ANNEX
to the Decision of the Council of
the Eurasian Economic Commission
No. dated 20
ANNEX No. 9
to the Rules for Conducting
Bioequivalence Studies of Medicinal
Products within the Eurasian Economic
Union

REQUIREMENTS
to conduct pharmacokinetic and clinical studies of bioequivalence of topical corticosteroid medicinal products

I. Introduction

1. These Requirements provide guidance how to demonstrate the *in vivo* bioequivalence of topical corticosteroid products with the help of pharmacodynamic studies based on a modified analysis of vasoconstriction (analysis of skin blanching, the Stoughton-McKenzie bioanalysis). This method involves the assessment of the duration of exposure to control the dose of topical corticosteroid that is delivered. The proposed methodology provides for a pilot study of the «dose duration – response» relationship to determine the acceptable dose duration for use in the pivotal study, followed by the pivotal *in vivo* bioequivalence study incorporating replicate design and confirmation of the acceptable «dose duration – response» of subjects. As all bioanalytic methods, this pharmacodynamic bioanalysis requires detailed validation, which is the responsibility of the sponsor.

2. Potent topical corticosteroid products may suppress the hypothalamic-pituitary-adrenal (HpA) axis, however, for medicinal products whose bioequivalence is confirmed, it is not required to submit the results of tests for the suppression of the hypothalamic-pituitary-adrenal axis.

3. The recommendations given in these Requirements apply to topical corticosteroid products, regardless of their potency level. Since the characteristics of the «dose duration – response» relationship may vary depending on the specific medicinal product, a pilot study is recommended to determine the relevant parameters of the main study.

II. Definitions

4. For the purposes of these Requirements, the following terms shall be used, their meanings set forth in the respective definitions below:

a nonresponder is defined as a subject who does not show any response to a single dose duration of the reference medicinal product under the same conditions - with or without obstruction (occlusion) – used in the pilot and pivotal studies;

a responder is defined as a subject shows a response to a single dose duration of the reference medicinal product under the same conditions - with or without obstruction (occlusion) – used in the pilot and pivotal studies.

III. General provisions

5. The confirmation of bioequivalence of two solid oral dosage forms of corticosteroid products based on a comparison of active substance and (or) metabolite concentrations in an accessible biologic fluid (for example, blood or urine) after administration of single or multiple doses of each

corticosteroid product to healthy volunteers. If this methodology is not feasible, alternative methods of *in vivo* and *in vitro* studies may be used to demonstrate bioequivalence, which include (in descending order according to the significance of the results obtained by these methods):

a) pharmacodynamic studies;

b) clinical studies;

c) *in vivo* animal studies;

d) *in vitro* studies.

6. A pharmacodynamic or *in vivo* clinical study is required to determine the bioequivalence of topical corticosteroid products if the concentration of the active substance or its metabolites cannot be determined in the available biological fluids. Clinical studies generally require large numbers of subjects and often do not have sufficient sensitivity. In contrast, pharmacodynamic studies offer the possibility to obtain acceptable bioequivalence data of corticosteroid products in a relatively small number of subjects.

7. The authorization of generic topical corticosteroid products should be based primarily on the availability of results of a comparative evaluation of the pharmacodynamic effects of the active substance intake from two potentially equivalent formulations of topical corticosteroid products. This approach is based on the property of corticosteroids to produce blanching or vasoconstriction in the microvasculature of the skin, which can be analyzed quantitatively and compared with the amount of active substance entering the skin.

8. Although there are many forms of the vasoconstrictor analysis, the general method is based on topical application of a corticosteroid-containing product for a period of 6 to 16 hours in healthy volunteers, followed by visual

estimation by a trained, blinded observer of the degree of blanching on a multiple unit scale (0 to 3 or 0 to 4 points) at a single time point (as a rule, in two hours after removal of the product).

9. These Requirements suggests conducting two *in vivo* studies – a pilot «dose duration – response» study and a pivotal *in vivo* bioequivalence study to compare test and reference products. The pilot study characterizes the «dose duration – response» relationship when building such relation in the Emax determination model and is conducted solely with the reference product. The dose duration method that is preferred to confirm the bioequivalence involves the use of three dose durations: ED_{50}, D_1 and D_2. The comparison of test and reference products in the pivotal study is conducted at the level of the dose duration, approximately equal to the population ED_{50} according to the results of the pilot study. Sensitivity in the pivotal study is established through application of the reference calibration standard at two levels of dose durations: D_1 (the calibration standard with shorter dose duration) and D_2 (the calibration standard with longer dose duration). It is recommended that D_1 equals approximately $0.5 \times ED_{50}$, and D_2 – $2 \times ED_{50}$ based on the results of the pilot study. Each subject acts as a detector in this study, therefore, only the data of the subjects whose D_2/D_1 ratio of pharmacodynamic responses meets a specified minimum value may be included in the analysis and subjected to statistical processing to confirm *in vivo* bioequivalence.

IV. Pharmacodynamic effect study: analysis of vasoconstrictive effects

10. When evaluating the results of equivalence studies, the authorized authorities (expert organizations) should ensure that the performer uses the method for the analysis of the vasoconstriction of skin when studying equivalence:

validation and standardization of the method of analysis of skin vasoconstriction in accordance with the requirements for the validation of bioanalytical methods should be performed;

personnel trained to properly assess vasoconstriction should be chosen as an observer.

1. Validation and standardization of the vasoconstriction analysis method

11. Application of the vasoconstrictor analysis to an assessment of bioequivalence of a topical corticosteroid products rests that on the assumption that the vasoconstrictor properties of a topical corticosteroids may be utilized to establish a standard, validated bioassay. The results of the development and validation of bioassay should be documented.

12. In the process of examination of the Registration Dossier for corticosteroid product, a comparison of the results of validation for a HPLC or GLC assay of the concentration of corticosteroid product in the blood after administration of a certain dose with the results of validation for the vasoconstrictor bioassay should be carried out. When using a vasoconstriction bioassay, the observed pharmacodynamic response (vasoconstriction) to the administered amount of the active substance of a

topical corticosteroid product is similar to the response of the HPLC or GLC detector to the same known administered amount of the active ingredient of the corticosteroid product.

13. Whereas only one instrument and detector are used in a standard blood or urine concentration level assay, each subject in a pharmacodynamic bioassay study acts as a «detector» responding to a known or unknown amount of active substance. Despite the fundamental differences between a standard blood or urine concentration level assay and a bioassay, many of the principles regarding standardization and validation are comparable.

Linearity

14. The pharmacodynamic relationships between either dose or concentration of corticosteroid product and its pharmacodynamic effect studied is applicable in the vasoconstriction bioanalysis in the presence of an assessment of its linearity. Despite the fact that various models are available to describe the relationship between «dose and effect», for the vasoconstrictor bioassay it may be especially useful to utilize the E_{max} assessment model, or the related sigmoid E_{max} model, which is calculated by the formula:

$$E = E_0 + \frac{E_{max} \times D}{ED_{50} + D}$$

and allows to measure the value of the effect (E) in terms of the administration of any dose (D) based on the values of the three constants: a baseline effect (E_0), a maximal effect (E_{max}) and a dose (D) at administration of which the effect is half-maximal (ED_{50}):

15. The *in vivo* vasoconstrictor response generally approaches a maximum. Thus, the main issue requiring resolution in the application of the vasoconstrictor analysis to assess bioequivalence is falling outside the limits of the linear range of the response of the microvasculature of the skin when applying the test products. Relatively high strengths of a topical corticosteroid product may have a minimal effect on vasoconstriction, regardless of the dose range. At relatively low strengths of a topical corticosteroid product, the determining of the minimal dose that produces a reliable and reproducible vasoconstrictor response becomes the major problem. Determination of the minimum dose is analogous to the determination of the lower threshold for the assay of the concentration level for the active substance in the blood or urine during the validation of bioanalytical methods to generate the standard pharmacokinetic curve. To evaluate the correctness of the choice of ED_{50}, D_1 and D_2, the generation and validation of a standard «dose – response» curve is necessary.

16. In standard analytical methods validation, establishing linearity in detector response is necessary. Linearity in response is also desirable in the development of a vasoconstrictor analysis. Since the intended generic and reference commercial products may be marketed at strengths corresponding to the horizontal part of the «dose – response» curve, the method should be optimized to assure that the products are compared in the linear portion of the curve.

17. Establishing a «dose – effect» relationship for topical corticosteroid products should be based on a reliable method to administer a predetermined

dose of the medicinal product to the skin. It is allowed to use one of the following three methods to ensure reliable application of the studied dose of corticosteroid product:

the dose duration method;

the dilution method;

the area method.

Both the dose duration method and the dilution method are well standardized and reproducible, however, the different characteristics in the product composition sometimes cannot be identified by the dilution method. The dose duration method is the most suitable to demonstrate the bioequivalence of topical corticosteroid products. Development of a «dose duration – response» relationship for a topical corticosteroid products will indicate points in the «response – time» relationship at which the vasoconstrictor response becomes insensitive. In general, the time course of response should be followed to return to baseline to insure that the maximal pharmacodynamic response is observed at each dose duration.

