In Vitro Release Test Studies for Topical Drug Products Submitted in ANDAs

Guidance for Industry

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2022
Generic Drugs

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Guidance for Industry[1]

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance is intended to assist applicants who are submitting abbreviated new drug applications (ANDAs) for liquid-based and/or other semisolid products applied to the skin, including integumentary and mucosal (e.g., vaginal) membranes, which are hereinafter called *topical products*.[2] Because of the complex route of delivery associated with these products, which are typically locally acting, and the potential complexity of certain formulations, topical products (other than topical solutions) are classified as complex products.[3] This guidance provides recommendations for in vitro release test (IVRT) studies that can be used to compare a proposed generic (test) topical product and its reference standard (RS) for the purpose of supporting a demonstration of bioequivalence (BE) to the reference listed drug (RLD). The reference standard ordinarily is the RLD.[4]

[1] This guidance has been prepared by the Office of Generic Drugs in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] Topical products in ANDAs within the scope of this guidance include ointments, creams, lotions, emulsions, pastes, shampoos, gels, suspensions, sprays, aerosols, foams, and other semisolid and/or liquid-based dosage forms dispensed with a structured arrangement of matter (which may include more than one phase state).

[3] A *complex product*, as defined in the GDUFA Reauthorization Performance Goals and Program Enhancements Fiscal Years 2023–2027 (GDUFA III Commitment Letter) (accessible at https://www.fda.gov/media/153631/download), includes, among others, products with complex formulations (e.g., colloids) and complex routes of delivery (e.g., locally acting drugs such as dermatological products).

[4] A reference listed drug "is the listed drug identified by FDA as the drug product upon which an applicant relies in seeking approval of its ANDA" (21 CFR 314.3(b)). A reference standard, which is selected by FDA, is the specific drug product that the ANDA applicant must use in conducting any in vivo bioequivalence testing required to support approval of its ANDA (see § 314.3(b)). We recommend that the reference standard also be used for in vitro testing. There may be circumstances (e.g., when the RLD is no longer marketed) in which the reference standard is a drug product other than the RLD. For more information on RLD and reference standard products, see the guidance for industry *Referencing Approved Drug Products in ANDA Submissions* (October 2020). We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

This guidance does not address drug products that are administered via ophthalmic, otic, nasal, inhalation, oral, or injection-based routes, or that are transdermal or topical delivery systems (including products known as patches, topical patches, or extended-release films).

It is beyond the scope of this guidance to discuss specific topical products to which this guidance applies. FDA recommends that applicants consult this guidance and any relevant product-specific guidances (PSGs)[5] and any other relevant guidances for industry,[6] when considering the design and conduct of IVRT studies that, in conjunction with other studies, as deemed necessary, may be appropriate to support a demonstration that a proposed generic topical product and its RLD are bioequivalent. FDA also recommends that applicants routinely refer to FDA's guidance web pages, because additional guidances may become available that could assist in the development of a generic topical product.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

This guidance has been developed as part of FDA's "Drug Competition Action Plan,"[7] which, in coordination with the Generic Drug User Fee Amendments (GDUFA)[8] program and other FDA activities, is intended to increase competition in the marketplace for prescription drugs, facilitate the entry of high-quality and affordable generic drugs, and improve public health.

The Federal Food, Drug, and Cosmetic Act (FD&C Act) generally requires an ANDA to contain, among other things, information to show that the proposed generic drug product (1) is the same as the RLD with respect to the active ingredient(s), conditions of use, route of administration, dosage form, strength, and labeling (with certain permissible differences); and (2) is

[5] Generic drug product-specific guidances are available at FDA's Product-Specific Guidances for Generic Drug Development web page at https://www.fda.gov/drugs/guidances-drugs/product-specific-guidances-generic-drug-development.

[6] Other relevant guidances include the draft guidances for industry *In Vitro Permeation Test Studies for Topical Drug Products Submitted in ANDAs* (October 2022) and *Physicochemical and Structural (Q3) Characterization of Topical Drug Products Submitted in ANDAs* (October 2022). When final, these guidances will represent the FDA's current thinking on these topics. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[7] See the FDA Drug Competition Action Plan (describing the FDA's Drug Competition Action Plan, implemented in 2017 and designed to, among other things, further encourage robust and timely market competition for generic drugs), available at https://www.fda.gov/drugs/guidance-compliance-regulatory-information/fda-drug-competition-action-plan.

