Statistical Approaches to Establishing Bioequivalence

An Overview of In Vitro Release Test (IVRT), In Vitro Permeation Test (IVPT), and Earth Mover's Distance (EMD) comparative studies

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Disclaimer

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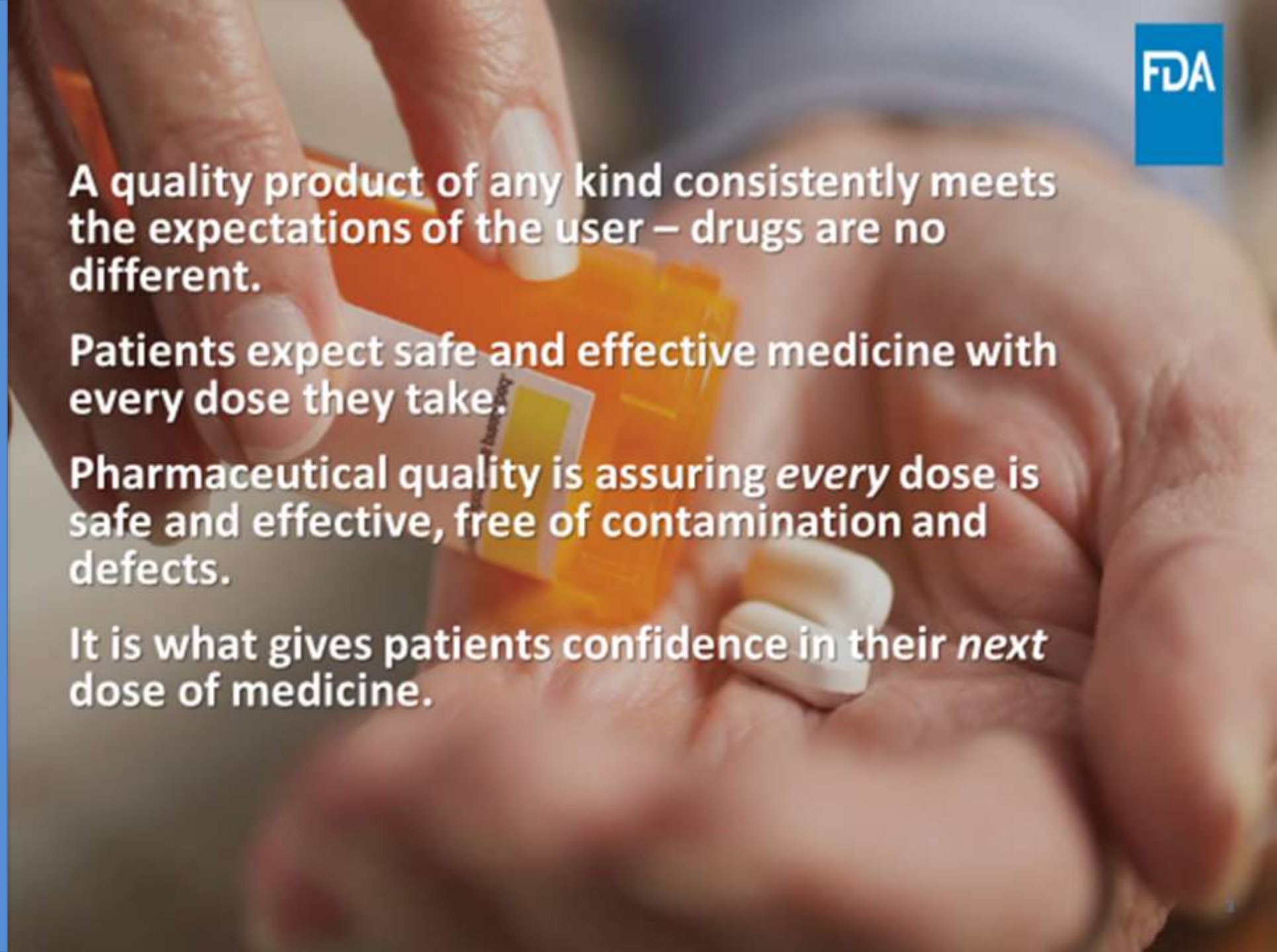
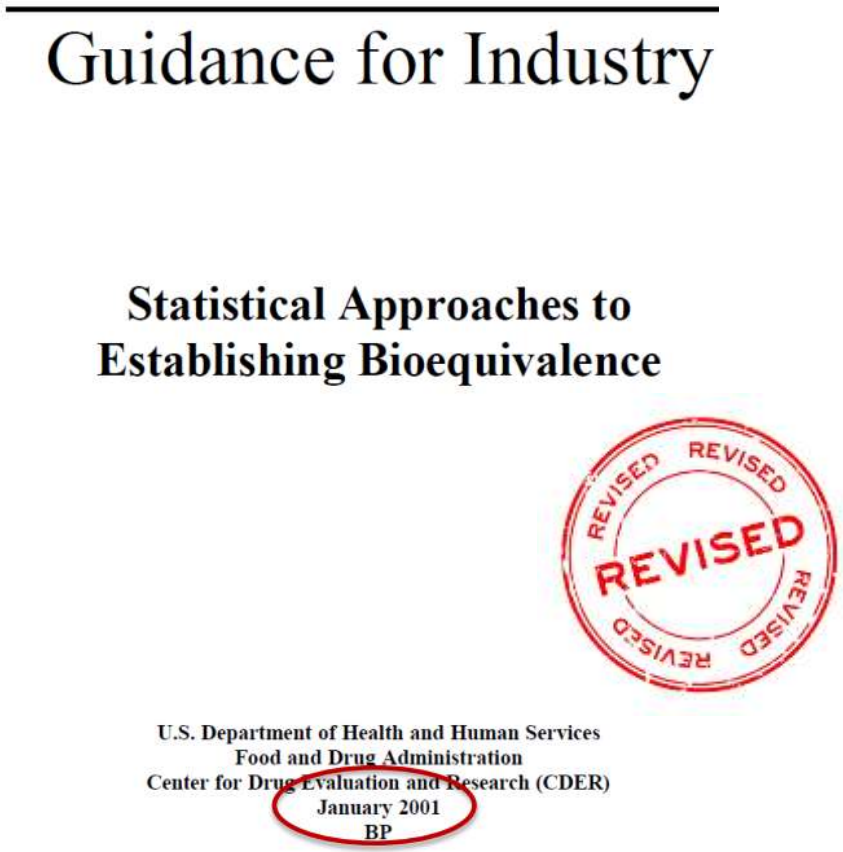

FDA
A quality product of any kind consistently meets the expectations of the user – drugs are no different.
Patients expect safe and effective medicine with every dose they take.
Pharmaceutical quality is assuring *every* dose is safe and effective, free of contamination and defects.
It is what gives patients confidence in their *next* dose of medicine.