Accuracy, precision and sensitivity

18. Development of methodology to establish the accuracy, precision, and sensitivity of a bioassay for a topical corticosteroid product should be coincident with the development of an acceptable standard curve for the vasoconstrictor analysis. For each population used in the study, it is necessary to develop such a methodology and develop a standard curve for the vasoconstrictor analysis. As with a standard analysis of the concentration

of the active substance in the blood or urine, this information is obtained by using the control group, the subjects of which are untreated, and calibration standards containing the test topical corticosteroid product. Replication of test results in untreated control group using calibration standards allows estimation of coefficients of variation. Similar to standard HPLC and GLC methods, the calibration standard, which involves the measurement of the detector response to a known concentration of the active substance, in pharmacodynamic bioanalysis of a topical corticosteroid product based on dose duration, the calibration standard involves the application of a standard dose of a topical corticosteroid product for different periods of time.

2. Assessment of vasoconstrictor response

19. Application of an instrumental chromameter or colorimeter to detect erythema offers the possibility of replacing subjective visual assessment in the vasoconstrictor analysis with objective, quantifiable measurements. The authorized authorities (expert organizations) accept the results of instrumental assessment of vasoconstriction analysis in bioequivalence studies along with visual assessments of the degree of vasoconstriction with appropriate validation of the latter with the establishment of a correlation between chromameter (colorimeter) measurements and visual assessments of the degree of vasoconstriction.

20. Compared with the visual assessment, the chromameter has a higher sensitivity to the skin blanching.

21. Due to a circadian rhythm, changes in the response of the skin vessels, the plasma cortisol content associated with the circadian rhythm, skin

blanching should be assessed for two consecutive 24-hour intervals (48 hours). The data of the area under the effect curve (AUEC) obtained for at least 24 hours from moment of medicinal product removal or medicinal product application are acceptable for bioequivalence assessment (see paragraph 45).

22. The indicators of the degree of vasoconstriction, measured with a chromameter and adjusted by the baseline value of the state of the vessels in the control sites of the skin without the application of the medicinal product (AUEC in the control group), should allow to confirm that there is no following indications within the subjects of the study:

a difference in response between left and right arms;

an effect on the response rate of the site of medicinal product application on the arm skin, that are no closer than 3 to 4 cm to the antecubital fossa or to the wrist.

If it is established that there is a difference in the responses or the effect on the response rate of the site where the medicinal product is applied, the design of the clinical study specified in paragraph 59 should be used, in which the scheme of product application on each arm is complementary (for example, the application site of the test product on one arm coincide with the application sites of the reference product on the other arm). This allows to minimize the difference in responses or the effect on the response of the product application site.

V. Pilot study of «dose duration – response» relation

23. The purpose of the pilot study is to determine the «dose duration – response relationship» of the topical corticosteroid product to be studied in

the pivotal *in vivo* bioequivalence study. The study is analogous to the development of a standard curve in the assay of an active substance in a biologic fluid. The result of the pilot study allows to obtain information on the «dose duration response» relationship necessary to determine the parameters ED_{50}, D_1, and D_2 to be used in the pivotal *in vivo* bioequivalence study, and an estimation of the proportion of subjects expected to meet the minimum D_2/D_1 ratio of AUEC values in the pivotal study. Since the results of a pilot study may functionally depend on the conditions of the study, including, among other factors subject, population characteristics, methodology used to assess skin blanching, and amount of medicinal product applied, the provisions of this Annex recommend the performance of a pilot study at each research center for each reference product in the study.

1. Study design and analysis

24. «Dose duration – response» study is carried out with reference product only, with randomization of application sites based on dose duration. Dose durations from 0.25 to 6.0 hours and untreated control sites on each arm to enable correction for color changes of skin sites affected by the medicinal product during the study of the duration of product exposure. Since the carrier (base) of the corresponding reference product is not generally available, untreated control sites refer to untreated areas of skin, not to areas of skin to which carrier (base) has been applied.

25. Chromameter assessment of the pharmacodynamic response to the topical corticosteroid product is carried out at certain time periods, rather than a single time point, following application and removal of each product dose.

26. The data of the «dose duration – response» correlation should be modeled using either a nonlinear mixed effect modelling method or a naive pooled data method to determine the population ED_{50} value which will serve as the approximate dose duration for the bioequivalence confirmation in the pivotal study.

27. The study includes 12 subjects;

28. For products marketed in multiple strengths, the pilot and pivotal studies should be conducted on the high strength product. Biowaiver of additional strengths for in vivo bioequivalence studies for lower strengths of a topical dermatologic corticosteroid product may be applied when two conditions are met:

positive results of the bioequivalence study in humans (*in vivo*) for a higher strength of the product;

submission of the comparative formulation data of excipients of lower strengths of the test product with the corresponding strengths of the reference product both in terms of their qualitative composition (Q_1 parameter) and in relation to the quantitative content of each component of the formulation (Q_2 parameter).

If the requirements of comparative formulation of excipients in Q_1 and Q_2 parameters for lower strengths of the test product with respect to the corresponding strengths of the reference product are not met, the biowaiver of additional strengths are possible only if the applicant presents the rationale that there is no effect on the efficiency and safety of the use of the test products due to the difference in formulations.

2. Criteria for subject inclusion in the study

29. When designing and conducting study, the following criteria for subject inclusion in the study are applied:

a subject with a «healthy» verified diagnosis;

a subject demonstrating pronounced vasoconstriction to topical corticosteroid product (responder); written informed consent;

a subject's willingness to follow study conditions.

3. Criteria for subject non-inclusion in the study and criteria for subject exclusion from the study

30. When designing and conducting study, the following criteria for subject non-inclusion in the study are applied:

clinically significant hypertension or circulatory disease;

smoking for one week before the study;

caffeine intake greater than 500 mg per day prior to or during the study (a cup of coffee contains about 85 mg of caffeine);

clinically significant history of alcohol intake (or ethyl alcohol containing products) or drug abuse;

use of topical dermatologic medicinal products applied on ventral forearms (including prior application of a topical corticosteroid product during a pharmacodynamic study to a particular skin site) within one month prior to the study;

adverse reactions to topical or systemic corticosteroid products;

any current disease or disease in history, including active dermatitis or any other dermatologic condition, which might significantly affect pharmacodynamic response to the administered medicinal product;

subjects who would require shaving ventral forearms to insure the application of an appropriate dose on skin surface;

use of any vasoactive (vasoconstrictor or vasodilator) medicinal products, that could modulate blood flow regardless of the procedure in which these products are dispensed (by prescription or OTC). Examples of such medicinal products include nitroglycerin, hypertensives, antihistamines, non-steroid anti-inflammatory drugs (including aspirin), cough syrups or syrups for the ARVI symptomatic treatment containing antihistamines and (or) either phenylpropanolamine or phentolamine;

any obvious difference in skin color between arms.

4. Restrictions imposed on subjects during the study

31. During the study, the following restrictions shall be imposed on the subjects:

no exercise with either arm, and no strenuous exercise overall is allowed for study duration;

no bathing or showering during the periods of medicinal product application and assessment of skin blanching is allowed; no use of cosmetic products in soft forms (creams, emollients, or similar products) to forearms for 24 hours prior to and throughout the study.

5. Subject screening for treatment response

32. Inclusion of the nonresponders reduces the ability of a study to detect true differences between test and reference products according to the results of the study. Therefore, for both the pilot «dose duration – response» study and the pivotal bioequivalence study, only responders, i.e., subjects

who have a pronounced vasoconstriction in response to the use of the reference product, should be included.

33. Quantitative assessment of skin blanching in the pilot and pivotal studies by the chromameter is considered as the most acceptable. However, due to the discrete multiple unit scale (0 to 3 or 0 to 4 points) for visual reading, the determination of the status of «respondent» may be based on visual assessment. The suggested dose duration is 4 hours (for a potency group III products) or 6 hours, with an assessment of skin blanching 2 hours after removal of the medicinal product. The subject who responded to the treatment demonstrates a change of at least one unit in the visual assessment.

34. To preserve skin sites on the forearm for use in the «dose duration – response» study or bioequivalence study, responder status may be based on studies conducted at sites other than the forearm.

35. Criteria for identification of responders, including dose duration, magnitude of response, and skin site tested, should be included in the study report. Responder status may also be confirmed from participation in a previous vasoconstriction analysis.

6. Validation of analytical procedure precision

36. Validation of analytical procedure precision within the site or intersites should be documented in four to six subjects who meet the criteria and restrictions of subsections 2 to 4. Four untreated control sites on each ventral forearm should be selected. Four chromameter readings of each site should be made within one hour period.

37. The validation study confirms acceptable precision by the bioequivalence testing firm in utilizing the chromameter for the measurement

the level of skin blanching. The study should be conducted prior to administration of the medicinal product.

38. Results should be included in the pilot study report, if submitted, and in the pivotal *in vivo* bioequivalence study report.

7. Use of occlusal and non-occlusive application conditions of medicinal product

39. Information about topical corticosteroid products allows the use of an occlusive film for the treatment of psoriasis or recalcitrant diseases. Information on the medicinal product (Summary of Product Characteristics, Basic Prescribing Information) provides details on the possibility or inadmissibility of the use of these medicinal products with occlusive films. Provided occlusion film is allowed based on the product information to the reference product, the pilot «dose duration – response» study and pivotal *in vivo* bioequivalence study may be conducted using occlusive film. However, such studies are not preferable, since the analysis of previously conducted pilot studies suggests that the ED_{50} (the dose duration to be used in the pivotal study) decreases with increasing potency of topical corticosteroid product. Evaluation of «dose duration – response» correlation requires dose duration data at times less than the ED_{50}. Very short dose duration is difficult to conduct during the study and contributes to high variability in response. Thus, occlusion may be appropriate only for the lower potency products, e.g., potency groups VI and VII. If occlusion is used for the pilot study, the same conditions should also be used for the pivotal study.