[8] In this guidance, *GDUFA* refers to the generic drug user fee program codified in the Generic Drug User Fee Amendments of 2012, Title III, Food and Drug Administration Safety and Innovation Act (Public Law 112-144), the Generic Drug User Fee Amendments of 2017, Title III, FDA Reauthorization Act of 2017 (Public Law 115-52), and the Generic Drug User Fee Amendments of 2022, Title III of Division F (the FDA User Fee Reauthorization Act of 2022) of the Continuing Appropriations and Ukraine Supplemental Appropriations Act, 2023 (Public Law 117-180).

bioequivalent to the RLD.[9] Thus, an ANDA will not be approved if the information submitted in the ANDA is insufficient to show that the test product is bioequivalent to the RLD.[10]

An IVRT study may be used to assess the rate of drug release (i.e., release of an active ingredient) from a topical product. Once validated, an IVRT study may also be useful in controlling product quality and/or establishing the acceptability of post-approval manufacturing changes. This guidance focuses on general considerations and recommendations for the method development, method validation, and conduct of IVRT studies that are submitted in ANDAs and intended to support a demonstration of BE.[11]

III. IVRT METHOD DEVELOPMENT

If an IVRT study is intended to support a demonstration of BE, the IVRT method development report should be submitted in the ANDA to show how the IVRT method was optimized, and to support a demonstration that the method parameters selected for the IVRT are appropriate or necessary, particularly in situations where the method parameters are different from the methods recommended in this guidance and described in the United States Pharmacopeia (USP) General Chapter <1724>.[12] The Agency's interest in reviewing the method development report is to understand why specific IVRT method parameters were selected and whether they are suitably sensitive and reproducible. This method development report should clearly indicate/distinguish the method parameters used for each set of data, illustrate the efforts made to optimize the IVRT method, and demonstrate that the method parameters selected for the IVRT are appropriate.

Applicants are encouraged to use the recommendations in this guidance, and if an applicant elects to use methods that are different from those recommended in this guidance, the IVRT method development report should demonstrate why it is scientifically justified to use an alternative approach than what is recommended in this guidance or USP <1724> to optimize the IVRT method.[13] Specific examples of procedures are described in subsequent sections, to help applicants identify circumstances when information should be submitted in the ANDA to explain why an alternative procedure was utilized.

The IVRT method development studies, being exploratory in nature, are often performed using a sample analytical method that is not validated (e.g., a high-performance liquid chromatography (HPLC) or ultrahigh performance liquid chromatography (UPLC) method); also, IVRT method development studies are often conducted in a manner that is not compatible with a quality management system which would otherwise make the evidence generated suitable to support

[9] See section 505(j)(2)(A), (j)(2)(C), and (j)(4) of the FD&C Act (21 U.S.C. 355(j)(2)(A), (j)(2)(C), (j)(4)); see also 21 CFR 314.94.

[10] 21 CFR 314.127(a)(4), (6).

[11] A demonstration of equivalent drug release rates for the test topical product and RS using an appropriately validated IVRT method can be used to support a demonstration of BE along with other data in the application (which may be specified in a PSG), as part of a comparative product characterization-based approach.

[12] Applicants may choose to use an approach different from the approach recommended in this guidance. However, the alternative approach must comply with relevant statutes and regulations (see 21 CFR 10.115(d)).

[1313] Ibid.

valid conclusions. Such method development studies would not be suitable to demonstrate the validity of an IVRT method, or the associated results. Therefore, although it may appear to be redundant, certain experiments performed during IVRT method development may need to be repeated during IVRT method validation, using appropriate controls, like a validated analytical method and procedures that are compatible with a suitable quality management system.

It is important to clearly segregate and consistently identify those experiments and results that were part of IVRT method development separately from those that were part of IVRT method validation. It is also important to consistently identify all relevant method parameters and experimental conditions/controls for each set of IVRT results. Information in the method development report should clearly identify/distinguish when the results for apparently similar sets of experiments may have been obtained using different method parameters. Method development reports should clarify which sets of diffusion cells were run in parallel or separately (e.g., on separate days). In addition, the sample analytical method (e.g., a HPLC or UPLC method) used to analyze the samples from each set of IVRT experiments should be specified, and the reports should indicate whether or not the sample analytical method was validated (either at the time of sample analysis or subsequently).

