42.1 | 57.8 | 65.3 | 70.0 | 72.4 | 76.5 | 80.4 | 82.6 | 87.1 | 87.3 | 97.2 |
| Mean before adjusting | 0.0 | 1.0 | 8.2 | 18.5 | 29.0 | 40.3 | 49.7 | 57.7 | 66.3 | 73.2 | 79.9 | 85.6 | 90.5 | 94.4 | 97.5 |

A lag time ($t_L$) is calculated by placing $d_A$ = 5% in formula (1). $t_A$ can be read from the curve (graph).

Using the medicinal product ① in Table 1 as an example, $t_L$ is calculated to be 7.7 min using $t_1$ = 5 min, $d_1$ = 1.3%, $t_2$ = 10 min, $d_2$ = 8.1%. Similarly, the lag times calculated for products No. 2 through No. 12 are shown in the third column of Table 2.

Step 2. Creating a dissolution curve adjusted for a lag time.

Subtract lag times from measurement times in individual products, and use the times obtained as adjusted measurement times. The dissolution rates and adjusted measurement times are shown in Table 2 and the dissolution curves before and after the adjustment are shown in Figs. 1 and 2.

Step 3. Calculating the average dissolution rates from the dissolution data of individual products whose lag times are adjusted.

The times ($t_{si}$) needed for calculating average dissolution rates are determined by the method described below. In Table 2, the slowest time among the first adjusted measurements (measurement at 10 min), 3.6 min, is obtained in product No. 12, and as a result, 4 min is set as the starting time for calculating the average dissolution rate, $t_{s1}$. Similarly, the fastest time among the last adjusted measurements (measurement at 90 min), 80.3 min, is obtained for product No. 10, and 80 min is set as the ending time, $t_{slast}$, to calculate the average dissolution rates. The time subtracted for an average lag time of 8.0 min from the actual measurement time is used as a medium measurement time to calculate

an average dissolution rate. Excluding zero, the original data have 14 measurement points (Table 1), and the data for calculating an average dissolution rate have 13 points (Table 2).

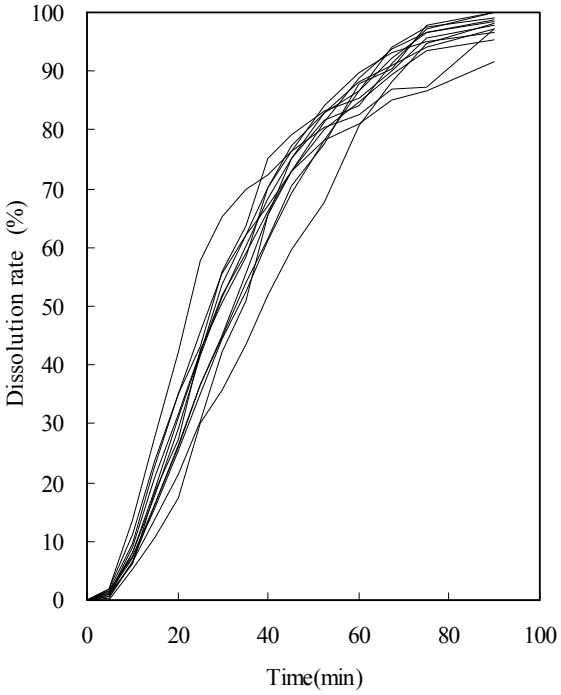


Figure 1: Dissolution curves of each product (measured values)