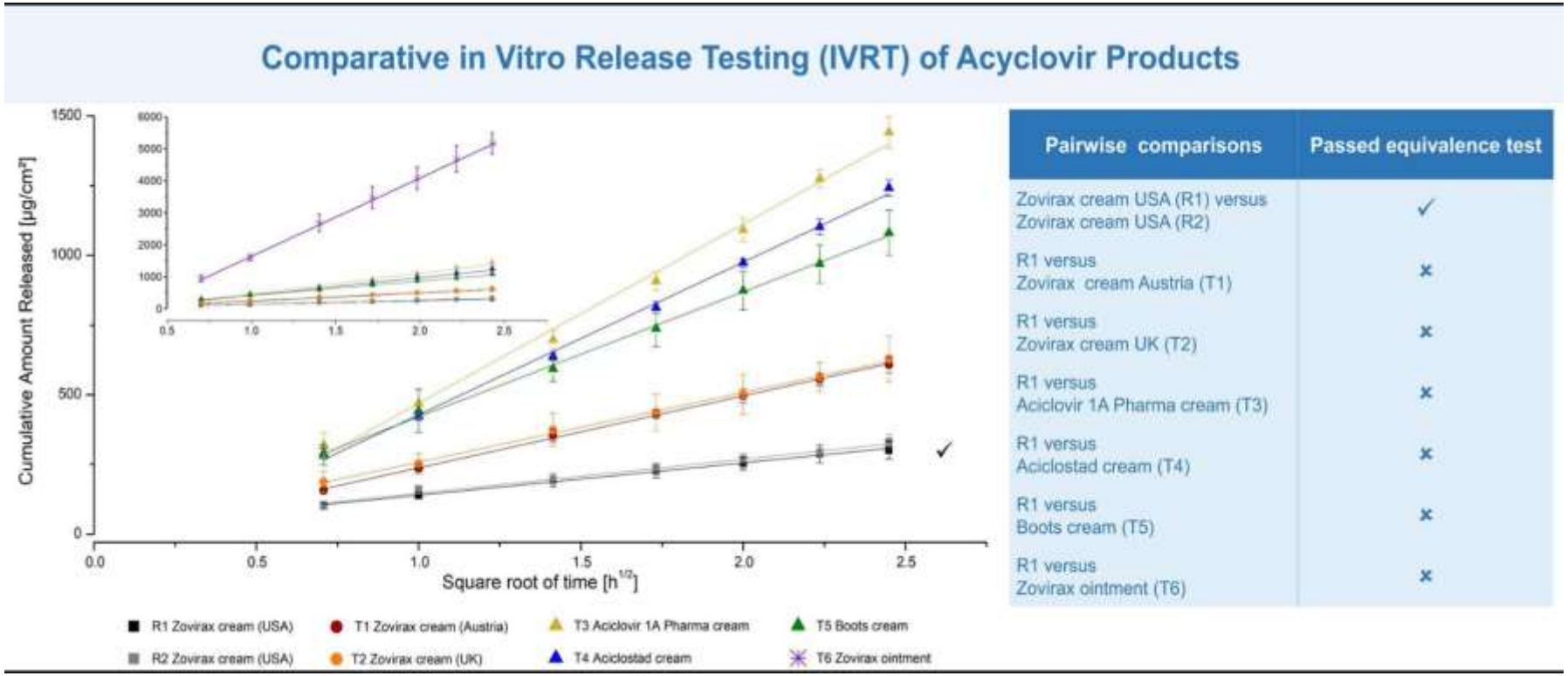
Objective



Highlight <u>content added</u> to the Statistical Approaches to Establishing Bioequivalence Guidance - Specific Situations

- In Vitro Release Test (**IVRT**)
- In Vitro Permeation Test (**IVPT**)
- *In Vitro Abuse Deterrent Formulation (**ADF**) comparative studies (not covered)*
- Earth Mover's Distance (**EMD**) probability distributions