A. IVRT Method Parameters

Theoretical or empirical information should be provided to explain the selection of IVRT method parameters such as the equipment, product dose amount, sampling times, stirring/agitation rate, etc. When the equipment selected is among the models of equipment in the USP <1724>, Semisolid Drug Products – Performance Tests, and when the product dose amount or stirring rate is a parameter that is fixed (not adjustable) with the selected equipment, it may be sufficient to explain these facts.

It is unconventional for IVRT sampling times to be selected within a study duration of less than 4 hours. This may occur in situations where the fixed product dose was depleted to such a great extent by 4 hours that the release kinetics were no longer linear thereafter (when plotted vs. the square root of time). In such instances, it would be appropriate to explain the efforts that were made to optimize the IVRT method (e.g., using a different diffusion cell equipment that allowed for a larger product dose to be used) so that the sustained steady state release kinetics could potentially be characterized over a conventional IVRT duration of 4 to 6 hours.

B. IVRT Receptor Solution

It is conventional to evaluate different receptor solutions during IVRT method development (all using the same membrane that has broad chemical compatibility with the receptor solutions evaluated); these receptor solutions are frequently binary hydro-alcoholic mixtures selected based upon the solubility and stability of the (frequently hydrophobic) drug in the receptor solution. The receptor solutions are conventionally sampled at least hourly across a 6-hour duration.

Information on the empirical solubility and stability of the drug in the receptor solution, as well as information on the linearity and precision of the resulting drug release rate in an IVRT should

be provided to help explain the selection of a receptor solution for the test method. The linearity of the drug release rate (slope) across all time points should ideally have an r^2 value of ≥ 0.97. In situations where the solubility of the drug in the receptor solution limits the release kinetics, causing a reduction in the release rate at the last time point(s), it may be appropriate to evaluate different receptor solutions. It may be appropriate to truncate the IVRT method to a 4- or 5-hour sampling duration if the linearity of the release rate in that truncated duration is improved (exhibiting a higher r^2 value), and if other aspects of the release kinetics (e.g., precision) in that receptor solution are optimized compared to other receptor solutions evaluated.

One advantage of selecting an optimal receptor solution as an initial step in IVRT method development is that it allows for the sample analysis method to be optimized for the selected receptor solution sample matrix before proceeding to an evaluation of different membranes using that receptor solution.

C. IVRT Membrane

It is conventional to evaluate different membranes during IVRT method development (all using the same receptor solution); these membranes are frequently synthetic membranes used for the filtration of particulate matter in solutions. IVRT membranes are selected based upon their effective pore size (e.g., 0.45 micrometers (µm)), as well as their expected inertness to binding the drug. Information should be provided in the IVRT method development report on each membrane's binding to the drug and its chemical compatibility with relevant receptor solution(s) selected for the IVRT method (based on the preceding phase of IVRT method development), as well as information on the linearity and precision of the resulting release rate when each membrane is used in an IVRT, as this information can help to explain why a specific membrane is optimal for the IVRT method.

IV. IVRT METHOD VALIDATION

The equipment, methodologies, and study conditions used in the IVRT pivotal study should be appropriately validated or qualified. It is conventional to initiate the validation of the sample analytical method (e.g., a HPLC or UPLC method) for the IVRT before initiating the IVRT method validation itself, although certain components of the sample analysis method validation (e.g., stability) often proceed in parallel with the IVRT method validation. If an applicant elects to use equipment, methodologies, or study conditions that are different from those recommended in this guidance or in USP <1724>, the applicant should demonstrate why the differences are scientifically justified.[14] It is important to consistently identify all relevant method parameters for each set of IVRT results, making it clear that the results were obtained using the same IVRT method parameters, and clarifying which sets of diffusion cells were run in parallel or separately. Detailed protocols and well-controlled test procedures are recommended to ensure the precise control of dosing, sampling, and other IVRT study parameters, and of potential sources of experimental bias.