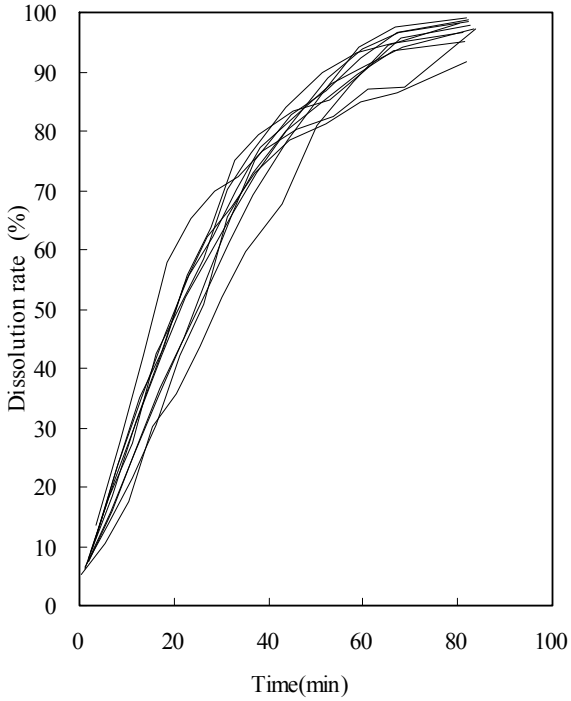


Figure 2: Lag-time adjusted dissolution curves of each product

Table 2 Adjusted time-points and dissolution rate for each reference product

| Products | Time (min) | $t_L$ | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 52.5 | 60 | 67.5 | 75 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ① | Adjusted time(min) | 7.7 | 2.3 | 7.3 | 12.3 | 17.3 | 22.3 | 27.3 | 32.3 | 37.3 | 44.8 | 52.3 | 59.8 | 67.3 | 82.3 |
| | Dissolution rate(%) | | 8.1 | 17.8 | 29.3 | 41.6 | 51.6 | 60.1 | 68.3 | 75.2 | 81.8 | 84.1 | 91.2 | 97.2 | 100.0 |
| ② | Adjusted time(min) | 7.6 | 2.4 | 7.4 | 12.4 | 17.4 | 22.4 | 27.4 | 32.4 | 37.4 | 44.9 | 52.4 | 59.9 | 67.4 | 82.4 |
| | Dissolution rate(%) | | 8.9 | 20.9 | 31.8 | 42.2 | 52.0 | 59.1 | 66.3 | 72.9 | 81.3 | 88.9 | 93.7 | 96.7 | 98.5 |
| ③ | Adjusted time(min) | 6.7 | 3.3 | 8.3 | 13.3 | 18.3 | 23.3 | 28.3 | 33.3 | 38.3 | 45.8 | 53.3 | 60.8 | 68.3 | 83.3 |
| | Dissolution rate(%) | | 11.3 | 23.7 | 35.0 | 45.8 | 55.7 | 62.2 | 70.3 | 77.3 | 82.8 | 88.1 | 91.0 | 94.1 | 97.2 |
| ④ | Adjusted time(min) | 7.9 | 2.1 | 7.1 | 12.1 | 17.1 | 22.1 | 27.1 | 32.1 | 37.1 | 44.6 | 52.1 | 59.6 | 67.1 | 82.1 |
| | Dissolution rate(%) | | 7.4 | 16.1 | 26.4 | 36.5 | 44.9 | 55.5 | 65.5 | 75.1 | 82.9 | 86.7 | 92.3 | 96.5 | 98.9 |
| ⑤ | Adjusted time(min) | 8.3 | 1.7 | 6.7 | 11.7 | 16.7 | 21.7 | 26.7 | 31.7 | 36.7 | 44.2 | 51.7 | 59.2 | 66.7 | 81.7 |
| | Dissolution rate(%) | | 7.1 | 15.6 | 25.5 | 35.0 | 44.3 | 52.6 | 61.3 | 69.3 | 78.4 | 86.7 | 94.2 | 97.5 | 99.1 |
| ⑥ | Adjusted time(min) | 8.7 | 1.3 | 6.3 | 11.3 | 16.3 | 21.3 | 26.3 | 31.3 | 36.3 | 43.8 | 51.3 | 58.8 | 66.3 | 81.3 |