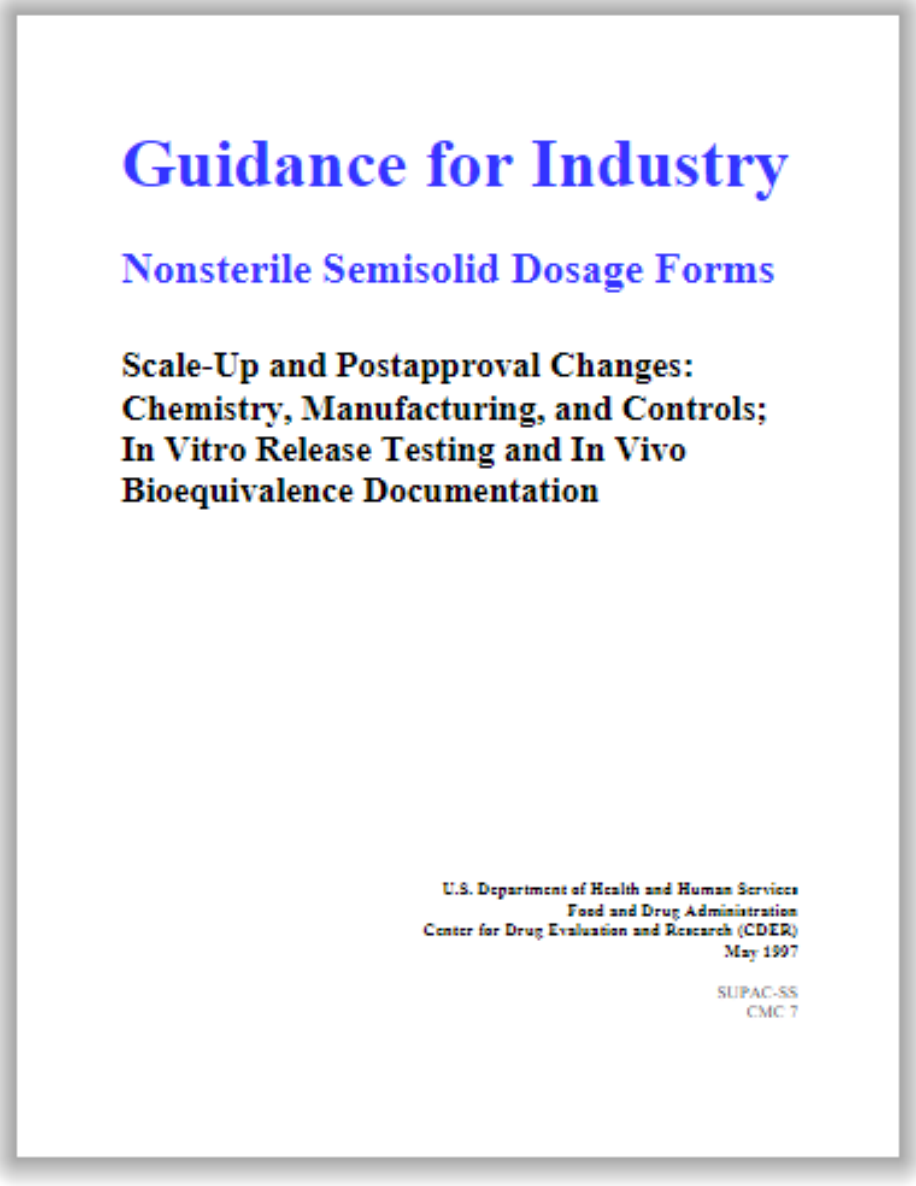
In Vitro Release Test (IVRT)



Pairwise comparisons	Passed equivalence test
Zovirax cream USA (R1) versus Zovirax cream USA (R2)	✓
R1 versus Zovirax cream Austria (T1)	×
R1 versus Zovirax cream UK (T2)	×
R1 versus Aciclovir 1A Pharma cream (T3)	×
R1 versus Aciclostad cream (T4)	×
R1 versus Boots cream (T5)	×
R1 versus Zovirax ointment (T6)	×

Katrin I. Tiffner, et. al, Comparative in vitro release testing (IVRT) of acyclovir products, International Journal of Pharmaceutics, Volume 609, 2021, 121186.

In-Vitro Release Test (IVRT)
SUPAC-SS Guidance (1997)



Guidance for Industry

Nonsterile Semisolid Dosage Forms

Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
May 1997

SUPAC-SS
CMC 7

The in vitro release comparison should be carried out as a two-stage test.

At the first stage, two runs of the (six cells) in vitro apparatus should be carried out, yielding six slopes (estimated in vitro release rates) for the prechange lot (R) and six slopes for the postchange lot (T). A 90% confidence interval (to be described below) for the ratio of the median in vitro release rate (in the population) for the postchange lot over the median in vitro release rate (in the population) for the prechange lot should be computed, expressed in percentage terms. If, at the first stage, this 90% confidence interval falls within the limits of 75% to 133.33%, no further in vitro testing is necessary.

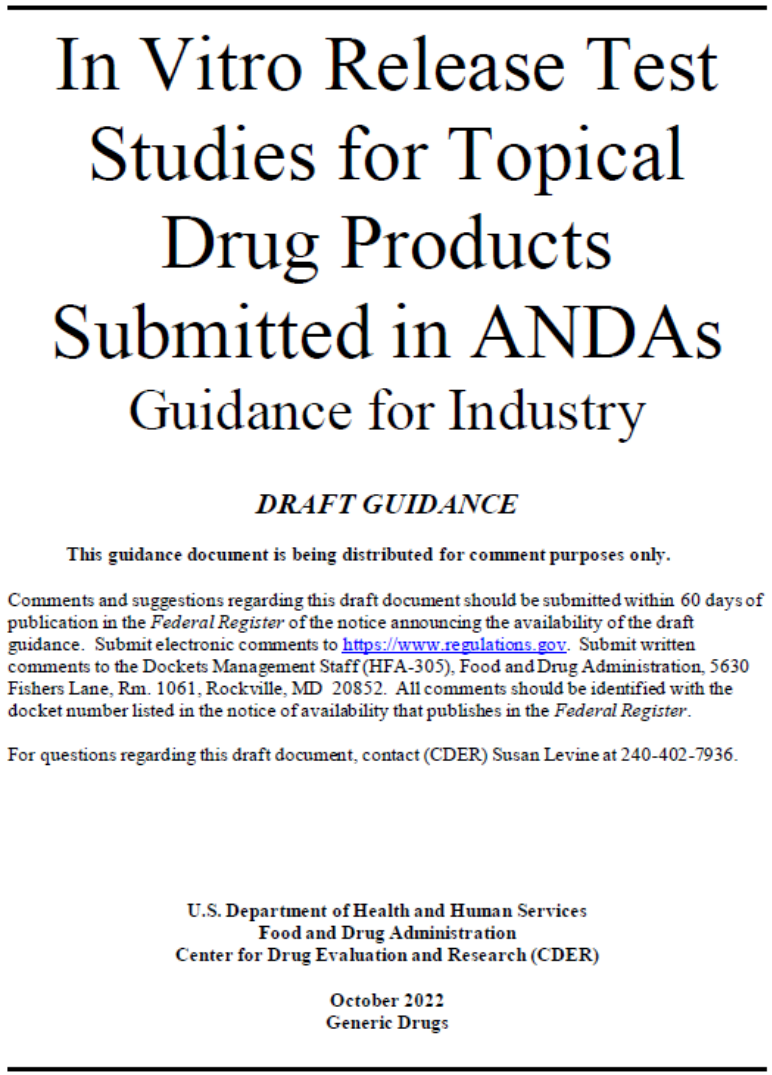
If the test is not passed at the first stage, 4 additional runs of the (six cells) in vitro apparatus should be carried out, yielding 12 additional slopes for each product, or 18 in all (including the first-stage results). The 90% confidence interval (to be described below) should be computed using all 18 slopes for each product, including the first-stage results. At the second stage, this 90% confidence interval should fall within the limits of 75% to