[14] Applicants may choose to use an approach different from the approach recommended in this guidance. However, the alternative approach must comply with relevant statutes and regulations (see 21 CFR 10.115(d)).

The qualification of an IVRT method parameter refers to the process of defining what attributes make it suitable to perform its function in the IVRT method. For example, when hourly measurements of the temperature at the membrane surface (when mounted in a diffusion cell) demonstrate that an IVRT equipment can maintain a membrane surface temperature in the range of 32°C ± 1°C across 6 hours, the results can support a demonstration that the equipment is qualified to perform its function in an IVRT method for which a method parameter is the control of membrane surface temperature in the range of 32°C ± 1°C across 6 hours. While an IVRT membrane surface temperature in the range of range of 32°C ± 1°C is appropriate for topical products applied on the skin, for topical products applied on mucosal membranes (e.g., a vaginal gel) the relevant IVRT membrane surface temperature would be 37°C ± 1°C. The validation of the IVRT method should incorporate the following qualifications and controls, performed using validated sample analytical procedures, as applicable.

A. Equipment Qualification

Suitable equipment for the IVRT method are described in USP General Chapter <1724>. These include different models of a vertical diffusion cell and an immersion cell. Other models of vertical diffusion cells and immersion cells that are essentially the same in design and/or operational principles as those described in USP General Chapter <1724> may also be suitable.

The operating principles and specific test procedures differ among the various equipment; relevant procedures from the manufacturer may be used for installation, operation, and performance qualifications. The laboratory qualification of each diffusion cell should, at minimum, include: (1) measurements of the diffusional area of the orifices of the donor and receptor compartments between which the membrane is mounte; (2) the empirically measured volume of the receptor solution compartment/vessel for each diffusion cell; (3) the stability of the temperature measured at the membrane surface (e.g., at 32°C ± 1°C), or just below the membrane, across a relevant duration (e.g., 6 hours); and (4) the rate of stirring or agitation, as applicable.

If information related to the diffusional area of the orifice and the volume of the receptor solution compartment for each diffusion cell is available from the manufacturer, that information should be provided for each relevant diffusion cell, in addition to the empirical measurements made by the laboratory. The equipment should control the diffusion cell thermoregulation so that the membrane surface temperature is verified to be stable (e.g., at 32°C ± 1°C) for each diffusion cell (e.g., measured by a calibrated infrared thermometer) before dosing. If it is not feasible to verify that the membrane surface temperature of a diffusion cell has equilibrated and stabilized (e.g., at 32°C ± 1°C) before dosing because of design and operating principles of a specific equipment, the qualification of that equipment should demonstrate that, under the specific conditions used for the IVRT method, the membrane surface temperature can be expected to be stable (e.g., at 32°C ± 1°C) for each diffusion cell throughout the test.

B. Membrane Qualification

Membrane inertness should be evaluated in relation to membrane binding of the drug in the receptor solution at a concentration relevant to the range of drug concentrations in the receptor solution during the test. Determinations should be based upon a minimum of three replicate membrane incubations for the IVRT duration at the relevant temperature (e.g., 6 hours at 32°C ± 1°C). Three replicate control incubations should be performed in parallel, without membranes, to monitor for drug loss that is not associated with membrane binding. Aliquots of these solutions should be collected before and after the duration of incubation, to assess any decrease in the amount of drug in solution. The recovery of drug in solution is recommended to be within the range of 100% ± 5% at the end of the test duration to qualify the inertness of the membrane.

C. Receptor Solution Qualification

The reason for selecting the composition of the receptor solution used for the IVRT study should be explained. The solubility of the drug in the IVRT receptor solution should be empirically determined in triplicate, to illustrate that the solubility of the drug in the receptor solution exceeds the highest sample concentration in the IVRT pivotal study, ideally by an order of magnitude, but demonstrably sufficient to facilitate a linear (steady state) release rate for the duration of the study (even when evaluating the relatively higher release rate of a formulation that is 150% of the nominal strength of the RS during the IVRT method validation).