| | Dissolution rate(%) | | 6.6 | 16.0 | 26.0 | 36.8 | 44.7 | 54.1 | 61.4 | 70.4 | 77.5 | 88.0 | 90.5 | 97.8 | 100.0 |
| ⑦ | Adjusted time(min) | 7.2 | 2.8 | 7.8 | 12.8 | 17.8 | 22.8 | 27.8 | 32.8 | 37.8 | 45.3 | 52.8 | 60.3 | 67.8 | 82.8 |
| | Dissolution rate(%) | | 9.5 | 22.7 | 35.1 | 43.3 | 55.8 | 63.8 | 75.0 | 79.3 | 83.3 | 85.3 | 90.2 | 95.8 | 97.7 |
| ⑧ | Adjusted time(min) | 8.0 | 2.0 | 7.0 | 12.0 | 17.0 | 22.0 | 27.0 | 32.0 | 37.0 | 44.5 | 52.0 | 59.5 | 67.0 | 82.0 |
| | Dissolution rate(%) | | 8.1 | 18.6 | 31.0 | 42.0 | 53.7 | 62.1 | 67.1 | 72.9 | 78.4 | 81.2 | 85.0 | 86.5 | 91.7 |
| ⑨ | Adjusted time(min) | 8.7 | 1.3 | 6.3 | 11.3 | 16.3 | 21.3 | 26.3 | 31.3 | 36.3 | 43.8 | 51.3 | 58.8 | 66.3 | 81.3 |
| | Dissolution rate(%) | | 6.6 | 13.8 | 21.5 | 30.4 | 42.3 | 50.8 | 65.4 | 73.0 | 80.1 | 84.9 | 89.4 | 93.6 | 95.2 |
| ⑩ | Adjusted time(min) | 9.7 | 0.3 | 5.3 | 10.3 | 15.3 | 20.3 | 25.3 | 30.3 | 35.3 | 42.8 | 50.3 | 57.8 | 65.3 | 80.3 |
| | Dissolution rate(%) | | 5.3 | 10.5 | 17.5 | 30.2 | 35.6 | 43.6 | 52.0 | 59.6 | 67.8 | 80.9 | 88.2 | 94.6 | 98.1 |
| ⑪ | Adjusted time(min) | 8.8 | 1.2 | 6.2 | 11.2 | 16.2 | 21.2 | 26.2 | 31.2 | 36.2 | 43.7 | 51.2 | 58.7 | 66.2 | 81.2 |
| | Dissolution rate(%) | | 6.3 | 18.2 | 27.3 | 42.5 | 50.5 | 58.4 | 70.3 | 76.4 | 84.1 | 89.9 | 93.3 | 94.9 | 96.5 |
| ⑫ | Adjusted time(min) | 6.4 | 3.6 | 8.6 | 13.6 | 18.6 | 23.6 | 28.6 | 33.6 | 38.6 | 46.1 | 53.6 | 61.1 | 68.6 | 83.6 |
| | Dissolution rate(%) | | 13.6 | 27.5 | 42.1 | 57.8 | 65.3 | 70.0 | 72.4 | 76.5 | 80.4 | 82.6 | 87.1 | 87.3 | 97.2 |