8. Methods of product application and removal

40. Either of two methods of application and removal may be utilized in the pilot and pivotal studies (see paragraph 60):

a) the first method. Staggered by time application with synchronized removal, in which the medicinal product is applied to skin sites at different times and removed at the same time. After synchronized measurement of the initial level of vasoconstriction, the product samples are applied in 6; 4 2; 1.5; 1; 0.75; 0.5; 0.25 hour before the synchronized removal of all applied samples of the product from the skin. Assessment of skin blanching is carried out after: 0 (at the moment of time immediately after removal of the product); 2, 4, 6, 19; 24 hours after removal of the product;

b) the second method. Synchronized application with staggered by time removal, in which the product is applied to skin sites at the same time and removed at different times. After synchronized measurement of the initial level of vasoconstriction, the product samples are applied simultaneously and then removed after 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6 hours after application. Assessment of skin blanching is carried out after 6, 8, 11, 24 and 28 hours after application of the product.

9. Standardization of the activity of study subjects during the study.

41. Subjects should begin all study phases at approximately the same time each day (deviations within one hour are allowed).

Verification of compliance with sufficient washout period relative for unauthorized products should be carried out.

42. The forearm should be free of any dirt or particulate matter that may interfere with proper application of the product or assessment of pharmacodynamic response. Cleansing of the skin is not recommended because of the possible effects on uptake of the active substance and pharmacodynamic response to the medicinal product. If necessary, cleansing should be performed not less than two hours prior to medicinal product application. If cleansing is performed, it should be noted in the study report.

43. Whether the study is conducted under occlusion or non-occlusion conditions, the protective agent, that does not produce the effect of an occlusive film to prevent smearing or removal of topical medicinal product from the skin site should be used. Care should be taken to avoid contact between the protective material and any medicinal product to prevent inadvertent contamination of untreated control sites or other test sites.

44. Skin sites should be no closer than 3 to 4 cm to the antecubital fossa or to the wrist.

45. Application of the reference product to skin sites of identical surface area on the ventral (internal) forearms. Suggested dose durations for the pilot study are 0.25, 0.5, 0.75, 1, 1.5, 2, 4 and 6 hours, but may vary depending on the test corticosteroid product. Eight dose of the medicinal product, (or eight skin sites with applied medicinal product (active skin sites), should be equally divided between the two arms of the study subject.

46. Amount of medicinal product, skin site size, and spacing between sites should be determined by the research center. In studies, doses of 2 to 10 mg/cm^2 of the skin surface and areas with a diameter of 1 cm should be used, unless otherwise specifically rationalized. Sites should be spaced as close as 2.5 cm center-to-center, or staggered pattern, depending on skin surface

suitability (for example, vascularity, nevi, etc.) and arm length. The subject should be excluded from the data analysis if the vasoconstrictor responses of two adjacent sites overlap, and the investigator cannot distinguish the vasoconstrictor response at each test site.

47. The inclusion of two untreated control skin sites on each arm for studies based on chromameter measurements.

48. Sites where eight doses of various durations (see paragraph 42) and four untreated control sites are applied should be randomly assigned among 12 sites selected in a subject, six sites should be per subject's arm: 2 untreated control sites and 4 dosed sites.

49. Studies based on visual assessment do not require the allocation of untreated control sites because the assessment involves a visual comparison of the treated site and the surrounding skin. The sites of application to each subject of 8 product doses with different exposure duration are randomly assigned between the two arms, while there are 4 dosed sites on each subject's arm.

50. Prior to measurement of the pharmacodynamic response at the end of the observation period, remaining topical corticosteroid product should be gently removed from the skin surface using any of the following methods:

a) 3 consecutive wipes of the skin with a dry cotton swab to remove the residual product. This method is suitable for applying the medicinal product at different times, followed by synchronized removal, as well as the synchronized application of the medicinal product to all sites, followed by removal through different periods of time;

b) washing all skin sites with mild skin cleanser and water, drying the sites dry with a nonabrasive paper towel, and subsequent drying in the open

air for at least five minutes prior to measuring the pharmacodynamic response. If five minutes after washing the treated skin sites, the subject has any visible cutaneous effects related to washing procedure itself, a longer waiting period may be necessary. Cleansing arm surfaces is carried out with a minimum amount of soft skin cleanser, for example, wetted hands are treated with one drop of a liquid cleanser, followed by rinsing.

51. Assessment of baseline skin color and skin blanching at each site. When applying the medicinal product at different times with subsequent synchronized removal for all dose exposure durations and untreated control sites, the baseline assessment is carried out within one hour before application of the medicinal product with the longest dose exposure duration and after 0, 2, 4, 6, 19 and 24 hours after removal of the product (see paragraph 37). Zero point corresponds to time of medicinal product removal.

When synchronized applying the medicinal product to all sites, followed by removal after different periods of time for all dose exposure durations and untreated control sites, the baseline assessment is carried out within one hour before applying the medicinal product to the active sites after 6, 8, 11, 24 and 28 hours after applying the medicinal product (see Addendum 2). Zero point corresponds to time of medicinal product application.

Optimal assessment time for either method of application and removal may require adjustment of these schedules for the particular medicinal product and test site. For either method, at least one result assessment should be scheduled between 17 and 24 hours.

10. Data analyses and pharmacodynamic modelling

52. If the data are obtained using a chromameter, then for each measured «skin blanching – time» relationship (for sites with applied medicinal product and untreated control sites), it is necessary to adjust the initial readings of the chromameter to the baseline of skin reaction at that site. Each site with applied medicinal product after the skin reaction baseline-adjustment should be corrected by the average value of the skin reaction in 2 control sites on the same arm with the skin reaction baseline-adjustment (Tables 1 to 3 of Addendum 4).

Using the trapezoidal method, it is necessary to calculate the following types of areas under the effect curve (AUEC) for each value that is adjusted based on the baseline of dose exposure duration of untreated control site (Tables 1, 4 of Addendum 4):

$AUEC_{0\text{-}24}$ the application of the medicinal product at different times with subsequent synchronized removal (see paragraph 37);

$AUEC_{6\text{-}28}$ for the synchronized application of the medicinal product to all sites with subsequent removal at different periods of time (see 37). In the general case, AUEC is calculated from the longest dose duration to 28 hours after medicinal product application.

53. Modeling of «dose duration – time» data by averaging over subjects at each time point of dose duration is not acceptable. Rather, the data should be modelled by using all observations of all individual subjects simultaneously.

The modeling software should calculate ED_{50} and E_{max} values for the data pooled from 12 subjects.

The following modeling methods are acceptable:

modeling within a nonlinear model with mixed effects (population model) using appropriate software (Fig. 1 of Addendum 1). The nonlinear model with mixed effects accounts for within- and among-subject variability;

modeling based on nonlinear regression by the least squares method with a pooling of individual observations of all subjects (naive pooled data method).

54. Based on the results of the assessment of the modeling data the following is determined for use in the pivotal study:

ED_{50} is the dose duration corresponding to half of maximal response;

D_1 is the value that corresponds to approximately half of the ED_{50};

D_2 is the value that corresponds to approximately twice the value of ED_{50}.

The observed ED_{50} value may be rounded to 15 minutes to obtain the ED_{50} value used in the pivotal study. It is acceptable to confirm the «dose duration – response" relation, based on D_1, which is 0.25 – 0.5 of the observed ED_{50} and D_2, which is 2 to 4 of the observed ED_{50}.

For potent corticosteroid products with short ED_{50} values, these recommendations may need the adjustment. These ED_{50} sampled values correspond to approximately 33% and 67%, respectively, of the maximal response, and represent the sensitive portion of the «dose duration – response» curve.

55. If the data are obtained by visual method (see subsection 2, section IV), the following should be calculated:

the area under the effect curve (AUEC) for each «vasoconstriction – time» profile;

the «dose duration – response» relationship as described in paragraph 66;

the ED_{50}, D_1, and D_2 values.

56. According to paragraph 26 of the Rules of marketing authorization and assessment of medicinal products for human use approved by Decision No. 78 of the Council of the Eurasian Economic Commission dated November 3, 2016, the sponsor of a clinical study may submit the proposed protocol of the pivotal study, data and summary of the results of the pilot study to the authorized authorities or expert organizations of the Member States for its assessment in the framework of scientific and pre-approval consultation in accordance with the legislation of the Member States if there are questions concerning:

validation of analytical procedure;

«dose duration – response» profile;

other aspects of the pilot study of «dose duration – response» prior to the pivotal *in vivo* bioequivalence study;

rationale for the choice of ED_{50}, D_1 and D_2 values.

When submitting the results of the pilot study, the sponsor may include all study data with the rationale of all data not included in the pharmacodynamic analysis. Sponsors may decide that they have sufficient information about the «dose duration – response» relationship of the topical corticosteroid product under study to proceed to the pivotal study without conduct of the pilot study. This decision should be based on the knowledge of ED_{50}, D_1, and D_2 values of the relevant reference product obtained in the clinical studies of this reference product as part of its pivotal study. According to paragraph 26 of the Rules of marketing authorization and

assessment of medicinal products for human use, in order to approve this decision, the sponsor may apply to the authorized authorities or expert organizations of the Member States for scientific and pre-approval consultation in accordance with the Member States' legislation.