Because outliers are expected to occur on occasion with this testing (for example, due to an air bubble between the product sample and the membrane), a nonparametric

method is proposed, whose performance tends to be resistant to the presence of outliers.

The first step in the computation of the confidence interval is to form the 36 (= 6 x 6) individual T/R ratios. This is illustrated in the following table, where the prechange lot slopes (R) are listed across the top of the table, the postchange lot slopes (T) are listed down the left margin of the table, and the individual T/R ratios are the entries in the body of the table:

In-Vitro Release Test (IVRT)



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

Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Susan Levine at 240-402-7936.

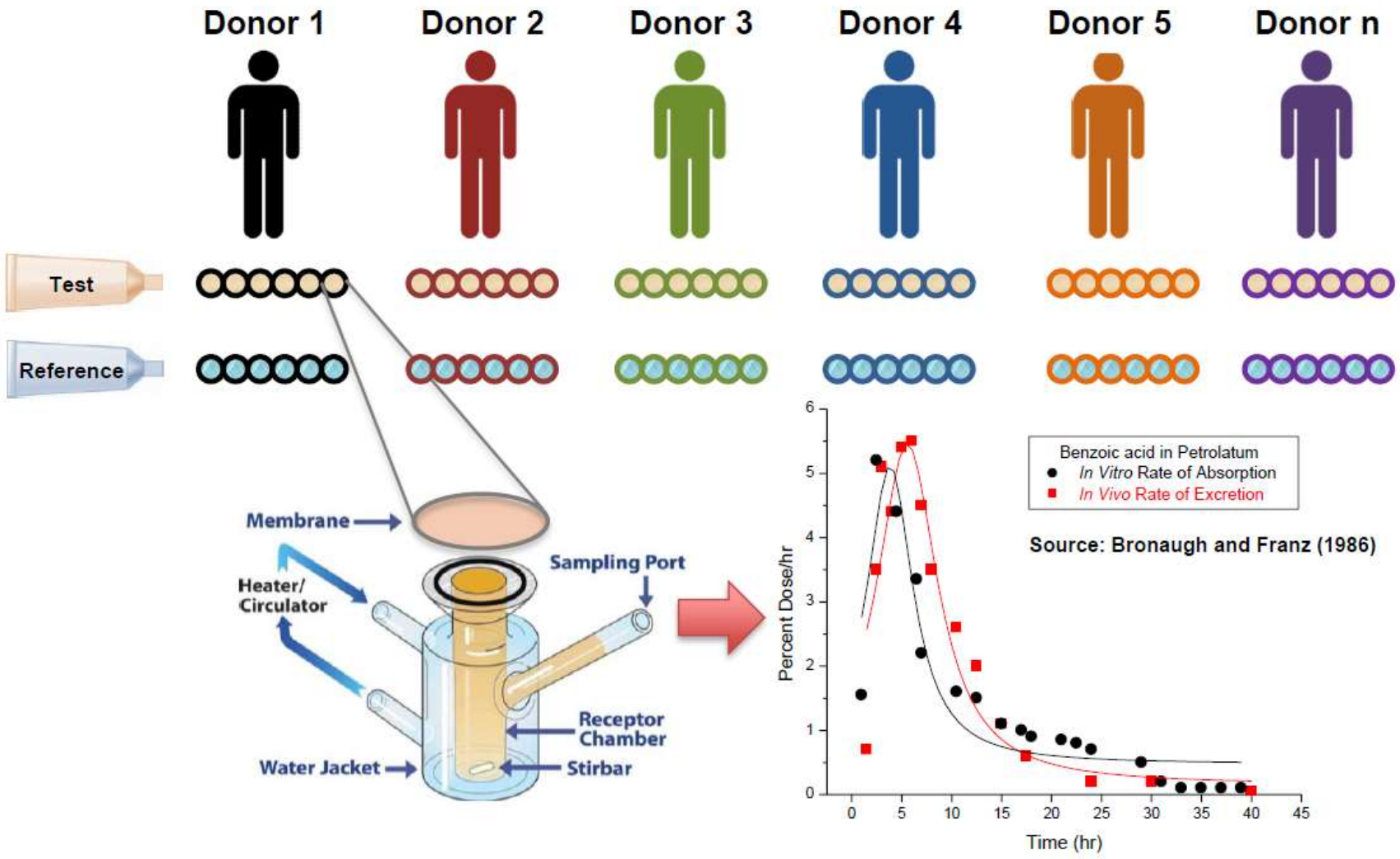
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2022
Generic Drugs

When an in-vitro release test (IVRT) is used to support a demonstration of BE for topical dermatological drug products as part of an in vitro characterization-based BE approach, a two-stage, nonparametric statistical approach is recommended.

The assessment of equivalence by an IVRT involves a comparison of the median in vitro drug release rates of two formulations using a non-parametric statistical test which is resistant to outliers that are expected to occur under the particular testing conditions.

In Vitro Permeation Test (IVPT)



In-Vitro Permeation Test (IVPT)

Contains Nonbinding Recommendations

Draft Guidance on Acyclovir

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the 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 Office of Generic Drugs.

