Scientific Considerations for In Vitro Bioequivalence Studies of Generic Cyclosporine Ophthalmic Emulsions

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BACKGROUND

Restasis®, a 0.05% cyclosporine ophthalmic emulsion (COE), is indicated to increase tear production in patients with suppressed production due to ocular inflammation associated with keratoconjunctivitis sicca (KCS).[1] Based on the clinical and critical formulation properties[2] of Restasis®, Office of Generic Drugs at the US Food and Drug Administration (FDA) posted a product specific guidance (PSG) that recommends two options for a generic sponsor to demonstrate bioequivalence (BE) of a generic COE product:[3] (1) a clinical endpoint study, or (2) in vitro studies with comparative physicochemical characterization and an in vitro drug release rate testing. As the in vitro option relies on demonstrating product sameness, FDA also recommends generics following the in vitro option to be formulated qualitatively and quantitatively similar to the reference product to ensure that any potential product differences are due to the manufacturing process, which are easily assessed by physicochemical testing.[2]

This study highlights the scientific considerations taken by the FDA in its recommendation of the in vitro bioequivalence studies for generic cyclosporine ophthalmic emulsion products. These in vitro studies include:

- **Qualitatively (Q1) and quantitatively (Q2) similarity to the RLD**
- **Comparative physicochemical properties**
- **Comparative *in vitro* drug release rate using a method that is able to discriminate the effect of process variability in the production of the test formulation.**

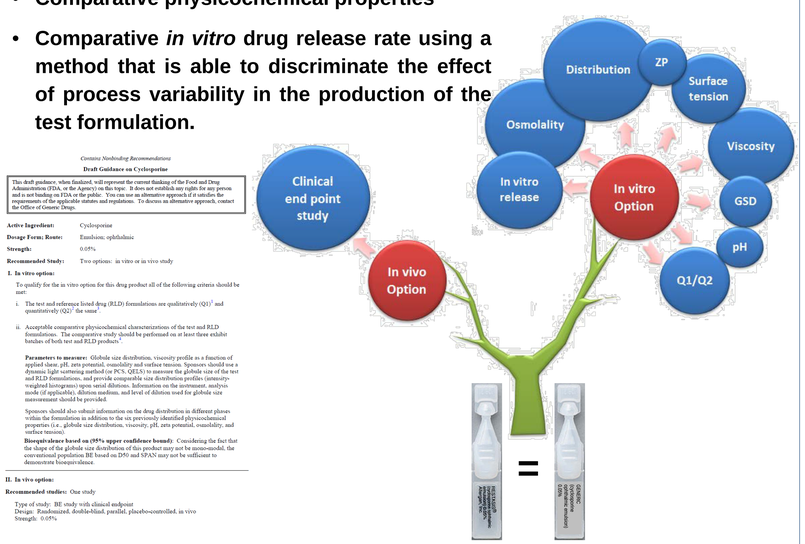
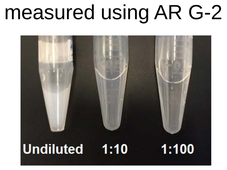
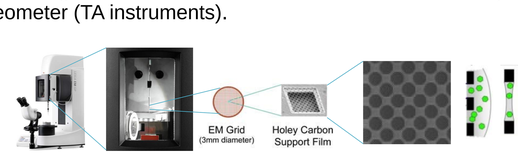


Fig. 1: FDA recommended in vivo and in vitro options to demonstrate the bioequivalence for a generic COE product. ZP: Zeta potential; GSD: globule size distribution; Q1/Q2: qualitative and quantitative sameness to the RLD.

MATERIALS AND METHODS

Globule size distribution of Restasis® was characterized by dynamic light scattering (DLS), using a Zetasizer Nano ZS (Malvern Instruments), NANO-flex nanoparticle size analyzer (Microtrac) and DynaPro plate reader II (Wyatt), laser diffraction (LD), using a Mastersizer 3000 with Hydro EV wet dispersion (Malvern), and by cryo Transmission Electron Microscopy (TEM), using a Jeol 1400 TEM/STEM at 120 kV. Viscosity was measured using AR G-2 rheometer (TA instruments).