The dissolution rate ($d_B$) at a particular time ($t_{si}$) for calculating an average dissolution rate is determined using the following formula:

$$d_B = d_1 + (d_2 - d_1) \times (t_{si} - t_1) / (t_2 - t_1) \quad (2)$$

Here, $t_1$: adjusted measurement time just before $t_{si}$.

$t_2$: adjusted measurement time just after $t_{si}$.

$d_1$: dissolution rate at $t_1$.

$d_2$: dissolution rate at $t_2$.

Table 3 shows the times for calculating the average dissolution rates and the dissolution rates calculated by interpolation for each product. Figure 3 shows the average dissolution curves before and after adjustment.

Table 3 Time-points used to calculate mean dissolution rate obtained by interpolation.

| Products | tsi | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 7 | 12 | 17 | 22 | 27 | 32 | 37 | 44.5 | 52 | 59.5 | 67 | 80 |
| ① | 11.4 | 17.2 | 28.6 | 40.9 | 51.0 | 59.6 | 67.8 | 74.8 | 81.5 | 84.0 | 90.9 | 97.0 | 99.6 |
| ② | 12.7 | 19.9 | 30.9 | 41.4 | 51.2 | 58.5 | 65.7 | 72.4 | 80.9 | 88.5 | 93.4 | 96.5 | 98.2 |
| ③ | 13.0 | 20.5 | 32.1 | 43.0 | 53.1 | 60.5 | 68.2 | 75.5 | 80.9 | 87.2 | 90.5 | 93.6 | 96.5 |
| ④ | 10.7 | 15.9 | 26.2 | 36.3 | 44.7 | 55.3 | 65.3 | 74.9 | 82.8 | 86.6 | 92.2 | 96.5 | 98.6 |
| ⑤ | 11.0 | 16.2 | 26.1 | 35.6 | 44.8 | 53.1 | 61.8 | 69.7 | 78.7 | 87.0 | 94.3 | 97.5 | 98.9 |
| ⑥ | 11.7 | 17.4 | 27.5 | 37.9 | 46.0 | 55.1 | 62.7 | 71.1 | 78.5 | 88.2 | 91.2 | 97.9 | 99.8 |
| ⑦ | 12.7 | 20.6 | 33.1 | 42.0 | 53.8 | 62.5 | 73.2 | 78.6 | 82.9 | 85.1 | 89.7 | 95.2 | 97.3 |
| ⑧ | 12.3 | 18.6 | 31.0 | 42.0 | 53.7 | 62.1 | 67.1 | 72.9 | 78.4 | 81.2 | 85.0 | 86.5 | 91.0 |
| ⑨ | 10.5 | 14.9 | 22.7 | 32.0 | 43.5 | 52.8 | 66.5 | 73.7 | 80.5 | 85.3 | 89.8 | 93.7 | 95.1 |
| ⑩ | 9.1 | 12.9 | 21.9 | 32.1 | 38.3 | 46.5 | 54.6 | 61.5 | 70.8 | 82.6 | 89.7 | 95.0 | 98.0 |
| ⑪ | 13.0 | 19.7 | 29.7 | 43.8 | 51.8 | 60.3 | 71.3 | 77.2 | 84.7 | 90.3 | 93.5 | 95.0 | 96.4 |
| ⑫ | 14.7 | 23.1 | 37.4 | 52.8 | 62.9 | 68.5 | 71.6 | 75.2 | 79.6 | 82.1 | 86.1 | 87.3 | 94.8 |
| Mean | 11.9 | 18.1 | 28.9 | 40.0 | 49.6 | 57.9 | 66.3 | 73.1 | 80.0 | 85.7 | 90.5 | 94.3 | 97.0 |

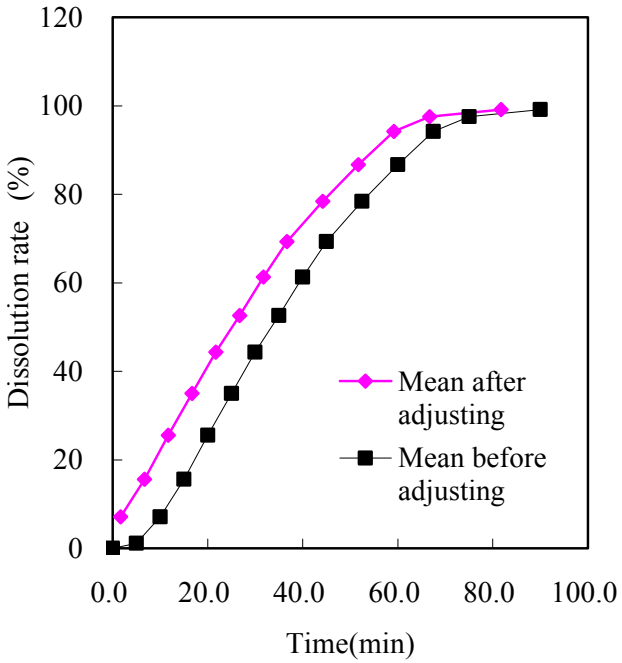


Figure 3: Mean dissolution curves before and after adjustment

Step 4. Determining the times for comparing dissolution profiles and the dissolution rates.