D. Receptor Solution Sampling Qualification

The accuracy and precision of receptor solution sample collection at each time point should be appropriately qualified. Evidence to qualify a sampling procedure should illustrate that the sampling technique can reliably collect a consistent volume of the sample from the well-mixed volume of the receptor compartment at each sampling event, and that no artifacts are likely to be created by the sampling technique (e.g., because of carryover between samples in automated sampling systems or because of sampling from an unmixed volume in the sampling arm of a vertical diffusion cell). Information should be included describing the equipment manufacturer's specification for the accuracy and precision of receptor solution sampling, when available.

E. Environmental Control

Ambient laboratory temperature and humidity during the study should be monitored and reported. An environmentally controlled temperature range of 21°C ± 2°C is recommended, and, if feasible, a humidity range of 50% ± 20% relative humidity is recommended.

F. Linearity and Range

The linearity (r^2 value) of the release rate (slope) should be plotted across the range of the sampling times, which corresponds to the IVRT study duration. The linearity of drug release should be calculated and reported for each diffusion cell and compared within and across all IVRT runs. For the release rate to be considered suitably linear, it should have an r^2 value ≥ 0.97

across the recommended IVRT study duration of 4–6 hours. An IVRT study duration of less than 4 hours may be insufficient to assess whether the release rates being compared for the test topical product and RS represent their steady state drug release kinetics, but an IVRT study duration of less than 4 hours (which is not recommended) may be justified if supported by compelling experimental data within the method development report to illustrate that reasonable and scientifically appropriate efforts were made to optimize the IVRT method. The IVRT method linearity and range should be established based upon the results of the precision and reproducibility runs, described further below.

G. Precision and Reproducibility

The intra-run and inter-run precision and reproducibility may be compared for the release rate (slopes) calculated for each diffusion cell. The mean, standard deviation, and percent coefficient of variation (%CV) among slopes may be calculated within and across all runs, and a minimum intra-run and inter-run %CV $\leq 15\%$ is recommended. Runs may be organized to facilitate a simultaneous evaluation of intra/inter-instrumentation and/or intra/inter-operator precision and reproducibility. A minimum of three independent precision and reproducibility runs is recommended.

H. Dose Depletion

The recovery of released drug in the receptor solution should be characterized in each diffusion cell as the cumulative amount of drug released into the receptor solution over the IVRT study duration. This may be expressed as a percentage of the amount of drug in the applied dose (which may be estimated based upon the nominal strength of the drug in the topical product and the approximate mass of topical product dosed on the membrane). For example, if 1 gram (g) of a topical product containing 5% drug was dosed on the membrane of each diffusion cell, the amount of drug in the applied dose may be estimated to be 50 mg. If a total of 10 mg of drug diffused into the receptor solution of each diffusion cell across the 6-hour duration of the IVRT, it would be possible to estimate that the 50 mg dose would have been depleted by 10 mg, amounting to a 20% dose depletion. The average percentage dose depletion may thereby be estimated and should be reported. While steady state release kinetics can typically be assumed under conditions when the dose depletion is less than 30%, for some topical products, steady state release kinetics may continue to be observed at higher percentage dose depletions. The IVRT method may be considered adequate despite a dose depletion of greater than 30% when experimental evidence illustrates that the release rate (slope) remains suitably linear for each diffusion cell when plotted versus the square root of time.

I. Discrimination Sensitivity, Specificity, and Selectivity

The IVRT method should be able to discriminate drug release rates from similar formulations. This should be evaluated by comparing the release rate from the test formulation with that from two comparable formulations in which the concentration of drug has been altered – one with a higher strength (150% of the nominal concentration of the RS) and one with a lower strength (50% of the nominal concentration of the RS). If precipitation of the active ingredient is observed when formulating a topical product at 150% compared to the nominal strength, it may

be necessary to use different strategies, which may be discussed with the Agency before the submission of an ANDA during a pre-ANDA product development meeting[15] or via a controlled correspondence.[16] The composition and procedures for preparation of these higher and lower strength formulations should be reported, although these formulations need not be prepared in a manner compatible with current Good Manufacturing Practices. The discrimination ability of the IVRT method should be described using three concepts of discrimination ability: sensitivity, specificity, and selectivity.