57. Format of computer data representation.

Primary chromameter data, baseline-adjusted data, baseline-adjusted, untreated control site-corrected data and AUEC calculation data should be attached to the study report as separate files.

VI. Pivotal *in vivo* bioequivalence study

58. The purpose of the pivotal study is to confirm *in vivo* bioequivalence of the test product to the corresponding reference product. The Annex specifies the minimum dose duration – response ratio which must be met by individual subjects for inclusion in the data analysis. Therefore, a pivotal study may generally be initiated without consultation with the regulatory body.

1. Study design

59. Pharmacodynamic bioequivalence study using replicate single dose durations of test and reference products and based on the population ED_{50} value identified in the pilot study.

60. Individual «dose duration – response» relationships based on an acceptable D_2/D_1 ratio for AUEC values of the reference product. The minimum value of the ratio should be 1.25. Compliance with this criterion is determined by the duplicate application of the reference product at a D_1 dose

that corresponds to approximately half of the population ED_{50}, and D_2 that corresponds to approximately doubled population ED_{50}.

61. 40 to 60 evaluable subjects, i.e.,subjects who meet the «responder» and «detector» criteria described in p. 27 and p. 66.

3. Inclusion criteria

62. The study applies the inclusion criteria described in subsection 2, section V of these Requirements.

4. Exclusion criteria

63. The study applies the exclusion criteria described in subsection 3, section V of these Requirements.

5. Study restrictions

64. The study applies limitations described in subsection 4, section V of these Requirements.

6. Subject response test
(subject screening for response)

65. The subject response study (subject screening for response) is described in subsection 5, section V of these Requirements.

7. Validation of analytical procedure precision

66. The validation of analytical procedure precision is described in subsection 6, section V of these Requirements.

8. Activities taken during the study day

67. The activity standardization of the subjects during the study is carried out in accordance with the requirements of subsection 9, section V of these Requirements.

68. Application of test dose durations to skin sites on the ventral forearms of each subject should be randomly assigned according to the recommendations described below. Sites may be occluded or non-occluded, based on the information given in paragraph 37, and the results of the pilot study. Untreated control skin sites left on each arm for studies based on chromameter assessments.

Dose durations and control sites on each arm should include:

the test product at the dose duration corresponding approximately to ED_{50}, as determined with the reference product in the pilot study (two sites per arm);

the reference product at the same dose duration corresponding approximately to ED_{50} as for the test product (two sites per arm);

D_1: the shorter dose duration of the reference product (one site per arm);

D_2: the longer dose duration of the reference product (one site per arm);

the untreated control sites (two sites per arm).

The total number of testing sites is 16 (eight sites per arm). The eight treatments should be randomized, as noted above. Application patterns on each arm should be complementary, i.e., D_2 is complementary to D_1, sites with the reference product is complementary to sites with test product, and control sites are also complementary. For example, where the reference product is applied to a specific skin site location on one arm, the reference

product should be applied to the corresponding skin site location on the other arm. When determining the skin on one arm as a control, the corresponding site on the second arm should also become a control.

A representative application sequence for a particular subject might be:

Antecubital fossa	
Left arm	Right arm
D_1	D_2
TMP	RMP
C	C
RMP	TMP
C	C
TMP	RMP
D_2	D_1
RMP	TMP
Wrist	
TMP is a test medicinal product; RMP is a reference medicinal product; C is a control site; D_1 is a dose duration of approximately $0,5xED_5$; D_2 is a duration of dose of approximately $2xED_{50}$.	

The sponsor’s responsibility is to describe the specific pattern of skin sites where medicinal products are applied that is, the medial (ulnar) and lateral (radial) locations of the sites with respect to the body axis, as well as the locations of the sites above and below with respect to each other.

69. Either the staggered by time application of medicinal product with synchronized removal or the synchronized application method with staggered removal, according to the methodology used in the pilot study, should be used for applying ED_{50}, D_1 and D_2 dose durations.

70. Examples of time periods for assessment of baseline skin color and skin blanching at each site are:

For staggered by time application with synchronized removal: for all dose durations and untreated control sites, baseline assessment is carried out within one hour prior to product application of the longest dose duration and after 0, 2, 4, 6, 19 and 24 hours after the removal of the medicinal product. Actual time will depend upon the time of dosing and the topical corticosteroid being studied. Zero point corresponds to time of medicinal product removal.

For synchronized application with staggered by time removal: for all dose durations and untreated control sites, baseline assessment is carried out within one hour prior to the time of product application to active sites; skin blanching is evaluated after application of the medicinal product at point D_2 and after 6, 8, 11, 24 and 28 hours. Actual time will depend upon the time of dosing and the topical corticosteroid being studied. Zero point corresponds to time of medicinal product application.

For example, if D_2 for a specific medicinal product equals 4 hours, the first post-baseline assessment of skin blanching at all sites, both with treated sites and untreated control sites, should be at 4 hours. For either method, at least the first post-baseline reading should be scheduled between 17.00 and 24.00.

9. Data and statistical analyses

Data analysis

71. The adjustment of the chromameter primary readings for each «skin blanching – time» profile (for treated sites and untreated control sites) is required for the baseline value at that site. The data of each product treated site adjusted based on the baseline should be corrected for the mean value of the two untreated control sites adjusted based on the baseline from the same arm.

The AUEC should be calculated for each baseline-adjusted, untreated control site-corrected dose duration:

$AUEC_{(0\text{-}24)}$ for the staggered application followed by synchronized removal;

$AUEC_{(D2\text{-}28)}$ (from time D_2 to 28 hours) for the synchronized application of product followed by staggered removal.

Only the data of «detectors», i.e., individual readings of subjects whose AUEC values at D_1 and D_2 are both negative and that meet the «dose duration – response» criterion below, should be included in the data analysis.

«Dose duration – response» criterion is:

$$\frac{AUEC \ \ at \ \ D_2}{AUEC \ \ at \ \ D_1} \geq 1.25$$

where:

AUEC at D_2 moment= 0.5×(AUEC at D_2 moment (left arm) + AUEC at D_2 moment (right arm);

AUEC at D_1 moment= 0.5×(AUEC at D_1 moment (left arm) + AUEC at D_1 moment (right arm).

72. Only subjects with a complete data set, i.e., duplicate values of D_1 and D_2, and quadruplicate values for test product, reference product, and control sites, should be included in the data analysis.

73. The bioequivalence comparison should be based on AUEC values calculated in accordance with paragraph 63 at the dose duration corresponding approximately to ED_{50} (for the test and reference medicinal product in accordance with the recommendations of paragraph 61).

74. All study data, including the data of «non-detectors», should be submitted. An explanation (for example, for a «non-detector», vasoconstrictor effect was overlapped due to an adjacent site, etc.) should accompany any data not used in the bioequivalence evaluation.

Statistical analysis

75. The statistical analysis requires the use of untransformed data because AUEC values for test and reference products, calculated from baseline-adjusted, untreated control site-corrected data, although generally negative, are sometimes positive. The presence of both positive and negative data eliminates the use of conventional statistical transformations. Previously used approximate methods, for example calculating a confidence interval for the difference between test and reference product averages, and calculating the ratio of the limits of a given confidence interval and estimating the average value of the reference product, are not applicable. Locke's method allows to calculate an exact confidence interval from untransformed data.

76. The 90% confidence interval should be calculated for the ratio of the average AUEC value due to the test product (average of four replicates)

to the average AUEC value due to the reference product (average of four replicates) using Locke's method. The formulae for the calculation are given in Addendum 3.

77. Currently there are no limits on recognition of bioequivalence. During the evaluation of the data submitted, it may be necessary to use the recognition levels of bioequivalence wider than 80-125%, which are standard.

When the equivalence interval is wider than 80.00 to 125.00%, established as a generally accepted standard, additional evaluation of data may be necessary.

78. A randomization code should be submitted with the study report, indicating specific skin sites for each dose duration and control sites.

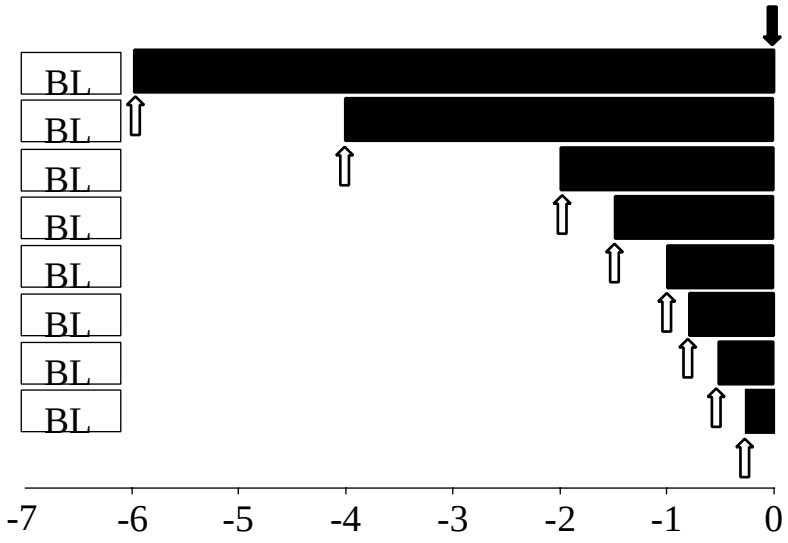
Format of computer data representation

79. Primary chromameter data, baseline-adjusted data, baseline-adjusted, untreated control site data and AUEC data should be attached as separate files.