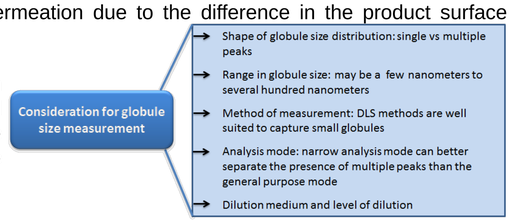


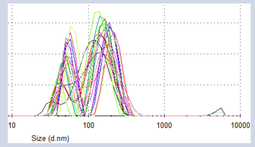
RESULTS

Globule size distribution (GSD):

GSD of a generic COE must be comparable to Restasis®, Changes in GSD may affect the ocular bioavailability of COE products due to changes in ocular clearance and altered drug diffusion and/or permeation due to the difference in the product surface area to volume ratio.[2]

Bioequivalence based on 95% upper confidence bound of GSD as assessed by the population bioequivalence approach. [4]



Dilution Ratio	Histogram (Intensity, % vs. Size, nm)	Z-Average (nm) 0.89 cP viscosity	PdI	Intensity Peaks (nm, % Intensity)
1:100		101.2 ± 1.3	0.27 ± 0.01	149.7 (78%) 46.3 (22%)

- DLS indicated that Restasis® is a polydisperse (multimodal) suspension with 50 nm and 150 nm globules. GSD parameters D50/SPAN and/or Z-average size/PdI are not appropriate GSD descriptors for BE assessment of multimodal GSD products.
- FDA recommends alternative methods, such as Earth Mover's Distance (EMD)[5] to compare the GSD of the generic and reference product. EMD computes the minimal cost needed to transform one distribution into the other using an optimization algorithm. The computed distance can then be used to evaluate the equivalence of the generic and reference product using the population BE test.
- Cryo-TEM provides a number-based GSD as well as morphology information about the sample. Cryo-TEM highlights the large number of small, 30 nm miscellular, particles in Restasis® in addition to the larger oil globules.

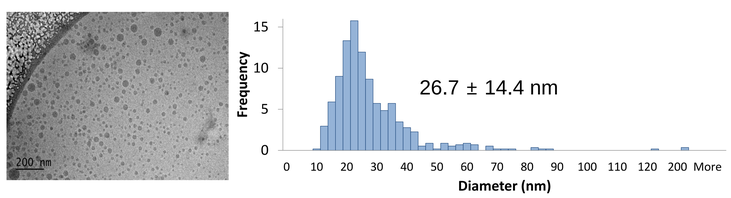


Fig. 2: Cryo-TEM Image of cyclosporine ophthalmic emulsion with image particle size analysis.

Viscosity profile as a function of applied shear:

Viscosity of a generic COE must be comparable to Restasis®. Increasing viscosity of ophthalmic formulations increases contact time between the formulation and eye tissues, potentially resulting in increased ocular bioavailability.

- Restasis® is a shear thinning (non-Newtonian) fluid. This can be attributed to carbomer copolymer A used in the formulation.
- FDA recommends viscosity profile as a function of applied shear as the viscosity changes and a single point viscosity does not accurately represent the product properties.

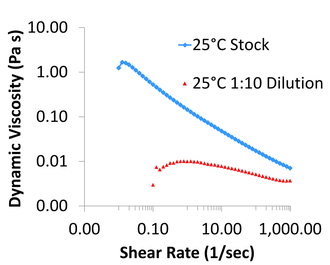


Fig. 3: Dynamic viscosity profile of cyclosporine ophthalmic emulsion.

Scientific rationale for other recommended physicochemical properties

A generic COE should be comparable to Restasis® in the following properties;

Parameters	Rationale
pH	• Irritation/Drug Absorption • Stability, solubility, permeability
Zeta Potential	• Product surface properties • Product stability
Osmolality	• Permeability • Irritation, tissue damage
Surface Tension	• Spreadability and corneal permeation • Irritation

Drug distribution in different phases within the formulation

- Within the formulation, cyclosporine can be distributed among the oil globules, micelles and the aqueous phase.
- The distribution of drug among these phases can influence the fraction of drug available for immediate absorption.
- A suitable analytical method needs to be applied to separate each phase for quantification of drug.

In vitro drug release

- The rate and extent of drug released from the emulsion can influence availability of drug for therapeutic effect.
- No validated method is currently available to test drug release from ophthalmic formulations, which necessitates developing and validating a sensitive method.
  - To account for the short ocular residence time, the method ideally should detect drug release at early time points.
  - The method should be able to discriminate the effect of process variability in the production. This can be done by comparing products that are intentionally manufactured with meaningful variations in formulation and manufacturing parameters, such as particle size, drug loading, and excipient amount.

CONCLUSIONS

- A weight of evidence approach is taken in the cyclosporine ophthalmic emulsion PSG in vitro BE option. The recommended in vitro tests represent a comprehensive evaluation of criteria formulation properties to demonstrate product sameness.
- Physicochemical characterization reveals that COE is a shear thinning fluid that contains a broad range (multimodal) GSD that require specific testing and analysis.
- An in vivo clinical endpoint study is recommended for COE products not formulated Q1/Q2 the same or with comparable physicochemical properties to the RLD.

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ACKNOWLEDGEMENTS

The authors would like to thank the FDA Advanced Characterization Facility and FDA/CDRH/OSEL for instrument access.

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