For the reference product in this example, the lag times are observed, and the dissolution rates do not reach 85% after 30 minutes of the lag time but do reach 85% by the specified time. Therefore, this example corresponds to criteria a, under No. 3, Item 4, Section 3-A of the Guideline. The criteria specify that the comparison time, $t_{ci}$, when the average dissolution rates are compared without $f_2$ functions, should be the reasonable time at which the reference product dissolution rates reach 40% and 85%. When there is no lag time adjustment, the average dissolution rates at the closest measurement point to 40% or 85% can be compared. When there is a lag time adjustment, the times at which the average reference product dissolution rates reach 40% and 85% are determined by interpolation, and the dissolution rates are then compared at those times. In this example, the time, $t_{c1}$, at which the reference product dissolution rates reach 40% is 17.0 min, as shown in Table 3. The time, $t_{c2}$, at which the rates of the reference product reach 85% is determined using formula (1). Data in Table 3 reveal that $d_A$ = 85.0 %, $d_1$ = 80.1%, $d_2$ = 85.7%, $t_1$ = 44.5 min, and $t_2$ = 52.0 min. Thus, using the following formula, the time at 85% dissolution is calculated to be 51.1 min:

$$t_A = 44.5 + (85.0-80.0) \times (52.0-44.5) / (85.7-80.0) = 51.1$$

When the $f_2$ function is applied, Ta/4, 2Ta/4, 3Ta/4, and Ta are comparison points if Ta is considered to be a time point at which the average reference product dissolution rates are approximately 85%. $t_{c2}$, determined above, is Ta, so in this example, the calculation method is not used, and Ta/4, 2 Ta/4, and 3 Ta/4 are calculated as 12.8, 25.5, and 38.3, respectively. The average reference product dissolution rates at each time point are determined using formula (2), and the following results are obtained:

$$= 28.9 + (40.0-28.9) \times (12.8-12.0) / (17.0-12.0) = 30.7\%$$
$$= 49.6 + (57.9-49.6) \times (25.5-22.0) / (27.0-22.0) = 55.4\%$$
$$= 73.1 + (80.0-73.1) \times (38.3-37.0) / (44.5-37.0) = 74.3\%$$

Step 5. Determining the dissolution rates of the test product at the comparison time point.

The average dissolution curves are determined using steps 1) to 3), although the data for the example are not shown. When the average dissolution rates are compared on the basis of those curves without f2 functions, the rates are determined to be 17.0 min and 51.1 min. When $f_2$ functions are applied, the rates are determined to be 12.8, 25.5, 38.3, and 51.1 min.

A-2 An example of when the average reference product dissolution rates do not reach 85% within the specified time.

Assuming that a dissolution test is performed using 12 units of the reference product, the results in Table 4 are obtained:

Table 4: Actual value of dissolution rates (%) of individual reference product

| Products | Time (min) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 37.5 | 45 | 60 | 90 | 120 | 240 | 360 |
| ① | 0.0 | 0.0 | 1.6 | 3.5 | 12.4 | 18.9 | 38.9 | 46.5 | 48.1 | 58.3 | 65.0 | 72.3 | 73.0 | 75.2 |
| ② | 0.0 | 0.0 | 0.0 | 7.4 | 11.1 | 19.4 | 29.9 | 44.7 | 52.0 | 60.9 | 70.2 | 74.2 | 72.9 | 74.9 |
| ③ | 0.0 | 0.0 | 0.7 | 6.0 | 15.5 | 24.0 | 31.9 | 45.1 | 52.5 | 60.3 | 70.7 | 72.8 | 73.6 | 76.7 |
| ④ | 0.0 | 0.0 | 1.1 | 5.7 | 16.5 | 24.5 | 35.7 | 43.3 | 48.4 | 58.8 | 71.7 | 74.4 | 75.0 | 77.8 |
| ⑤ | 0.0 | 0.0 | 1.3 | 8.0 | 10.5 | 20.9 | 34.3 | 47.3 | 52.4 | 56.5 | 65.9 | 73.8 | 73.7 | 74.8 |
| ⑥ | 0.0 | 0.0 | 3.0 | 3.3 | 12.9 | 22.3 | 39.8 | 41.8 | 47.8 | 62.0 | 69.9 | 70.7 | 73.7 | 75.3 |
| ⑦ | 0.0 | 0.4 | 1.3 | 6.9 | 10.1 | 24.8 | 29.2 | 41.4 | 47.0 | 63.6 | 73.5 | 73.5 | 76.5 | 77.6 |
| ⑧ | 0.0 | 0.2 | 0.2 | 5.5 | 12.6 | 27.4 | 28.7 | 43.0 | 48.9 | 58.7 | 70.6 | 71.4 | 72.0 | 76.6 |
| ⑨ | 0.0 | 0.0 | 1.8 | 6.8 | 18.6 | 19.4 | 32.9 | 37.5 | 49.1 | 61.6 | 69.2 | 71.8 | 72.9 | 78.0 |
| ⑩ | 0.0 | 0.7 | 1.0 | 4.9 | 14.2 | 20.2 | 27.8 | 41.2 | 54.9 | 61.1 | 71.2 | 72.5 | 75.0 | 75.1 |
| ⑪ | 0.0 | 0.0 | 0.1 | 7.6 | 16.1 | 21.5 | 38.4 | 38.6 | 50.0 | 58.7 | 66.8 | 71.0 | 73.2 | 74.9 |
| ⑫ | 0.0 | 0.4 | 2.8 | 5.4 | 10.9 | 22.5 | 33.4 | 45.2 | 48.4 | 61.2 | 66.5 | 72.4 | 73.0 | 73.4 |
| Mean before adjustment | 0.0 | 0.1 | 1.3 | 5.9 | 13.5 | 22.1 | 33.4 | 43.0 | 50.0 | 60.2 | 69.3 | 72.6 | 73.7 | 76.1 |