ADDENDUM 1
to Annex No. 9
to the Rules for Conducting
Bioequivalence Studies of Medicinal Products
within the Eurasian Economic Union

SCHEME
for a suggested pilot study protocol staggered by time application with synchronized removal of a medicinal product

Fig. 1
Evaluation diagram of the baseline skin color, application and removal of the medicinal product.



Time before removal of the medicinal product, h.

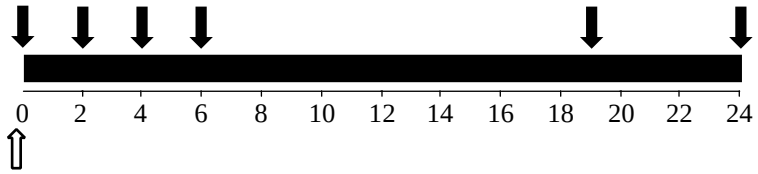
Legend: BL is baseline of vasoconstriction;

⇧ – application of the medicinal product;

⬇ – removal of the medicinal product;

Fig. 2

Skin blanching evaluation diagram



Time after removal of the medicinal product, h.

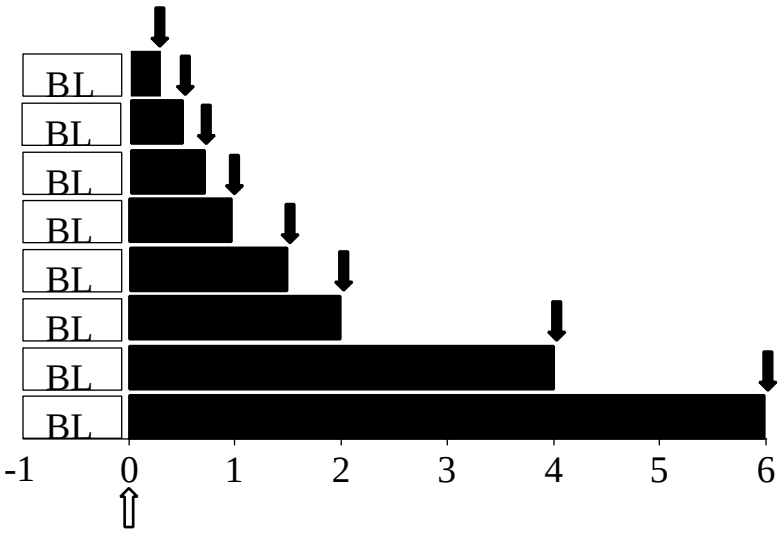
- ⇧ medicinal product removal time;
- ⬇ results evaluation time.

ADDENDUM 2
to Annex No. 9
to the Rules for Conducting
Bioequivalence Studies of Medicinal Products
within the Eurasian Economic Union

SCHEME
for a suggested pilot study protocol with synchronized application and staggered by time removal of the medicinal product

Fig. 3

Evaluation diagram of the baseline skin color, application and removal of the medicinal product



Time after application of the medicinal product, h.

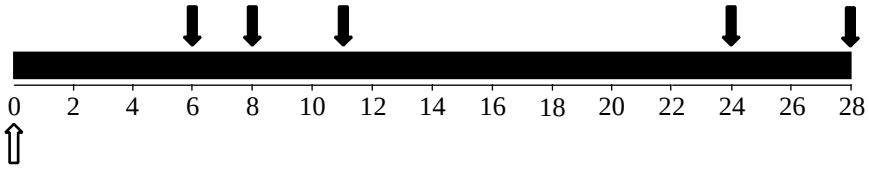
Legend: BL is baseline of vasoconstriction;

⇧ – application of the medicinal product

⬇ – removal of the medicinal product.

Fig. 4

Skin blanching evaluation diagram



Time after application of the medicinal product, h.

Legend:

⇧ – medicinal product application time;

- ⬇ – results evaluation time.

ADDENDUM 3
to Annex No. 9
to the Rules for Conducting
Bioequivalence Studies of Medicinal Products
within the Eurasian Economic Union

EXAMPLE
of tabular and graphical representation of pilot study results

1. Studies based on the staggered by time application of the medicinal product followed by synchronized removal, and based on synchronized application of the medicinal product to all sites, followed by staggered removal are equally acceptable.

2. Table 1 presents chromameter data for 1 subject within 24 hours. Baseline-adjusted data are presented in Table 2, and baseline-adjusted, untreated control site corrected data are presented in Table 3. In this example, each site treated with the medicinal product was corrected for its corresponding untreated control site.

3. In this example, the results of the study using only two untreated control sites per arm are given. Correction of skin color values in the remaining sites of the arm was carried out by subtracting the average value of these two control sites from each value of the site with the application of the medicinal product on the same arm.

Table 4 presents $AUEC_{0-24}$ values. The E_{max} model based on the pooled data presented in Fig. 5.

Table 1

Chromameter readings for a subject

Subject	Dose duration, hours	Site	Base line	Hours after the medicinal product removal					
				0	2	4	6	19	24
1	0.25	unt*	9.86	9.99	10.10	9.52	10.03	10.40	9.65
1	0.25	trt*	10.36	9.89	10.38	10.32	10.51	10.86	10.04
1	0.5	unt*	9.27	8.20	9.78	8.54	9.61	9.87	9.59
1	0.5	trt*	9.59	8.77	9.35	9.27	8.78	10.87	9.59
1	0.75	unt*	8.45	8.75	8.24	8.16	8.92	8.43	8.22
1	0.75	trt*	8.46	8.66	8.53	8.04	8.26	8.72	8.56
1	1	unt*	9.00	9.63	8.45	8.03	8.94	9.33	9.66
1	1	trt*	8.52	8.80	8.87	8.53	8.05	8.66	8.21
1	1.5	unt*	9.44	9.39	9.46	9.27	9.92	9.59	9.01
1	1.5	trt*	9.59	9.60	9.99	9.93	9.18	10.23	9.24
1	2	unt*	10.12	10.13	9.50	9.93	9.39	10.95	10.84
1	2	trt*	10.28	10.25	10.68	10.15	10.31	11.46	8.92
1	4	unt*	8.89	8.01	8.78	8.89	9.76	8.48	9.18
1	4	trt*	8.21	8.28	8.36	7.98	7.96	8.15	8.30
1	6	unt*	9.18	9.46	8.79	8.03	9.29	10.11	9.52
1	6	trt*	9.37	9.61	9.30	8.92	9.20	10.16	9.63

* Unt means skin site not treated with the medicinal product, trt means skin site treated with the medicinal product.

Table 2

Baseline-adjusted chromameter readings for a subject

Subject	Dose duration, hours	Site	Base line	Hours after the medicinal product removal					
				0	2	4	6	19	24
1	0.25	unt*	-	0.13	0.24	-0.34	0.17	0.54	-0.21
1	0.25	trt*	-	-0.47	0.02	-0.04	0.15	0.50	-0.32
1	0.5	unt*	-	-1.07	0.51	-0.73	0.34	0.60	0.32
1	0.5	trt*	-	-0.82	-0.24	-0.32	-0.81	0.81	0.23
1	0.75	unt*	-	0.30	-0.21	-0.29	0.47	-0.02	-0.23
1	0.75	trt*	-	0.20	0.07	-0.42	-0.20	0.26	0.10
1	1	unt*	-	0.63	-0.55	-0.97	-0.06	0.33	0.66
1	1	trt*	-	0.28	0.35	0.01	-0.47	0.14	-0.31
1	1.5	unt*	-	-0.05	0.02	-0.17	0.48	0.15	-0.43
1	1.5	trt*	-	0.01	0.40	0.34	-0.41	0.64	-0.35
1	2	unt*	-	0.01	-0.62	-0.19	-0.73	0.83	0.72
1	2	trt*	-	-0.03	0.40	-0.13	0.03	1.18	-1.36
1	4	unt*	-	-0.88	-0.11	0.00	0.87	-0.41	0.29
1	4	trt*	-	0.07	0.15	-0.23	-0.25	-0.06	0.09
1	6	unt*	-	0.28	-0.39	-1.15	0.11	0.93	0.33
1	6	trt*	-	0.24	-0.07	-0.45	-0.17	0.79	0.26

* Unt means skin site not treated with the medicinal product, trt means skin site treated with the medicinal product.

Table 3

Baseline-adjusted, untreated control site-corrected chromameter readings for a subject

Subject	Dose duration, hours	Site	Base line	Hours after the medicinal product removal					
				0	2	4	6	19	24
1	0.25	unt*	-	0.13	0.24	-0.34	0.17	0.54	-0.21
1	0.25	trt*	-	-0.47	0.02	-0.04	0.15	0.50	-0.32
1	0.5	unt*	-	-1.07	0.51	-0.73	0.34	0.60	0.32
1	0.5	trt*	-	-0.82	-0.24	-0.32	-0.81	0.81	0.23
1	0.75	unt*	-	0.30	-0.21	-0.29	0.47	-0.02	-0.23
1	0.75	trt*	-	0.20	0.07	-0.42	-0.20	0.26	0.10
1	1	unt*	-	0.63	-0.55	-0.97	-0.06	0.33	0.66
1	1	trt*	-	0.28	0.35	0.01	-0.47	0.14	-0.31
1	1.5	unt*	-	-0.05	0.02	-0.17	0.48	0.15	-0.43
1	1.5	trt*	-	0.01	0.40	0.34	-0.41	0.64	-0.35
1	2	unt*	-	0.01	-0.62	-0.19	-0.73	0.83	0.72
1	2	trt*	-	-0.03	0.40	-0.13	0.03	1.18	-1.36
1	4	unt*	-	-0.88	-0.11	0.00	0.87	-0.41	0.29
1	4	trt*	-	0.07	0.15	-0.23	-0.25	-0.06	0.09
1	6	unt*	-	0.28	-0.39	-1.15	0.11	0.93	0.33
1	6	trt*	-	0.24	-0.07	-0.45	-0.17	0.79	0.26

* Unt means skin site not treated with the medicinal product, trt means skin site treated with the medicinal product.

