

Dose uniformity of topical corticosteroid preparations: difluprednate ophthalmic emulsion 0.05% versus branded and generic prednisolone acetate ophthalmic suspension 1%

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Purpose: To compare the dose uniformity of difluprednate ophthalmic emulsion 0.05% (Durezol®) with both branded and generic prednisolone acetate ophthalmic suspension 1% under different simulated patient usage conditions.

Methods: Drug concentrations of difluprednate emulsion, branded prednisolone acetate suspension (Pred Forte®) and generic prednisolone acetate suspension following three storage conditions (upright, then shaken; upright, not shaken; inverted, not shaken) were analyzed by high performance liquid chromatography assay and results were reported as percent of declared concentration. Two drops were dispensed every four hours four times daily.

Results: Regardless of bottle orientation and shaking, all difluprednate emulsion concentrations at each time point analyzed were within 15% of declared concentration. Both branded and generic prednisolone acetate suspension concentrations varied substantially throughout the study. For the bottle stored upright and not shaken, 46% of the branded concentrations were not within 15% of declared concentration; for the bottle stored upright and shaken prior to use, 60% failed to meet this criterion. None of the branded concentrations from the inverted and not shaken bottle was within 15% of declared concentration. Generic prednisolone concentrations demonstrated the poorest dose uniformity, with 96% of the concentrations from the inverted and not shaken bottle not within 15% of declared concentration; 94% of the concentrations from the upright and shaken bottle and 87% from the upright and not shaken bottle similarly failed to meet this criterion.

Conclusions: Dose uniformity of Durezol emulsion was predictable in all simulated patient usage conditions, whereas the drop concentrations of Pred Forte and generic prednisolone acetate suspensions were highly variable throughout the study. Drop concentrations are more predictable with Durezol emulsion than with either prednisolone acetate suspension.

Keywords: difluprednate, dose uniformity, emulsion, prednisolone acetate

Introduction

The dose uniformity of any ophthalmic medication is critically dependent on the homogeneity of the formulation. Most topical ophthalmic preparations containing lipid-soluble and aqueous components are suspensions, in which the active drug is suspended in an aqueous vehicle. Homogeneity is a concern for suspensions because sedimentation and aggregation occurs upon storage of the medication.¹ Because of a suspension's tendency to settle over time, suspensions must be shaken prior to use in order to redisperse the drug throughout the contents of the bottle. The need for shaking

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to achieve dose uniformity raises several potential concerns. First, the type, intensity, and duration of shaking behavior vary among people, and no studies have identified the shaking characteristics required for optimal redispersibility.² Second, in a study of corticosteroid preparations, Apt et al determined that patient compliance with shaking instructions was only 37%.³ Third, even with “optimal” shaking, suspensions may potentially have poor homogeneity due to formulation issues with particle size, caking, and agglomeration. Each of these issues may contribute to a lack of dose uniformity, which produces the potential for decreased efficacy with lower-than-expected dosing or increased safety concerns with higher-than-expected dosing.

Technologic advances have now provided ophthalmic preparations consisting of oil in water emulsions, with the drug molecule dissolved in the oil phase and aqueous-compatible surfactants providing emulsion stability, obviating the need for shaking prior to use. Thus, emulsion formulations have improved dose uniformity compared with suspensions. Ophthalmic emulsions were first introduced nearly a decade ago, with the commercial availability of two dry eye products, ie, over-the-counter (OTC) Refresh Endura® (Allergan, Inc., Irvine, CA) and prescription formula Restasis® (Allergan, Inc.). Since that time, another artificial tear product with a lipid emulsion formulation, the OTC Soothe® XP Emollient eye drops (Bausch and Lomb, Rochester, NY), has been introduced. Difluprednate ophthalmic emulsion 0.05% (Durezol®, Alcon Laboratories, Inc., Fort Worth, TX) is the only other ophthalmic lipid emulsion commercially available in the US. This corticosteroid was approved by the US Food and Drug Administration in 2008 for the treatment of pain and inflammation associated with ocular surgery.⁴