Active Ingredient: Acyclovir

Dosage Form; Route: Cream; topical

Recommended Studies: Two options: in vitro

I. In vitro option:

To qualify for the in vitro option for this drug prod

A. The test and Reference Listed Drug (RLD) [1] quantitatively (Q2) the same as defined in th – *Refuse-to-Receive Standards*, Revision 1 (

B. The test and RLD products are physically an acceptable comparative physicochemical ch the test and three lots (as available) of the R

C. The test and RLD products have an equivale acceptable in vitro release test (IVRT) comp and RLD products using an appropriately va

D. The test and RLD products are bioequivalen permeation test (IVPT) comparing the rate a excised human skin from a minimum of one an appropriately validated IVPT method.

Additional comments: Specific recommendations

[1] Guidance for Industry ANDA Submissions – Refuse-to-Re

Recommended Dec 2014; Revised Dec 2016

14. The flux (rate of acyclovir permeation) should be plotted as J on the mass/area/time (e.g., ng/ cm^2/hr) versus time on the X-axis. Flux prc resemble plasma pharmacokinetic profiles, although the flux is a rate concentration. The extent of acyclovir permeation should also be plc amount of acyclovir permeated on the Y-axis in units of mass/area (e on the X-axis.

15. The flux should calculated based upon: the receptor sample concentr each time point; the precise, empirically measured volume of that spe 6.0 mL) which may vary between individual cells; the area of dose ap and the duration for which the receptor volume was accepting the ac the sample exemplified here represented a two hour period following calculated based upon the values above as:

$$J = [(2.0\ ng/mL) \times (6.0\ mL)]/(1\ cm^2)/(2\ hrs) = 6\ ng/cm$$

16. This flux should be calculated and reported for each diffusion cell fo and plotted across the entire study duration to generate the flux profil The rate calculated above may be plotted at the 2 hr time point, or at and 2 hrs (i.e., 1 hr). A similar approach is utilized to calculate the c of acyclovir that has penetrated during each sampling interval.

17. The maximum flux (J_{max}) at the peak of the acyclovir flux profile sho test and RLD products. This is analogous to the comparison of the C products in the case of plasma pharmacokinetics. Similarly, the cum of acyclovir across the study duration should be compared for the tes corresponds to the area under the curve (AUC) of the incremental ac profile.

18. A confidence interval (CI) should be calculated for each pharmacoki

    a. the log-transformed maximum flux (J_{max})
    b. the log-transformed total (cumulative) penetration (AUC)

19. The statistical analysis should consider a sample of n donors, for whi sections from each one of the n donors are available for each treatme (i) from each donor (j) should have been randomly assigned to each p treatment groups would correspond to the test acyclovir cream 5% (T

20. The replicate skin sections from donor 1 dosed with the test product may be denoted as T11, T21, …, Tr1, and likewise from donor 2, T12, T22, …, Tr2, and so forth up to n donors; T1n, T2n, …, Trn. Similarly, the replicate skin sections dosed with the RLD product may be denoted as R1n, R2n, …, Rrn.

21. For each donor, $I_j = \frac{1}{r}\sum_{i=1}^{r}(T_{ij} - R_{ij})$ should be calculated, which the point estimate,

$$\bar{I}_\cdot = \frac{1}{n}\sum_{j=1}^{n} I_j$$

the estimate of inter-donor variability,

$$S_I^2 = \frac{1}{(n-1)}\sum_{j=1}^{n}(I_j - \bar{I}_\cdot)^2$$

and the estimate of within-reference variability:

$$S_{WR}^2 = \frac{\sum_{j=1}^{n}\sum_{i=1}^{r}(R_{ij} - \overline{R_{\cdot j}})^2}{(r-1)n}$$

where $\overline{R_{\cdot j}}$ is the average across all r replicates for donor J of R.

22. Under normality assumptions, the following distributional results l

$$\bar{I}_\cdot \sim N(\mu_T - \mu_R,\ \frac{\sigma_I^2}{n})$$

$$\frac{(r-1)n\,S_{WR}^2}{\sigma_{WR}^2} \sim \chi^2_{(r-1)n}$$

and the two quantities are considered statistically independent. Fu assume a balanced design in which no donor-by-product interactio

23. The recommended statistical methodology to evaluate BE includes uses the within - reference variability as a cutoff point.

24. For $S_{WR} \leq 0.294$, the test and RLD products are declared bioequi confidence interval:

$$\bar{I}_\cdot \pm t_{(n-1),\alpha} * \sqrt{\frac{S_I^2}{n}}$$

is contained within the limits $[\frac{1}{m},\ m]$.