Table 4

Baseline-adjusted, untreated control site-corrected chromameter readings and $AUEC_{(0-24)}$ data for a subject

Subject	Dose duration, hours	Site	Base line	Hours after the medicinal product removal						AUEC(0-24)**
				0	2	4	6	19	24	
1	0.25	trt*	-	-0.60	-0.22	-0.30	-0.02	-0.04	-0.11	-1.23
1	0.5	trt*	-	0.25	-0.75	0.41	-1.15	0.21	-0.09	-7.39
1	0.75	trt*	-	-0.10	0.28	-0.13	-0.67	0.28	0.33	-1.48
1	1	trt*	-	-0.35	0.90	0.98	-0.41	-0.19	-0.97	-3.80
1	1.5	trt*	-	0.06	0.38	0.51	-0.89	0.49	0.08	-0.23
1	2	trt*	-	-0.04	1.02	0.06	0.76	0.35	-2.08	5.77
1	4	trt*	0.95	0.26	-0.23	-1.12	0.35	-0.20	-4.74	1
1	6	trt*	-0.04	0.32	0.70	-0.28	-0.14	-0.07	-1.53	1

* Unt means skin site not treated with the medicinal product, trt means skin site treated with the medicinal product.

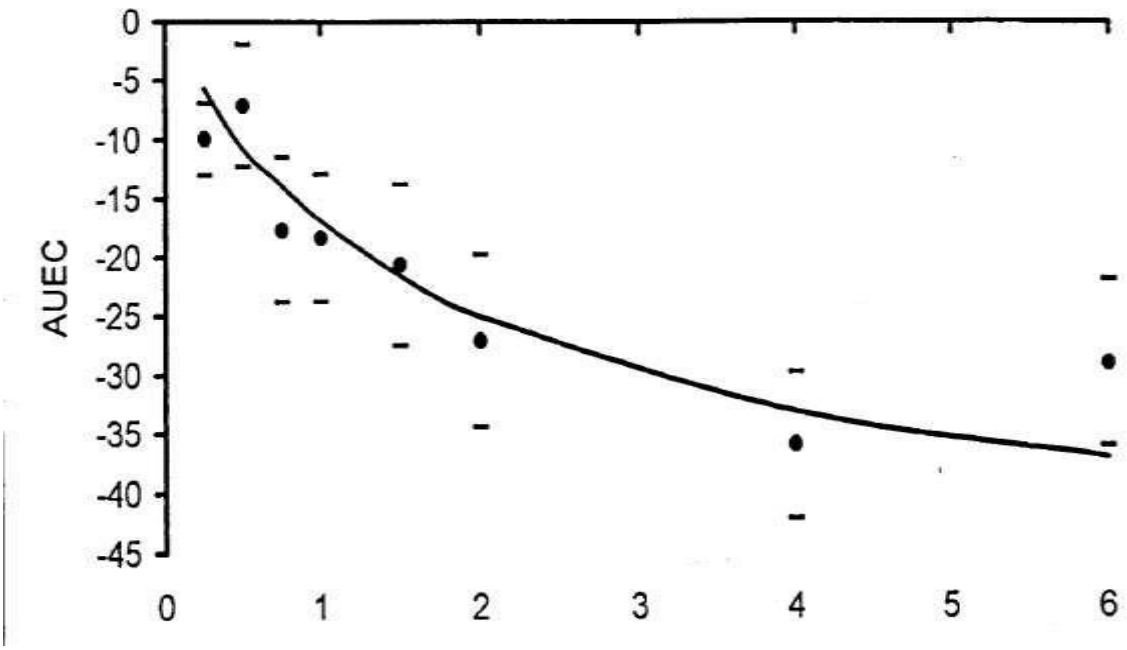
** $AUEC_{(0-24)}$ values are calculated from the data adjusted relative to the baseline of the vascular response and the color change of the control skin sites at the appropriate time points.

Table 5

$AUEC_{(0-24)}$ values for all 12 subjects at each dose duration

Dose duration, hours	Subject number											
	1	2	3	4	5	6	7	8	9	10	11	12
0.25	-1.23	-0.02	-13.87	-27.7	-10.65	-10.41	4.20	-11.95	-12.36	1.15	-30.03	-7.25
0.5	-7.39	-6.13	-15.03	-3.71	7.72	-5.94	-12.31	7.45	12.95	-39.45	-39.56	14.73
0.75	-1.48	-8.92	-18.39	-43.82	-23.42	-2.29	1.34	5.95	1.88	-40.68	-61.06	-21.09
1	-3.80	-24.56	-16.25	-44.39	-20.37	-8.92	-18.84	8.78	-43.35	-16.19	-43.58	10.81
1.5	-0.23	-19.21	-15.44	-77.04	-19.95	-20.64	-42.70	1.26	-20.97	6.87	-40.73	0.51
2	5.77	-1.80	-23.74	-66.80	-32.00	-19.52	-37.29	-48.83	-39.79	10.75	-62.01	-10.51
4	-4.74	-43.07	-24.80	-62.96	-32.81	-8.52	-45.46	-71.77	-57.55	-37.64	-27.82	-14.89
6	-1.53	-41.56	-21.79	-71.60	-61.51	-19.01	-37.24	-8.14	-34.18	-35.01	-33.60	16.14

Observed AUEC(0-24) mean values (black circles) and standard error of the mean (upper and lower limits), and Emax model (solid line) based on the pooled «dose duration – response data» of all 12 subjects in the pilot study



In the example presented in this Addendum:

data are baseline-adjusted and untreated control-site corrected, thus AUEC value is assumed to be zero at dose duration equal to zero;

the Emax modelling was carried out using a software foe population pharmacokinetic-dynamic data modeling. The modeled population values were: ED_{50} = 1.89 hours E_{max} = -48.80 standard units of the instrument scale depending on the dose duration.

based on these data of the pilot study, the dose durations selected as the approximate ED50 for the comparison of test and reference products, and as D1 and D2 in the pivotal in vivo bioequivalence study were: approximated ED_{50} = 2.0 hours, D_1 = 1.0 hour and D_2 = 4.0 hours.

ADDENDUM 4
to Annex No. 9
to the Rules for Conducting
Bioequivalence Studies of Medicinal Products
within the Eurasian Economic Union

EXAMPLE
of tabular and graphical representation,
and analysis of pivotal study data

This example presents the chromameter data and AUEC(0-24) values obtained in the in vivo pivotal bioequivalence study based on the results of a pilot study, the data of which are given in Addendum 3. In the pivotal study, the comparison was based on the «dose duration – response» relationship in accordance with the study design shown in Figure 1 of Addendum 1. Table 1 presents initial data for one subject within 24 hours. Table 2 presents baseline-adjusted data. Table 3 presents baseline-adjusted, untreated control site-corrected data. In this example, each site treated with the medicinal product was corrected for its corresponding untreated control site. The Addendum recommends use of only two untreated control sites per arm, and subtracting their average from each value of treated sites of the same arm. Table 4 presents the baseline-adjusted and untreated control site-corrected values for test and reference products for all subjects. Table 5 presents right and left arm $AUEC_{(0-24)}$ data, and the two arm average data for D_1 and D_2 for all subjects. Table 5 identifies «detectors,» i.e., evaluable subjects, who meet the «dose duration –

response» criterion. Table 6 presents right and left arm $AUEC_{(0-24)}$ data, and the two arm average data for test and reference products for all subjects at a dose duration of 2.0 hours, and highlights the two arm average $AUEC_{(0-24)}$ data of the «detectors». Only the data of «detectors» are included in the bioequivalence assessment, as described in Addendum 5.