In order to verify the expected dose uniformity of the difluprednate emulsion, the concentration of difluprednate was measured under varying simulated patient conditions. It was also compared with two active controls, branded prednisolone acetate ophthalmic suspension 1% (Pred Forte®, Allergan, Inc., Irvine, CA), one of the most commonly used formulations of ophthalmic prednisolone in the US, and generic prednisolone acetate ophthalmic suspension 1%. Prednisolone acetate is indicated for the treatment of steroid-responsive inflammation of the palpebral and bulbar conjunctiva, cornea, and anterior segment of the globe.⁵

Methods

Test articles

Commercially available difluprednate ophthalmic emulsion 0.05% (Durezol), branded prednisolone acetate ophthalmic

suspension 1% (Pred Forte), and generic prednisolone acetate ophthalmic suspension 1% (Falcon Pharmaceuticals Ltd) in their originally manufactured containers were evaluated in this study. Five mL of difluprednate was contained in a 5 mL nominal volume bottle and 5 mL each of branded and generic prednisolone acetate was contained in 10 mL bottles.

Study procedure

This study evaluated the effects of bottle orientation during storage and the presence or absence of shaking on the uniformity of dispensed drops. Three bottles of each test article were stored in a defined orientation for 12 hours, ie, two bottles of each test article were stored upright and one each was stored inverted. Following storage, one of the two upright bottles of each test article was shaken for five seconds using a wrist-action mechanical shaker at six cycles/sec, with an amplitude of 0.7 cm (Table 1). After shaking the two test article bottles, two drops of all nine bottles were dispensed into a tared glass vial. The drug concentration of the drops was analyzed by high performance liquid chromatography (HPLC), and results were reported as percent of declared concentration. The assay for determining difluprednate concentration was a specific, stability-indicating, normal-phase HPLC method. The procedure used gradient elution and ultraviolet detection. The assay method in the monograph for prednisolone acetate ophthalmic suspension in the US Pharmacopeia was used for determining prednisolone acetate concentration. A slight modification in sample preparation was made and validated to account for the small sample size used in this study. In all cases, HPLC instrumentation from Waters Corporation (Milford, MA) was utilized. The dispensing protocol was repeated three times at four-hour intervals each day to simulate four times daily patient dosing. On days 0, 5, 10, and 15, the drug concentrations from each of the four time points were analyzed. On the other days, data from the first and fourth time points were analyzed.

Table 1 Study design evaluating the effect on ophthalmic steroid dose uniformity of varying simulated patient usage conditions, specifically bottle storage orientation and shaking

Test article	Storage orientation	Shaking
Pred Forte®	Upright	No
	Upright	Yes
	Inverted	No
Durezol®	Upright	No
	Upright	Yes
	Inverted	No
Generic prednisolone	Upright	No
	Upright	Yes
	Inverted	No

Results

The dose uniformity of all three steroid preparations after upright bottle storage and no shaking prior to dispensing is shown in Figure 1. The percentage of declared concentration of the difluprednate emulsion was near 100% at 97.6% (40/41) of the data points for this test condition. The percentage of declared concentration of the branded prednisolone acetate suspension was more variable over time, ranging from 20.5% to 181.4%. At day 0, prednisolone concentrations were greater than the declared concentration, but then dropped below declared concentration and did not consistently reach concentrations greater than 100% until day 5. Concentrations remained primarily above 100% through day 7, then were near or below 100% through day 11, at which time concentrations remained consistently above 100% until the bottle was emptied on day 14. On days 0, 5 and 10, when data from all four time points were analyzed, branded prednisolone concentrations were lowest at the first time point and peaked at the third or fourth time point; each of these data series showed substantial intraday variability, with day 5 containing both the lowest (20.5%) and the highest (181.4%) concentrations. The generic prednisolone acetate concentrations were the most varied of all the steroid preparations, ranging from 7.0% to 231.5% of declared concentration. Most time points prior to day 13 were less than 100% of declared concentration, whereas each time point at day 14 and beyond was greater than 100%, ranging from 123.6% to 231.5%.