25. If $S_{WR} > 0.294$, the hypotheses to be tested are:

$$H_0: \frac{(\mu_T - \mu_R)^2}{\sigma_{WR}^2} > \theta$$

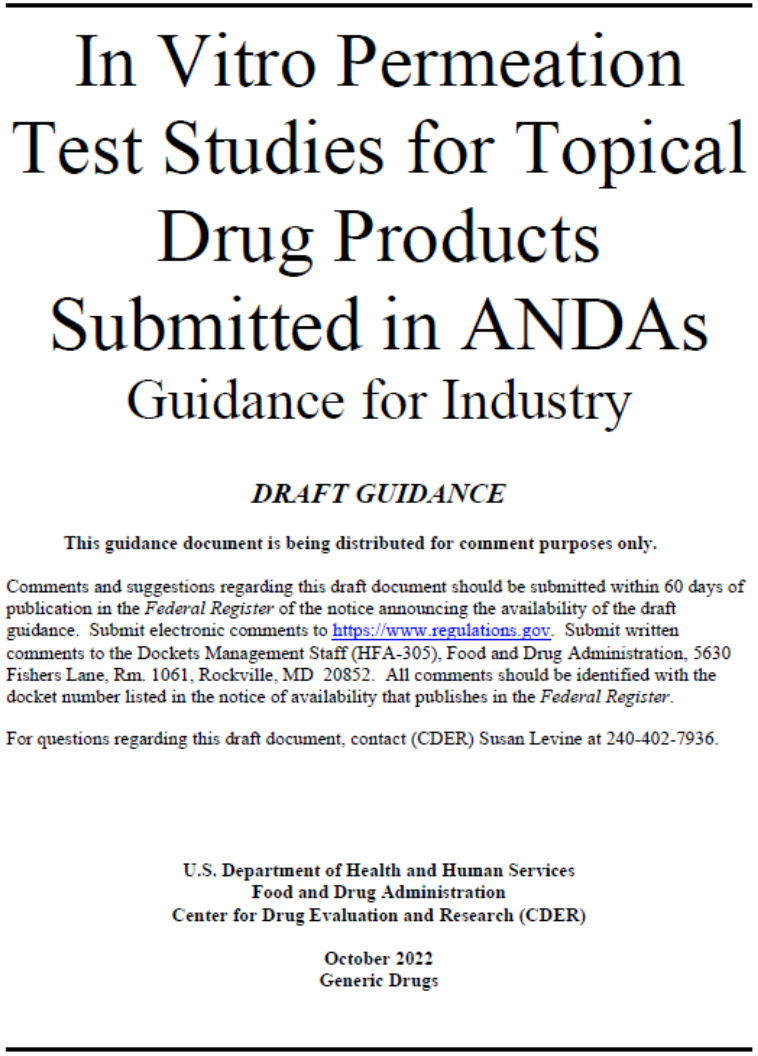
$$H_a: \frac{(\mu_T - \mu_R)^2}{\sigma_{WR}^2} \leq \theta$$

where σ_{WR}^2 is the reference population within-subject variance and θ is equal to $\frac{(\ln(m))^2}{(0.25)^2}$, and where m represents the BE limit (1.25). The aim should be to construct a (1-α) *100% CI for the quantity $(\mu_T - \mu_R)^2 - \theta\,\sigma_{WR}^2$. If the upper bound of this CI is less than or equal to zero, the null hypothesis should be rejected. Rejection of the null hypothesis, H_0, supports BE. This criterion should be accompanied by a point estimate constraint according to which the geometric mean ratio (point estimate of the log-transformed response has to fall within the pre-specified limits: $[\frac{1}{m},\ m]$.

26. One possible way to perform the analysis in order to derive the upper bound of this CI is to use an approach similar to that described in the FDA Draft Guidance on Progesterone (recommended Apr 2010; revised Feb 2011), with appropriate modifications for this replicate experimental design.

27. The method of randomization should be described in the protocol and the randomization schedule provided, preferably in a SAS data set in .xpt format (created using the SAS XPORT procedure). It is recommended that an independent third party generate and hold the randomization code throughout the conduct of the study in order to minimize bias. The sponsor may generate the randomization code if not involved in the packaging and labeling of the test and RLD products dosed in the study. A sealed copy of the randomization scheme should be retained at the study site and should be available to FDA investigators at the time of site inspection to allow for verification of the treatment identity of each skin section.

In-Vitro Permeation Test (IVPT)



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U.S. Department of Health and Human Services
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October 2022
Generic Drugs

When an in-vitro permeation test (IVPT) is used to support a demonstration of BE for topical dermatological drug products as part of an in vitro characterization-based BE approach, a mixed scaled criterion is recommended, and described in detail in the draft guidance for industry *In Vitro Permeation Test Studies for Topical Drug Products Submitted in ANDAs* (October 2022). According to that methodology, a confidence interval is calculated for each of the endpoints, log-transformed maximum flux (Jmax) and log-transformed total (cumulative) amount (AMT) permeated. The permeation test is performed with excised skin sections from patients undergoing a surgical procedure or from cadaver donors and the statistical test uses the within-reference standard deviation, Swr, as the threshold that prompts use of either the unscaled or scaled confidence interval.

Earth Mover's Distance (EMD) in the guidance

6. *Earth Mover's Distance Based Profile Comparison Approach*

EMD is a statistical metric that measures the discrepancy (distance) between distributions without a prior assumption of the distribution.[35] The EMD has been recommended in a profile comparison approach to assess equivalence of particle size distribution profile,[36] where the profile exhibits complex distribution (i.e., multiple peaks) that cannot be accurately described by some conventional descriptors (e.g., the D50 and SPAN). The EMD-based profile comparison approach is briefly described as follows. To assess equivalence between the T and R product formulations in the particle size distribution shape, an average profile of all R product samples (i.e., R center) is calculated and serves as the reference profile to compute the distance between an R or a T product sample to the R center using the EMD algorithm. After obtaining the profile distances between each R product sample and the R product average (R – R center distance), and the profile distances between each T product sample and the R product average (T – 'R center' distance), a statistical equivalence method, e.g., the PBE, is then applied to the two groups of distances to indicate whether the T and R products are statistically equivalent in the particle size distribution shape. For details, refer to Rubner et al. (2000).[37]

Importantly, considering the increasingly emerging technologies and methods for in vitro BE studies, applicants are encouraged to contact the Agency early to discuss their proposed study designs and statistical methods via the controlled correspondence, pre-ANDA meeting, pre-IND meeting, or pre-NDA meeting pathway.[38]

EMD can be used for profile comparison

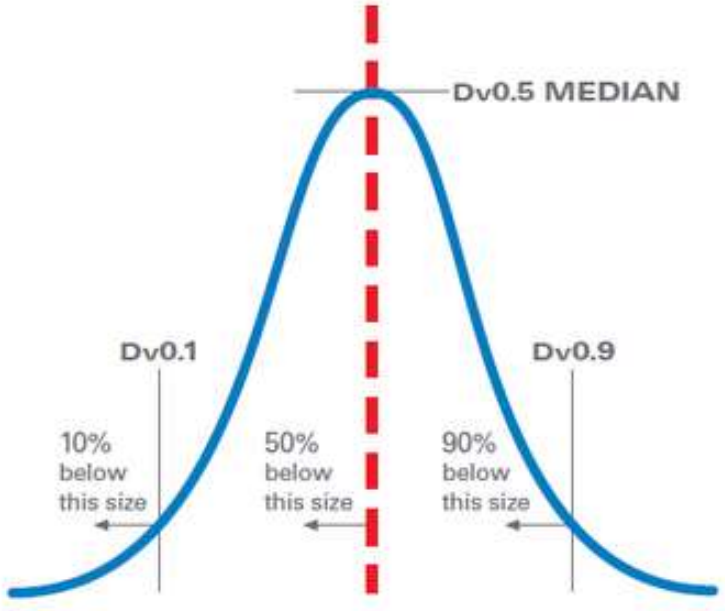
- The EMD is a widely used tool in pattern recognition, machine learning, computer vision, etc., especially for discriminant analysis of the histogram-type data.