1. IVRT Sensitivity

IVRT sensitivity is the ability to detect changes in the release rate, as a function of drug concentration in the formulation. If the IVRT method consistently identifies higher or lower rates of release for test formulations with increased or decreased drug concentrations, respectively, relative to the formulation at the nominal strength of the RS run in parallel on the same day, the IVRT method would generally be considered sensitive.

2. IVRT Specificity

IVRT specificity is the ability to accurately monitor the proportionality of changes in the release rate as a function of drug concentration in the formulation. This proportionality may be illustrated in a plot of the relationship between the formulation concentration and the average IVRT release rate (slope). The specificity of the IVRT method should be calculated, plotted with a linear trendline, and the linearity quantified and reported as an r^2 value. To be considered suitably specific, an IVRT method should be proportionally linear in its response to differences in release rates, with a minimum r^2 value ≥ 0.95 for the correlation of the formulation concentration to the average IVRT release rate (slope).

IVRT specificity is a function of the proportionality of release rates across different strengths of the product, some, or all of which may be formulated as small-scale laboratory batches, with each strength having a slightly different formulation composition to accommodate for the different amount of the active ingredient in that strength of the product. These slight formulation differences across the different strengths of the product may impact the ideal proportionality of release rates across the different strengths of the product.

Thus, the proportional linearity of release rates across different strengths of the product may be impacted by formulation differences across the strengths that are independent of the proportional responsiveness of the IVRT method. The minimum r^2 value ≥ 0.95 for the correlation of the formulation concentration to the average IVRT release rate (slope) takes into account that the IVRT method's response to differences in release rates may not appear to be perfectly proportional because of formulation differences that are independent of the IVRT method.

[15] See the guidance for industry *Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA* (November 2020) for information on the enhanced pathway for discussions between FDA and a prospective applicant preparing to submit an ANDA for a complex product as defined in that guidance.

[16] See the guidance for industry *Controlled Correspondence Related to Generic Drug Development* (December 2020) for information on the types of inquiries accepted as controlled correspondence and on how to submit controlled correspondence to OGD.

Note that the linearity of release rates across different strengths of the product (which assesses the specificity of the IVRT method, with a minimum r^2 value ≥ 0.95) is fundamentally different and has different scientific considerations than the linearity of the release rate for a single strength of the product across the range of the sampling times (which assesses the IVRT method's ability to monitor the steady state release kinetics of the active ingredient, with a minimum r^2 value ≥ 0.97). Despite the potential for different scientific considerations to impact the linearity of the IVRT results in each context, for well-developed and suitably controlled IVRT methods, the r^2 value ≥ 0.99 is routinely observed in both contexts.

3. IVRT Selectivity

IVRT selectivity is the ability of the IVRT method to discriminate the drug release rates between the reference topical product and the altered (50% and 150% nominal strength) concentration test formulations such that their release rates are determined to be statistically inequivalent compared to that from the nominal reference strength formulation. Determination of inequivalence between release rates should be evaluated using the statistical approach described in USP General Chapter <1724>.

Specifically, the release rates from six cells dosed with the nominal reference strength formulation should be compared with the release rates from 6 cells dosed with the formulation at 150% the nominal reference strength, using the statistical approach described in USP General Chapter <1724>. All 12 cells being compared should have been run in parallel on the same day, and the release rate from the formulation at 150% the nominal reference strength should fail to show equivalence to the release rate from the nominal reference strength formulation.

The release rates from 6 cells dosed with the nominal reference strength formulation should also be compared with the release rates from 6 cells dosed with the formulation at 50% the nominal reference strength, using the statistical approach described in USP General Chapter <1724>. All 12 cells being compared should have been run in parallel on the same day, and the release rate from the formulation at 50% the nominal reference strength should fail to show equivalence to the release rate from the nominal reference strength formulation.

4. IVRT Supplemental Selectivity

IVRT supplemental selectivity is the ability of the IVRT method to discriminate the drug release rates between the reference topical product and an altered formulation with the same nominal reference strength, such that their release rates are determined to be statistically inequivalent.

The demonstration of IVRT selectivity (distinct from supplemental selectivity) validates the ability of the IVRT method to discriminate differences in release rates under conditions when the release rate is expected to differ in a predictable manner (i.e., when there are different concentrations of drug in the formulation).