Table 1

Subject	Treatment*	Arm	Location of application	Site	Base line	Hours after the medicinal product removal					
						0	2	4	6	19	24
1	A	R	1	unt	7.34	7.23	8.09	7.64	7.82	7.68	8.71
1	A	R	1	trt	7.11	7.86	7.59	5.92	6.23	6.32	7.30
1	B	R	2	unt	6.18	7.38	7.26	6.85	7.35	7.14	7.87
1	B	R	2	trt	6.79	6.29	6.12	4.45	5.88	6.01	7.26
1	C	R	3	unt	6.28	7.32	7.80	6.77	7.75	6.59	7.55
1	C	R*	3	trt	7.78	9.26	9.30	7.42	8.24	7.40	8.59
1	D	R*	4	unt*	9.31	10.19	10.61	9.56	10.88	9.52	10.13
1	D	R*	4	trt*	7.38	8.22	6.94	5.07	6.98	7.24	7.91
1	C	L*	1	unt*	7.62	7.98	7.56	7.48	7.24	6.73	7.49
1	C	L*	1	trt*	6.97	5.42	5.39	4.39	4.79	5.76	6.45
1	B	L*	2	unt*	7.12	6.32	6.76	6.25	6.74	6.80	7.58
1	B	L*	2	trt*	7.46	4.48	4.38	4.11	4.39	6.27	7.25
1	A	L*	3	unt*	7.69	7.03	7.73	7.21	7.87	7.89	8.38
1	A	L*	3	trt*	8.99	8.75	8.07	6.74	6.53	7.14	8.25
1	D	L*	4	unt*	8.99	8.28	8.95	8.50	9.10	9.05	9.93
1	D	L*	4	trt*	8.80	8.04	6.71	5.51	5.14	7.05	7.96

*Note:
R means right arm, L means left arm;
unt means skin site not treated with the medicinal product (without application of any medicinal product) corresponding to each treated site; trt means treated site of the skin;
treatment A means a reference product at dose duration D1 (1.0 hour)

treatment B means a reference product at dose duration D_2 (4.0 hours)
treatment C means a test product at dose duration of 2.0 hours
treatment D means a reference product at dose duration of 2.0 hours

Table 2

Baseline-adjusted chromameter readings for a subject

Subject	Treatment*	Arm	Location of application	Site	Base line	Hours after the medicinal product removal					
						0	2	4	6	19	24
1	A	R*	1	unt*	-	-0.11	0.75	0.30	0.48	0.34	1.37
1	A	R*	1	trt*	-	0.75	0.48	-1.19	-0.88	-0.79	0.19
1	B	R*	2	unt*	-	1.20	1.08	0.67	1.17	0.96	1.69
1	B	R*	2	trt*	-	-0.50	-0.67	-2.34	-0.91	-0.78	0.47
1	C	R*	3	unt*	-	1.04	1.52	0.49	1.47	0.31	1.27
1	C	R*	3	trt*	-	1.48	1.52	-0.36	0.46	-0.38	0.81
1	D	R*	4	unt*	-	0.88	1.30	0.25	1.57	0.21	0.82
1	D	R*	4	trt*	-	0.84	-0.44	-2.31	-0.40	-0.14	0.53
1	C	L*	1	unt*	-	0.36	-0.06	-0.14	-0.38	-0.89	-0.13
1	C	L*	1	trt*	-	-1.55	-1.58	-2.58	-2.18	-1.21	-0.52
1	B	L*	2	unt*	-	-0.80	-0.36	-0.87	-0.38	-0.32	0.46
1	B	L*	2	trt*	-	-2.98	-3.08	-3.35	-3.07	-1.19	-0.21
1	A	L*	3	unt	-	-0.66	0.04	-0.48	0.18	0.20	0.69
1	A	L*	3	trt*	-	-0.24	-0.92	-2.25	-2.46	-1.85	-0.74
1	D	L*	4	unt*	-	-0.71	-0.04	-0.49	0.11	0.06	0.94
1	D	L*	4	trt*	-	0.76	-2.09	-3.29	-3.66	-1.75	-0.84

*Notes:
treatment A means a reference product at dose duration D_1 (1.0 hour)
treatment B means a reference product at dose duration D_2 (4.0 hours)
treatment C means a test product at dose duration of 2.0 hours
treatment D means a reference product at dose duration of 2.0 hours
R means right arm L means left arm
unt means skin site not treated with the medicinal product (without application of any medicinal product) corresponding to each treated site;
trt means treated site of the skin;

Table 3

Baseline-adjusted, untreated control site-corrected chromameter readings and AUEC(0-24) data for a subject

Subject	Treatment*	Arm	Location of application	Site	Base line	Hours after the medicinal product removal						$AUEC_{(0-24)}$
						0	2	4	6	19	24	
1	A	R*	1	trt*	-	0.86	-0.27	-1.49	-1.36	-1.13	-1.18	-25.98
1	B	R*	2	trt	-	-1.70	-1.75	-3.01	-2.08	-1.74	-1.22	-45.53
1	C	R*	3	trt*	-	0.44	0.00	-0.85	-1.01	-0.69	-0.46	-16.20
1	D	R*	4	trt*	-	-0.04	-1.74	-2.56	-1.97	-0.35	-0.29	-27.29
1	C	L*	1	trt*	-	-1.91	-1.52	-2.44	-1.80	-0.32	-0.39	-27.19
1	B	L*	2	trt*	-	-2.18	-2.72	-2.48	-2.69	-0.87	-0.67	-42.26
1	A	L*	3	trt*	-	0.42	-0.96	-1.77	-2.64	-2.05	-1.43	-46.87
1	D	L*	4	trt*	-	-0.05	-2.05	-2.80	-3.77	-1.81	-1.78	-58.77

*Note:
Treatment A means a reference product at dose duration D_1 (1.0 hour)
Treatment B means a reference product at dose duration D_2 (4.0 hours)
Treatment C means a test product at dose duration of 2.0 hours
Treatment D means a reference product at dose duration of 2.0 hours
R means right arm, L means left arm, trt means treated site of the skin

Table 4

Baseline-adjusted, untreated control site-corrected chromameter readings for 12 subjects

Test medicinal product

Subject	Treatment*	Arm	Application site	Hours after the medicinal product removal					
				2	2	2	2	2	2
1	C	R*	3	0.44	0.00	-0.85	-1.01	-0.69	-0.46
1	C	L*	1	-1.91	-1.52	-2.44	-1.80	-0.32	-0.39
2	C	R*	3	-1.51	-3.29	-3.45	-4.11	-0.89	-1.26
2	C	L*	1	0.23	-1.09	-0.94	-2.15	-2.05	-0.66
3	C	L*	3	-1.29	-1.75	-0.96	-0.90	-3.06	-1.05
3	C	R*	2	0.02	-1.43	-2.24	-1.16	-1.56	-1.72

Subject	Treatment*	Arm	Application site	Hours after the medicinal product removal					
				2	2	2	2	2	2
4	C	R*	1	-0.02	-0.19	-0.52	-1.00	-0.43	-0.50
4	C	L*	3	-0.12	0.15	-0.29	-0.06	-0.07	0.12
5	C	L*	3	-0.36	-0.01	-0.19	-0.06	-0.72	-0.28
5	C	R*	1	-0.02	-0.63	-1.13	-0.90	-0.88	-0.03
6	C	R*	4	-0.80	0.60	0.32	0.30	-1.09	-1.53
6	C	L*	3	-1.08	-0.45	-0.98	-0.83	-1.18	-0.07
7	C	R*	4	-0.28	0.25	-0.34	-0.64	-0.64	-0.41
7	C	L*	1	0.67	0.74	0.72	1.03	0.33	-0.11
8	C	R*	4	-0.40	0.49	0.46	0.00	-0.35	0.78
8	C	L*	3	0.30	0.05	0.07	0.19	-0.05	-0.28
9	C	R*	1	-0.71	-1.13	-1.94	-2.40	-1.70	-1.41
9	C	L*	3	-0.34	-0.52	-1.46	-1.41	-0.31	-1.10
10	C	R*	1	-0.49	-0.43	-0.63	-0.10	-0.50	-1.10
10	C	L*	3	0.10	-0.66	-0.44	-0.68	-0.34	-0.86
11	C	L*	2	-0.58	-0.93	-1.60	-2.29	-0.24	-0.54
11	C	R*	3	0.12	-1.67	-1.71	-2.34	0.15	-1.28
12	C	L*	3	0.05	-0.08	-0.18	-0.35	-1.28	-0.46
12	C	R*	3	-0.60	0.15	0.19	-0.42	-0.40	-0.32
Mean value				-0.30	-0.57	-0.86	-0.96	-0.76	-0.62
Standard deviation, SD				0.65	0.91	1.01	1.12	0.76	0.59
Standard error of mean, SE				0.13	0.19	0.21	0.23	0.16	0.12
Coefficient of variation, %CV				217	161	118	117	100	96

*Note:
Treatment C means a test product at dose duration of 2.0 hours
R means right arm, L means left arm;

Reference medicinal product

Subject	Treatment*	Arm	Application site	Hours after the medicinal product removal					
				2	2	2	2	2	2
1	D	R*	4	-0.04	-1.74	-2.56	-1.97	-0.35	-0.29

1	D	L*	4	-0.05	-2.05	-2.80	-3.77	-1.81	-1.78
2	D	R*	4	-0.23	-1.58	-2.53	-2.53	0.00	-0.49
2	D	L*	4	-2.30	-2.88	-2.15	-3.05	2.09	0.27
3	D	R*	1	1.25	-0.10	-1.99	-1.52	0.24	-1.24
3	D	L*	4	-0.04	-0.28	-1.30	-1.23	-0.77	-1.07
4	D	R*	4	-0.43	-0.34	-1.50	-1.80	-0.74	-0.96
4	D	L*	2	-0.47	-0.22	-0.49	-0.83	-0.89	-0.82
5	D	L*	2	-0.71	-1.77	-1.62	-2.62	-0.76	-0.60
5	D	R*	3	0.46	-1.23	-1.23	-1.61	-1.70	-0.47
6	D	R*	1	-0.11	0.20	1.35	0.86	-0.77	-1.00
6	D	L*	4	-0.95	-1.07	-0.52	-1.17	-2.33	-1.52
7	D	R*	2	-0.22	-0.30	-0.42	-0.18	-0.74	-1.00
7	D	L*	4	-0.51	0.03	-0.76	-0.12	-0.42	-1.24
8	D	R*	2	0.51	0.30	0.92	0.63	0.56	0.34
8	D	L*	1	-0.44	0.08	-0.16	-0.95	-2.00	-1.49
9	D	R*	4	-0.40	-1.15	-2.25	-2.57	-1.20	-1.55
9	D	L*	2	-1.16	-1.05	-1.90	-1.80	-1.06	-1.42
10	D	L*	3	0.28	-0.31	-1.16	-1.40	-0.64	-0.57
10	D	R*	1	-0.14	-0.05	-0.24	-0.63	-0.41	-1.09
11	D	R*	1	-0.46	-0.82	-1.10	-2.15	-0.47	-0.59
11	D	L*	4	-0.15	-1.45	-1.66	-1.61	-1.14	0.55
12	D	R*	1	-0.25	-0.76	-1.35	-2.29	-1.23	-0.99
12	D	L*	4	1.89	0.73	2.07	0.82	-0.59	0.70
Mean value				-0.19	-0.74	-1.06	-1.40	-0.71	-0.76
Standard deviation, SD				0.79	0.87	1.23	1.20	0.90	0.68
Standard error of mean, SE				0.16	0.18	0.25	0.25	0.18	0.14
Coefficient of variation, %CV				405	117	116	86	126	89