Step 1. Calculating a lag time.

The adjusted measurement time obtained from the calculation of the dissolution lag times for each product using formula (1), using the same method as in example A-1, is shown in Table 5. In this example, all values adjusted for lag times are rounded to whole minutes.

Table 5: Adjusted time-points and dissolution rates

| Products | $t_i$ (min) | Time (min) | 20 | 25 | 30 | 37.5 | 45 | 60 | 90 | 120 | 240 | 360 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ① | | Adjusted time(min) | 4 | 9 | 14 | 22 | 29 | 44 | 74 | 104 | 224 | 344 |
| | 16 | Dissolution rate(%) | 12.4 | 18.9 | 38.9 | 46.5 | 48.1 | 58.3 | 65.0 | 72.3 | 73.0 | 75.2 |
| ② | | Adjusted time(min) | 7 | 12 | 17 | 24 | 32 | 47 | 77 | 107 | 227 | 347 |
| | 13 | Dissolution rate(%) | 11.1 | 19.4 | 29.9 | 44.7 | 52.0 | 60.9 | 70.2 | 74.2 | 72.9 | 74.8 |
| ③ | | Adjusted time(min) | 6 | 11 | 16 | 23 | 31 | 46 | 76 | 106 | 226 | 346 |
| | 14 | Dissolution rate(%) | 15.5 | 24.0 | 31.9 | 45.1 | 52.5 | 60.3 | 70.7 | 72.8 | 73.6 | 76.7 |
| ④ | | Adjusted time(min) | 6 | 11 | 16 | 23 | 31 | 46 | 76 | 106 | 226 | 346 |
| | 14 | Dissolution rate(%) | 16.5 | 24.5 | 35.7 | 43.3 | 48.4 | 58.8 | 71.7 | 74.4 | 75.0 | 77.8 |
| ⑤ | | Adjusted time(min) | 7 | 12 | 17 | 24 | 32 | 47 | 77 | 107 | 227 | 347 |
| | 13 | Dissolution rate(%) | 10.5 | 20.9 | 34.3 | 47.3 | 52.4 | 56.5 | 65.9 | 73.8 | 73.7 | 74.8 |
| ⑥ | | Adjusted time(min) | 4 | 9 | 14 | 22 | 29 | 44 | 74 | 104 | 224 | 344 |
| | 16 | Dissolution rate(%) | 12.9 | 22.3 | 39.8 | 41.8 | 47.8 | 62.0 | 69.9 | 70.7 | 73.7 | 75.3 |
| ⑦ | | Adjusted time(min) | 7 | 12 | 17 | 24 | 32 | 47 | 77 | 107 | 227 | 347 |
| | 13 | Dissolution rate(%) | 10.1 | 24.8 | 29.2 | 41.4 | 47.0 | 63.6 | 73.5 | 73.5 | 76.5 | 77.6 |
| ⑧ | | Adjusted time(min) | 5 | 10 | 15 | 23 | 30 | 45 | 75 | 105 | 225 | 345 |
| | 15 | Dissolution rate(%) | 12.6 | 27.4 | 28.7 | 43.0 | 48.9 | 58.7 | 70.6 | 71.4 | 72.0 | 76.6 |
| ⑨ | | Adjusted time(min) | 7 | 12 | 17 | 24 | 32 | 47 | 77 | 107 | 227 | 347 |
| | 13 | Dissolution rate(%) | 18.6 | 19.4 | 32.9 | 37.5 | 49.1 | 61.6 | 69.2 | 71.8 | 72.9 | 78.0 |
| ⑩ | | Adjusted time(min) | 5 | 10 | 15 | 23 | 30 | 45 | 75 | 105 | 225 | 345 |
| | 15 | Dissolution rate(%) | 14.2 | 20.2 | 27.8 | 41.2 | 54.9 | 61.1 | 71.2 | 72.5 | 75.0 | 75.1 |
| ⑪ | | Adjusted time(min) | 7 | 12 | 17 | 24 | 32 | 47 | 77 | 107 | 227 | 347 |
| | 13 | Dissolution rate(%) | 16.1 | 21.5 | 38.4 | 38.6 | 50.0 | 58.7 | 66.8 | 71.0 | 73.2 | 74.9 |
| ⑫ | | Adjusted time(min) | 6 | 11 | 16 | 23 | 31 | 46 | 76 | 106 | 226 | 346 |
| | 14 | Dissolution rate(%) | 10.9 | 22.5 | 33.4 | 45.2 | 48.4 | 61.2 | 66.5 | 72.4 | 73.0 | 73.4 |