When upright bottles were shaken slowly for five seconds prior to dispensing, difluprednate emulsion concentrations were consistently near 100% of declared concentration, with the range of concentrations varying 8.1% (Figure 2). The branded prednisolone acetate suspension concentrations were primarily higher than 100% of the declared concentration, with the initial concentration 312.0% of declared concentration; only two data points were below 100%, but the lowest reached only 51.0% of declared concentration. In contrast, generic prednisolone acetate concentrations were primarily below 100% through day 14, with most time points below 70% of declared concentration. From day 15 and beyond, only three time points were below 100%, with concentrations on the final two days spiking to 504.6% of declared concentration.

Figure 3 illustrates that the inverted bottle of difluprednate emulsion, not shaken prior to dispensing, approximated declared concentration throughout the study, with a high of 103.5% and a low of 96.2%. The branded prednisolone acetate suspension produced concentrations above the declared concentration (up to 201.4%) for the first two days, after which concentrations dropped below 100%, with values ranging from 80.1% to 10.7% of declared concentration. The generic prednisolone acetate suspension followed a similar pattern, with most time points in the first three days far above 100% of declared concentration, after which they dropped far below 100%. None of the time points for the

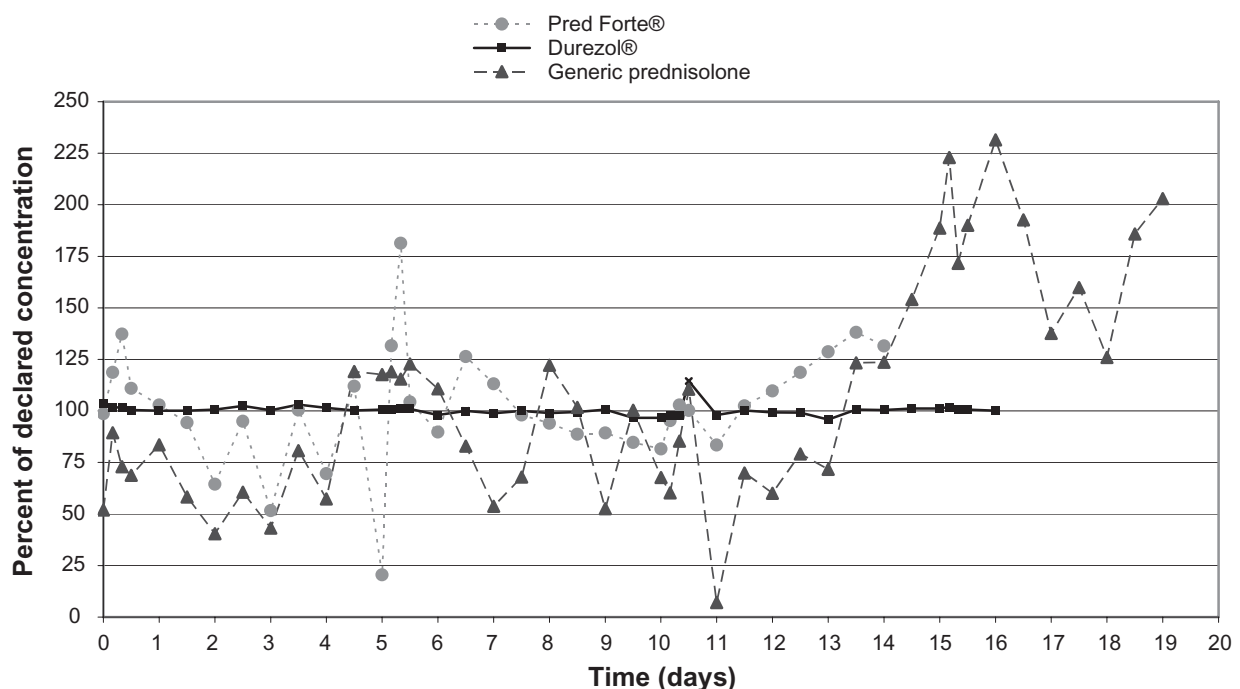


Figure 1 Dose uniformity of difluprednate emulsion and prednisolone acetate suspensions with upright storage orientation and no shaking of contents.