A separate and supplemental demonstration of the selectivity of an IVRT method, when feasible, independently validates the ability of the IVRT method to discriminate differences in release

rates under the conditions of the pivotal IVRT study, in which the test and reference topical products are compared at the same strength. Thus, the supplemental demonstration of the selectivity of the IVRT method validates that it can detect differences in the release rate that are associated with aspects of the formulation other than the strength, and this is ideal, when feasible.

Determination of inequivalence between release rates should be evaluated using the statistical approach described in USP General Chapter <1724>. Specifically, the release rates from 6 cells dosed with the nominal reference strength formulation should be compared with the release rates from 6 cells dosed with an altered formulation, also at the nominal reference strength, using the statistical approach described in USP General Chapter <1724>. All 12 cells being compared should have been run in parallel on the same day, and the release rate from the altered formulation at the same nominal reference strength should fail to show equivalence to the release rate from the nominal reference strength formulation.

The altered formulation used in the assessment of supplemental selectivity should have the same nominal strength as the reference topical product, and may include changes in inactive ingredients, changes in inactive ingredient concentration(s), changes in the manufacturing processes, or combinations thereof. However, not all variations in a formulation will necessarily produce a difference in the release rate compared to the reference formulation, and if two similar formulations are found to have equivalent release rates, the demonstration of supplemental selectivity may be inconclusive. Therefore, applicants are encouraged to develop or select an altered formulation for the demonstration of supplemental selectivity based on differences in physicochemical and structural properties of the formulation (relative to the reference formulation) that are likely to alter the release rate of the active ingredient from the formulation. The altered formulation may be a marketed topical product, such as a different dosage form at the same strength of the same drug (e.g., a 5% gel versus a 5% ointment). Product batch information for all topical product lots used in IVRT method development, and validation studies, as applicable, should be submitted in the study reports. The topical product information should include, but not be limited to, information about the batch formula, manufacturing date, batch size, altered manufacturing processes (if applicable) and, if available, potency and content uniformity.

J. Robustness

The IVRT method may be considered robust to a variation in the test method if the average slope of an IVRT run under the altered IVRT method parameters is within ± 15% of the average slope of the precision and reproducibility IVRT runs. Robustness testing may encompass variations in the IVRT method that are relevant to the equipment and test method, for example:

- Temperature variations (e.g., - 1°C and +1°C relative to 32°C ± 1°C)
- Dose volume variations (e.g., +10% and -10% in the dose volume)
- Receptor solution variations (e.g., slight change in composition and/or pH)
- Mixing rate variation (e.g., slight change in stirring speed, as applicable)

V. SAMPLE ANALYTICAL METHOD VALIDATION

While exploratory studies performed during IVRT method development may use an unvalidated sample analytical method, it is essential that all studies conducted as part of the IVRT method validation use a validated sample analytical method. A validated IVRT method should use a validated receptor solution sample analytical method. Therefore, a discussion of the sample analytical method for the IVRT method is included in this guidance under this section.

It is important to note that the study protocols and reports related to the IVRT method are distinct from those for the sample analytical method that is used to quantify drug concentrations in IVRT receptor solution samples. The validation of a sample analytical method, in and of itself, does not demonstrate the validity of an IVRT method. Separate and specific reports should be submitted for the validation of the sample analysis (e.g., HPLC or UPLC) method and for the validation of the IVRT method.

Any results from studies of the IVRT method that are performed (during method development) using a different sample analytical method than that which is ultimately validated, cannot support a demonstration of the validity of the IVRT method. Information should be provided in the IVRT method validation report referencing the (separate) sample analytical method validation, and clearly indicate that all relevant results in the IVRT method validation report were obtained using a validated sample analytical method (as opposed to an analytical method with different parameters than those which were validated).

The receptor sample analysis procedures, typically involving HPLC or UPLC, should be performed using chromatography software (e.g., a chromatography data system) with audit trails, and should include a multi-point (6–8 concentration) calibration curve with suitable quality control samples, and should be validated in a manner compatible with the FDA guidance for industry *Bioanalytical Method Validation* (May 2018).