Step 2. Creating a dissolution curve adjusted for a lag time.

Similar to A-1, the values obtained by subtracting lag times from measurement times are considered

to be an adjusted measurement time. Table 5 shows the dissolution rates and adjusted measurement times of individual product units.

Step 3. Calculating the average dissolution rates from the dissolution data of individual product units where lag times are adjusted.

When the dissolution rates do not reach 85% within the specified time, the time point for comparing average dissolution rates should be determined using the dissolution rate at the final measurement time for a reference product as a criterion. When a lag time is observed, the dissolution testing time for each product unit varies depending on the lag time. The shortest testing time is used as the final measurement time for all product units because the product with the longest lag time has the shortest testing time.

For example, the shortest testing time is 344 minutes in products No. 1 and No. 6, and thus 344 minutes is used as a final time, $t_{slast}$, for calculating an average dissolution rate. For other time points, most products show the adjusted measurement times, such as 7, 12, 17,…227; those times are then used as the time ($t_{si}$) for calculating average dissolution times, and the calculation procedure can be skipped. The individual product dissolution rates at $t_{si}$ using formula (2) are calculated, and the results are shown in Table 6. The average dissolution curves before and after the adjustment are also shown in Figure 4.

Table 6: Time tsi for calculating the mean dissolution rate, and dissolution rates(%)

| Products | tsi | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7 | 12 | 17 | 24 | 32 | 47 | 77 | 107 | 227 | 344 |
| ① | 16.3 | 30.9 | 41.8 | 47.0 | 51.1 | 58.9 | 65.7 | 72.3 | 73.0 | 75.1 |
| ② | 11.1 | 19.4 | 29.9 | 44.7 | 52.0 | 60.9 | 70.2 | 74.2 | 72.9 | 74.9 |
| ③ | 17.2 | 25.6 | 33.7 | 45.6 | 53.4 | 60.7 | 70.8 | 72.8 | 73.6 | 76.6 |
| ④ | 18.1 | 26.7 | 37.0 | 43.8 | 49.7 | 59.3 | 71.8 | 74.4 | 75.0 | 77.7 |
| ⑤ | 10.5 | 20.9 | 34.3 | 45.7 | 52.4 | 56.5 | 65.9 | 73.8 | 73.7 | 74.8 |
| ⑥ | 18.5 | 32.8 | 40.6 | 43.8 | 51.6 | 62.8 | 70.0 | 70.8 | 73.7 | 75.3 |
| ⑦ | 10.1 | 24.8 | 29.2 | 41.4 | 47.0 | 63.6 | 73.5 | 73.5 | 76.5 | 77.6 |
| ⑧ | 18.5 | 27.9 | 32.3 | 43.8 | 50.9 | 59.3 | 70.7 | 71.4 | 72.1 | 76.6 |
| ⑨ | 18.6 | 19.4 | 32.9 | 37.5 | 49.1 | 61.6 | 69.2 | 71.8 | 72.9 | 77.9 |
| ⑩ | 16.6 | 23.2 | 31.4 | 43.9 | 56.4 | 61.8 | 71.3 | 72.5 | 75.0 | 75.1 |
| ⑪ | 16.1 | 21.5 | 38.4 | 38.6 | 50.0 | 58.7 | 66.8 | 71.0 | 73.2 | 74.9 |
| ⑫ | 13.2 | 24.7 | 35.1 | 45.6 | 49.6 | 61.5 | 66.7 | 72.4 | 73.0 | 73.4 |
| Mean after adjustment | 15.4 | 24.8 | 34.7 | 43.5 | 51.1 | 60.5 | 69.4 | 72.6 | 73.7 | 75.8 |