- PSD (intensity) is the typical histogram data.

- The EMD can be used to compare the PSD profiles for equivalence test.

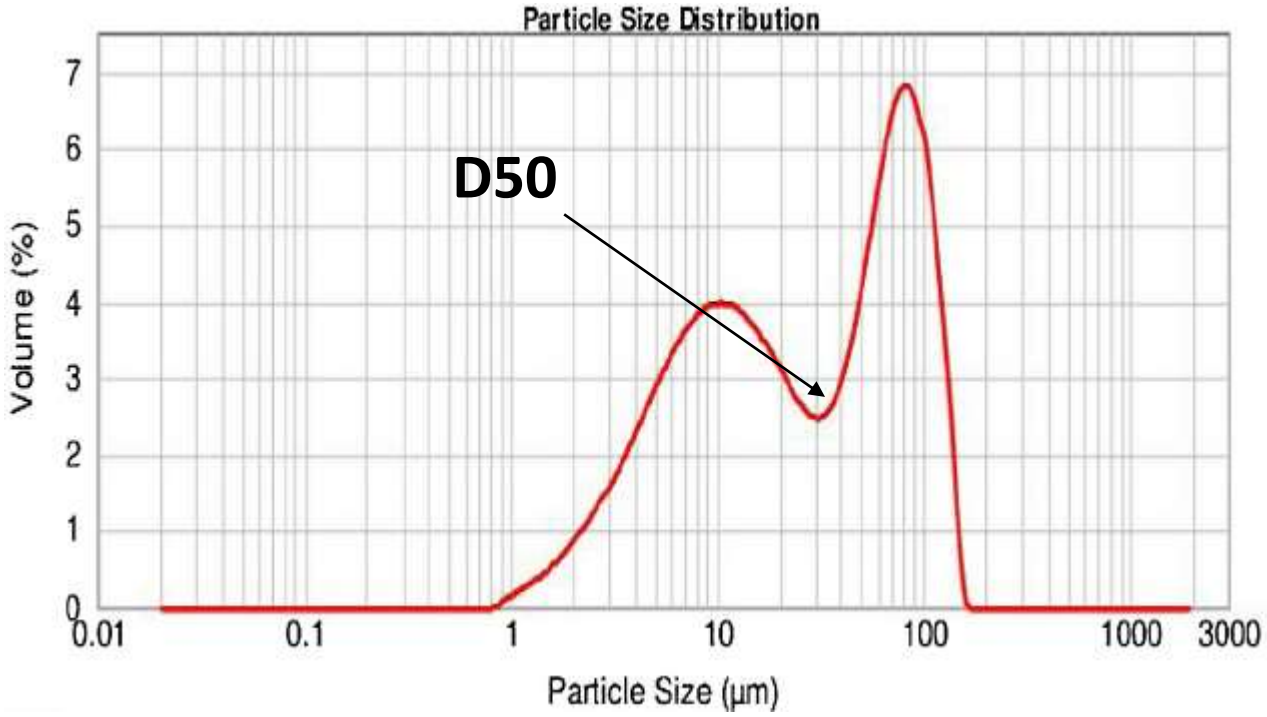
When is EMD needed?