The validation of the receptor sample analytical method should include relevant qualifications of dilution integrity, if applicable, as well as stability assessments with the highest relevant temperature in the receptor solution for the longest relevant duration; the highest relevant temperature may be warmer than the IVRT membrane surface temperature because the temperature of the receptor solution is often higher than the temperature at the surface of the membrane (e.g., the temperature of the receptor solution may be 34°C when the temperature of membrane surface is 32°C, so stability assessments with the IVRT receptor solution may be performed at 34°C for 6 hours; the temperature would be higher for an IVRT with a vaginal gel, for example).

VI. IVRT PIVOTAL STUDY

The IVRT pivotal study comparing the drug release rates between the test and reference topical products should be performed in a manner compatible with the general procedures and statistical analysis method specified in USP General Chapter <1724>. The cumulative amount of drug

released at each sampling time point should be reported for each diffusion cell. Relevant summary statistics for the IVRT study should also be reported.

A. Handling and Retention of Samples

Refer to 21 CFR 320.38, 320.63 and the guidances for industry *Handling and Retention of BA and BE Testing Samples* (May 2004) and *Compliance Policy for the Quantity of Bioavailability and Bioequivalence Samples Retained Under 21 CFR 320.38(c)* (August 2020), as applicable, regarding considerations for retention of study drug samples and to 21 CFR 320.36 for requirements for maintenance of records of BE testing. Retention samples should be randomly selected from the drug supplies received before dispensing during the IVRT study in which the test topical product and RS are compared. Experimental observations that may have the potential to influence the interpretation of the study results, as well as any protocol deviations, should be reported.

B. Control of Study Procedures

Study procedures that have the potential to influence the results of the study should be appropriately controlled. Also, experimental observations that may have the potential to influence the interpretation of the study results, as well as any protocol or standard operating procedure (SOP) deviations, should be reported.

In addition, investigators should perform the IVRT validation and pivotal studies within a quality management system that includes, but is not limited to, documented procedures for:

- Study personnel identification, training, qualification, and responsibilities
- Study management and study management personnel responsibilities
- Quality control (QC) and QC personnel responsibilities
- Quality assurance (QA) and QA personnel responsibilities
- Use of SOPs
- Use of study protocols
- Use of study reports
- Maintenance and control of the study facility environment and systems
- Qualification and calibration of instruments and computerized systems
- Good documentation practices including, but not limited to, contemporaneous documentation of study procedures and recording of experimental observations or deviations from procedures specified in the study protocol or in relevant SOPs

- Maintenance of suitable records that facilitate the reconstruction of study events and procedures, including study sample handling and storage records (e.g., sample tracking logs), audit trails for sample analysis procedures, control of study materials and reagents, and electronic data control

- Archival of study records

C. Blinding Procedure

A detailed description of the blinding procedure should be provided in the study protocol and final report for the IVRT pivotal study. The packaging of the test topical product and RS should be similar in appearance to maintain adequate blinding of the investigator and any experimental operators. Once blinded, the test topical product and RS should be identified by a random designation, e.g., "A" or "B."

D. Dosing

In the IVRT pivotal study, the test topical product and RS should be dosed in an alternating pattern on successive diffusion cells. There are two possible sequences for the alternating pattern (either ABABAB or BABABA). One of these two dosing sequences should be randomly selected.

VII. SUBMITTING INFORMATION ON IVRT STUDIES IN AN ANDA

For IVRT studies with topical products submitted in ANDAs that are intended to support a demonstration of BE, detailed study protocols, relevant SOPs, and detailed reports should be submitted for the IVRT method validation and the IVRT pivotal study. In addition, a detailed report describing the IVRT method development should be submitted. These protocols, SOPs, and reports should be submitted in module 5.3.1.2 of the electronic Common Technical Document (eCTD) and should describe experimental procedures, study controls, quality management procedures, and data analyses.

Note that the study protocols, SOPs, and reports related to the IVRT method are distinct from those for the sample analytical method that is used to quantify drug concentrations in IVRT receptor solution samples (e.g., a HPLC or UPLC method). Separate protocols and SOPs should be submitted for the sample analytical method validation. Sample analytical method development and validation reports, pivotal IVRT study sample analysis reports, as well as associated SOPs and protocols relevant to the sample analysis for an IVRT study should be submitted in Module 5.3.1.4 of the eCTD.