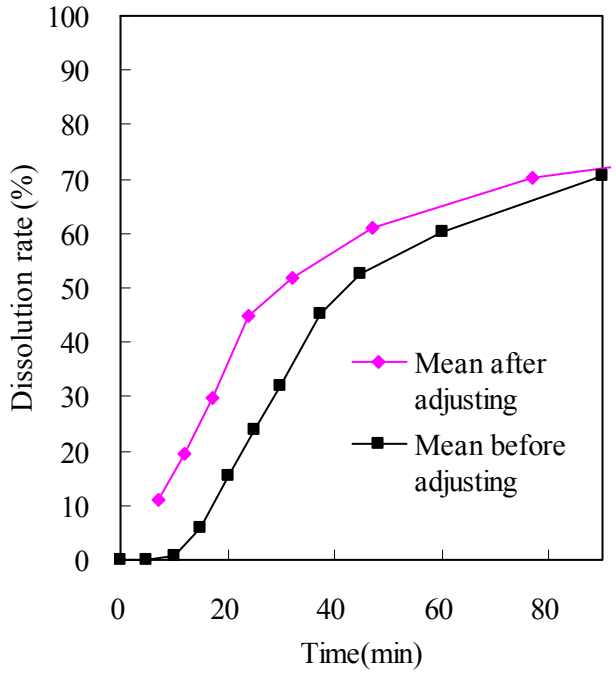


Figure 4: Mean dissolution curves before and after adjustment

Step 4. Determining the times for comparing dissolution profiles and dissolution rates.

When comparing average dissolution rates without $f_2$ functions, the comparison time point ($t_{ci}$) is the time showing half of the final average dissolution rate and the final testing time. The average dissolution rate at the final testing time is 75.8%, and half of that is 37.9%. The time, $t_{s1}$, at which the average dissolution rate is 37.9% is determined using interpolation, and the calculated time is 19 minutes.

When the $f_2$ function is applied, Ta/4, 2Ta/4, 3Ta/4, and Ta are comparison time points if Ta is considered as a time point at which the final dissolution rate of the reference product is 85%. The average dissolution rate of the reference product at Ta is 64.4% (75.8 × 0.85), and Ta of 46 minutes is calculated using interpolation. Ta/4, 2Ta/4, and 3Ta/4 are calculated to be 12, 23, and 35 minutes, respectively. Since the average dissolution rates at 12 minutes are in shown in Table 6, those at 23 minutes and 35 minutes are calculated using interpolation to be 42.3% and 52.7%, respectively.

Step 5. Determining the dissolution rate of the test product at the comparison time.

The average dissolution curves are determined using steps (1) to (3), but the sample data are not shown. When the average dissolution rates are compared on the basis of the curves without $f_2$ functions, the rates are determined to be 19 and 344 minutes. Note that when the last measurement time of a test product is shorter than 344 minutes, $t_{c1}$ should be 19 minutes and $t_{c2}$ should be the last test product measurement time. This means that the average test product dissolution rates at $t_{c2}$ should be determined using interpolation. When $f_2$ functions are applied, the dissolution rates are determined to be 12, 23, 35, and 46 minutes.