*Note:
Treatment D means a reference product at dose duration of 2.0 hours
R means right arm, L means left arm

Table 5

AUEC $_{(0-24)}$ values for the right arm, left arm and average value for two arms at dose durations equal to D1 and D2, as well as the ratio of the average AUEC value at D_2 to the average AUEC value at D_1 for 12 subjects

AUEC$_{(0-24)}$ at D_1				AUEC$_{(0-24)}$ at D_2				AUEC at D_2/ AUEC at D_1
Subject	Arm	AUEC	AUEC (mean)	Subject	Arm	AUEC	AUEC (mean)	
1	R*	-25.98	-36.42	1	R*	-45.53	-43.90	1.21
1	L*	-46.87		1	L*	-42.26		
2	R*	-62.43	-45.09	2	R*	-69.72	-59.96	1.33
2	L*	-27.76		2	L*	-50.20		
3	R*	-22.53	-28.41	3	R*	-31.87	-64.04	2.25
3	L*	-34.29		3	L*	-96.21		
4	R*	-7.49	-11.70	4	R*	-27.48	-23.30	1.99
4	L*	-15.91		4	L*	-19.12		
5	L*	-16.59	-17.36	5	L*	-25.01	-16.58	0.95
5	R*	-18.14		5	R*	-8.15		
6	R*	-8.24	-10.44	6	R*	-27.36	-9.33	0.89
6	L*	-12.64		6	L*	8.70		
7	R*	-10.89	-13.36	7	R*	-20.44	-23.68	1.77
7	L*	-15.83		7	L*	-26.92		
8	L*	7.08	4.69	8	L*	-26.16	-21.02	-4.48

8	R*	2.31		8	R*	-15.88		
9	L*	-34.22	-13.82	9	L*	-33.80	-21.39	1.55
9	R*	6.58		9	R*	-8.97		
10	L*	-4.10	3.06	10	L*	-52.60	-43.79	-14.29
10	R*	10.23		10	R*	-34.97		
11	R*	-33.30	-37.30	11	R*	-57.00	-52.20	1.40
11	L*	-41.30		11	L*	-47.40		
12	R*	-0.55	-21.06	12	R*	-29.24	-28.22	1.34
12	L*	-41.57		12	L*	-27.20		

*Note: R means right arm, L means left arm, circled value indicates AUEC ratio > 1.25

Table 6

$AUEC_{(0-24)}$ values for right and left arms, the two arm average value for test and reference products of 12 subjects at dose duration of 2.0 hours

$AUEC_{(0-24)}$ of test product					$AUEC_{(0-24)}$ of reference product				
Subject	Arm	Application site	AUEC	AUEC (mean)	Subject	Arm	Application site	AUEC	AUEC (mean)
1	R*	3	-16.20	-21.69	1	R*	4	-27.29	-43.03
1	L*	1	-27.19		1	L*	4	-58.77	
2	L*	3	-56.98	-48.52	2	R*	4	-28.65	-22.20
2	R*	1	-40.06		2	L*	4	-15.75	

3	L*	3	-43.63	-38.99	3	R*	1	-15.27	-18.65
3	R*	2	-34.36		3	L*	4	-22.03	
4	R*	1	-14.06	-7.62	4	R*	4	-26.67	-22.42
4	L*	3	-1.18		4	L*	2	-18.18	
5	L*	3	-8.39	-13.34	5	L*	2	-35.48	-34.25
5	R*	1	-18.29		5	R*	3	-33.01	
6	R*	4	-9.51	-15.23	6	R*	1	0.01	-18.83
6	L*	3	-20.96		6	L*	4	-37.68	
7	R*	4	-12.05	0.98	7	R*	2	-12.17	-10.96
7	L*	1	14.01		7	L*	4		
8	R*	4	0.30	0.56	8	R*	2	13.57	-7.94
8	L*	3	0.81		8	L*	1	-29.45	
9	R*	1	-43.68	-32.05	9	R*	4	-41.15	-37.40
9	L*	3	-20.42		9	L*	2	-33.65	
10	R*	1	-10.61	-11.51	10	L*	3	-20.35	-16.10
10	L*	3	-12.41		10	R*	1	-11.86	
11	L*	2	-26.33	-26.18	11	R*	1	-26.13	-26.73
11	R*	3	-26.04		11	L*	4	-27.33	
12	L*	3	-15.77	-11.62	12	R*	1	-35.19	-12.56
12	R*	3	-7.47		12	L*	4	10.08	
Mean value			-18.77	-18.77	Mean value			-22.59	-22.59
Standard deviation, SD			16.45	15.28	Standard deviation, SD			16.14	10.92
Standard error of mean, SE			3.36	3.12	Standard error of mean, SE			3.30	2.23
Coefficient of variation, %CV			88	81	Coefficient of variation, %CV			71	48

Note: R means right arm, L means left arm, circled AUEC data of seven subjects whose AUEC relations (see table 5) $\geq$ 1.25, i.e. suitable assessment subjects. This AUEC data is used in the calculation of the 90% confidence interval in Addendum 5.

ADDENDUM 5
to Annex No. 9
to the Rules for Conducting
Bioequivalence Studies of Medicinal Products
within the Eurasian Economic Union

Locke's method: formulae and an example

The calculation of the 90% confidence interval for the pivotal bioequivalence data based on Table 6 is given below. The data used to calculate the confidence interval are the average AUEC values of «detectors» only.

Table 7

Average AUEC values of subjects in the pivotal study meeting the «dose duration – response» criterion, given in paragraph 64 of the Annex

Subject	$AUEC_{(0-24)}$ of test product (mean)	$AUEC_{(0-24)}$ of reference product (mean)
2	-48.52	-22.20
3	-38.99	-18.65
4	-7.62	-22.42
7	0.98	-10.96
9	-32.05	-37.40
11	-26.18	-26.73
12	-11.62	-12.56

To calculate the confidence interval, the following intermediate values should be calculated:

$$\bar{X}_T = \frac{1}{n}\sum_{i=1}^{n} X_{Ti} \qquad \bar{X}_R = \frac{1}{n}\sum_{i=1}^{n} X_{Ri}$$

where:

n – is the number of evaluable subjects (7 in this example).

$$\hat{\sigma}_{TT} = \frac{\sum_{i=1}^{n} (X_{Ti} - \bar{X}_T)^2}{n - 1} \qquad \hat{\sigma}_{RR} = \frac{\sum_{i=1}^{n} (X_{Ri} - \bar{X}_R)^2}{n - 1}$$

$$\hat{\sigma}_{TR} = \frac{\sum_{i=1}^{n} (X_{Ti} - \bar{X}_T)(X_{Ri} - \bar{X}_R)}{n - 1}$$

Formulae for calculating the sample means, sample variances, and sample covariance for average AUEC values of individual evaluable subjects are presented. In this example, these are:

$$\bar{X}_T = -23.43 \quad \bar{X}_R = -21.56 \quad \hat{\sigma}_{TT} = 323.13 \quad \hat{\sigma}_{RR} = 80.10 \quad \hat{\sigma}_{TR} = 78.83$$

Student's t-test is determined for n - 1 degrees of freedom.

For example, for n = 7, t (6 degrees of freedom) is 1.9432. Then determine

$$G = \frac{t^2\ \hat{\sigma}_{RR}}{n\ \bar{X}_R^{\,2}}$$

G < 1 is required to have a proper confidence interval. If G≥1, the study does not meet the *in vivo* bioequivalence requirements.

If G < 1, calculate:

$$K = \left(\frac{\bar{X}_T}{\bar{X}_R}\right)^2 + \frac{\hat{\sigma}_{TT}}{\hat{\sigma}_{RR}}(1 - G) + \frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\left(G\frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}} - 2\frac{\bar{X}_T}{\bar{X}_R}\right)$$

In this example, K is 2.791.

The confidence interval limits may then be calculated:

$$\frac{\left(\frac{\bar{X}_T}{\bar{X}_R} - G\frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\right) \mp \frac{t}{\bar{X}_R}\sqrt{\frac{\hat{\sigma}_{RR}}{n}\,K}}{1 - G}$$

In this example, 90% confidence interval limits are 53.6% and 165.9%, based on the data of 7 evaluable subjects.