D50: Median
SPAN: (D90-D10)/D50



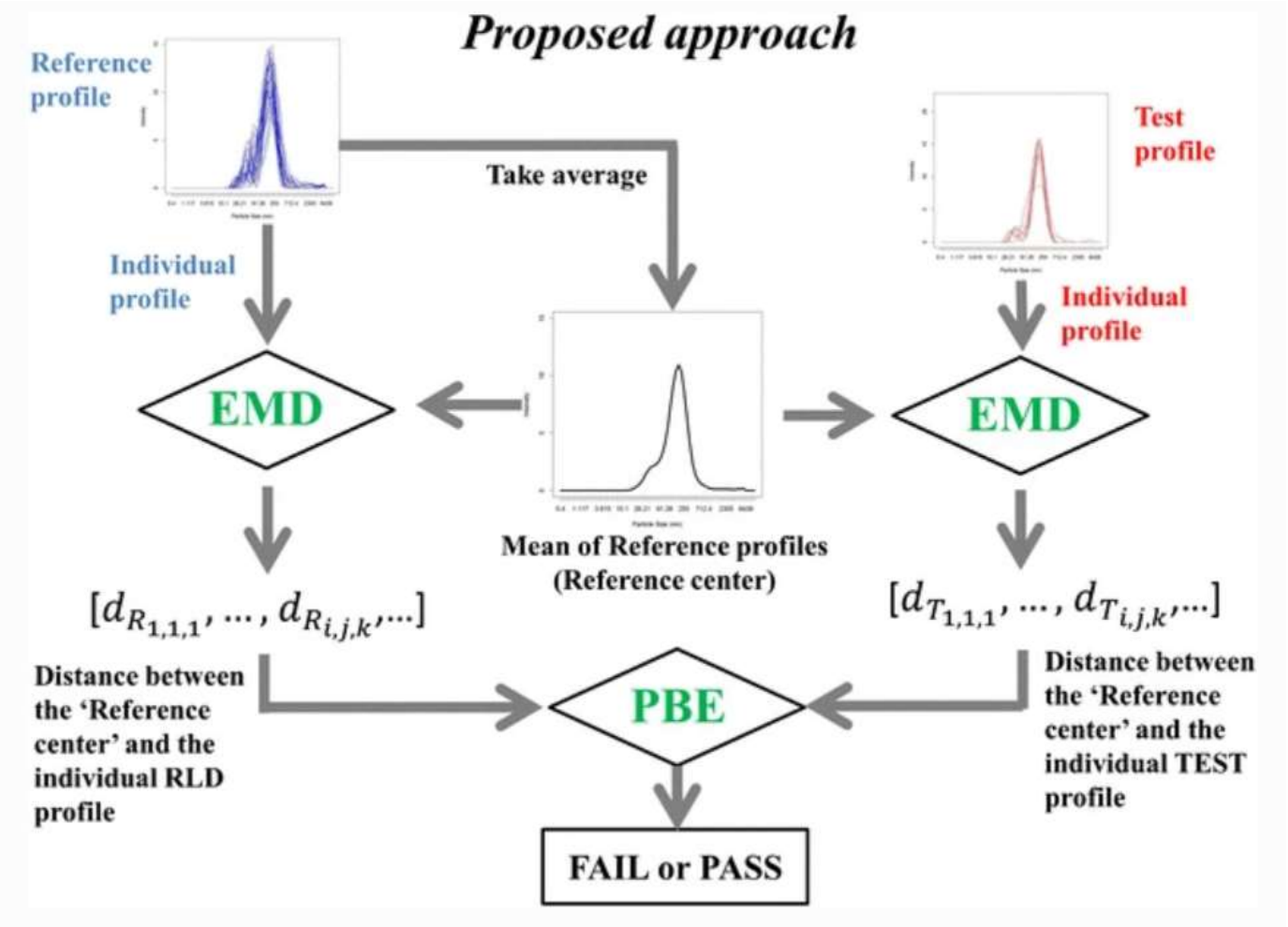
Mono-modal (single-peak) assumption is applied.

For a complex (e.g., multimodal) PSD profile, D50 and SPAN may not be appropriate metrics for the profile analysis.



Here is the place where the **EMD** comes into play for whole profile comparison.

A use case: EMD methodology details for particle size distribution comparison



Hu M, et. al., Equivalence Testing of Complex Particle Size Distribution Profiles Based on Earth Mover's Distance. AAPS J. 2018 Apr 12;20(3):62.

Currently recommended in the Product-Specific Guidances (PSGs) for PSD analysis

Contains Nonbinding Recommendations

Draft Guidance on Cyclosporine

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Active Ingredient:	Cyclosporine
Dosage Form; Route:	Emulsion; ophthalmic
Strength:	0.05%
Recommended Study:	Two options: in vitro or in

I. In vitro option:

To qualify for the in vitro option for this drug produc
met:

i. The test and reference listed drug (RLD) formula
quantitatively (Q2)[2] the same[3].

ii. Acceptable comparative physicochemical charact
formulations. The comparative study should be t

Contains Nonbinding Recommendations

Draft Guidance on Barium Sulfate

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Active Ingredient:	Barium sulfate
Dosage Form; Route:	For suspension; oral
Strength:	98% (334 g / bottle)
Recommended Studies:	In vitro study

Additional Comments:

- The proposed test drug product should be qualitatively (Q1)[1] and quantitatively (Q2)[2] the same as the reference listed drug (RLD).
- Test and reference drug products should have comparable physicochemical properties, including but not limited to, viscosity across a range of shear rates (e.g., low, medium, and high), and pH.
- The comparative analyses should be performed on at least three lots of the test drug product and three lots of the reference drug product.

The EMD-based approach described in the PSG for cyclosporine ophthalmic emulsion

Bioequivalence based on (95% upper confidence bound): Considering the fact that the shape of the globule size distribution of this product may not be mono-modal, the conventional population BE based on D50 and SPAN may not be sufficient to demonstrate bioequivalence.

Instead, the equivalence between the test and RLD formulations in the shape of the globule size distribution (such as the presence of multiple peaks) should be demonstrated by a method proposed by the sponsor. A statistical metric is preferred to assess the difference (e.g., in terms of distance) between the shapes of distribution profiles. One suggested approach is the earth mover's distance (EMD)[5] method, which computes the minimal cost needed to transform one distribution into the other using an optimization algorithm. An average profile of all RLD samples (i.e., RLD center) is calculated and served as the reference profile to compute the distance between a RLD or a test sample to the RLD center. After obtaining the profile distances between each RLD sample and the RLD average ('RLD' – 'RLD center' distance), and the profile distances between each test sample and the RLD average ('TEST' – 'RLD center' distance), a statistical metric should be employed to quantify the difference between the two categories of distances. One suggested method is the population BE test[6,7]. In order to properly account for variability of the reference product and to achieve adequate power, a sufficient number of samples and replicates should be used.

THANK YOU

Acknowledgements

- FDA BE Stat Guidance Revision Working Group
  - Elena Rantou, Ph.D.
  - Meng Hu, Ph.D.

References

- Guidance for Industry Immediate Release Solid Oral Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation, Center for Drug Evaluation and Research (CDER) November 1995 CMC 5, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/supac-ir-immediate-release-solid-oral-dosage-forms-scale-and-post-approval-changes-chemistry

- Nonsterile Semisolid Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) May 1997 SUPAC-SS CMC 7, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/supac-ss-nonsterile-semisolid-dosage-forms-scale-and-post-approval-changes-chemistry-manufacturing

- FDA Draft Guidance on Acyclovir, December 2016, https://www.accessdata.fda.gov/drugsatfda_docs/psg/Acyclovir_topical%20cream_RLD%2021478_RV12-16.pdf

- Shah, V.P., Tsong, Y., Sathe, P. and Liu, J.P., 1998. In vitro dissolution profile comparison—statistics and analysis of the similarity factor, f2. *Pharmaceutical research*, *15*(6), pp.889-896.