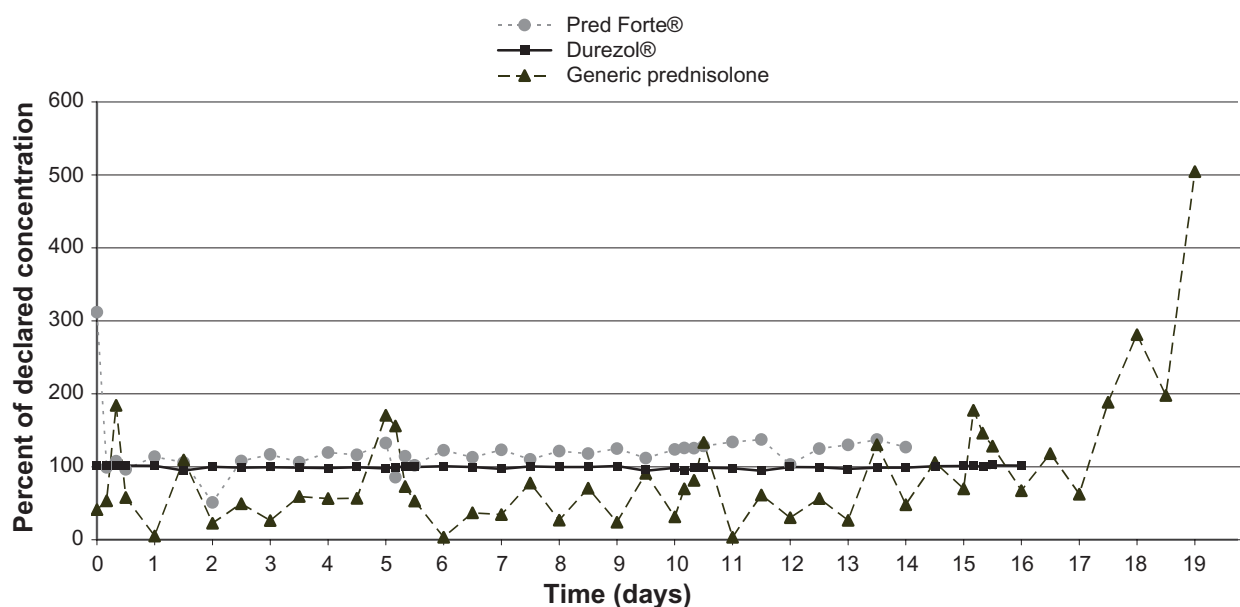


Figure 2 Dose uniformity of difluprednate emulsion and prednisolone acetate suspensions with upright storage orientation after 5 seconds of shaking of contents.

final 10 days of the experiment reached greater than 20% of declared concentration.

As shown in Table 2, difluprednate emulsion concentration remained within 15% of declared concentration at each time point analyzed and under all conditions tested. In contrast, branded prednisolone acetate suspension concentrations met this criterion for 54% of the upright and not shaken time points and 40% of the upright and shaken time points. None of the prednisolone acetate concentrations from the inverted bottle was within 15% of declared concentration.

Generic prednisolone acetate concentrations were within 15% of declared concentration for only 4%–13% of the time points, depending on the condition tested. Measurement of drug concentrations within 10% of declared concentration remained nearly identical for difluprednate emulsion, with only one datum point from the upright and not shaken bottle outside this range (98% of the data points were within 10% of declared concentration). One hundred percent of concentrations analyzed from the other two difluprednate bottles were within 10% of declared concentration.

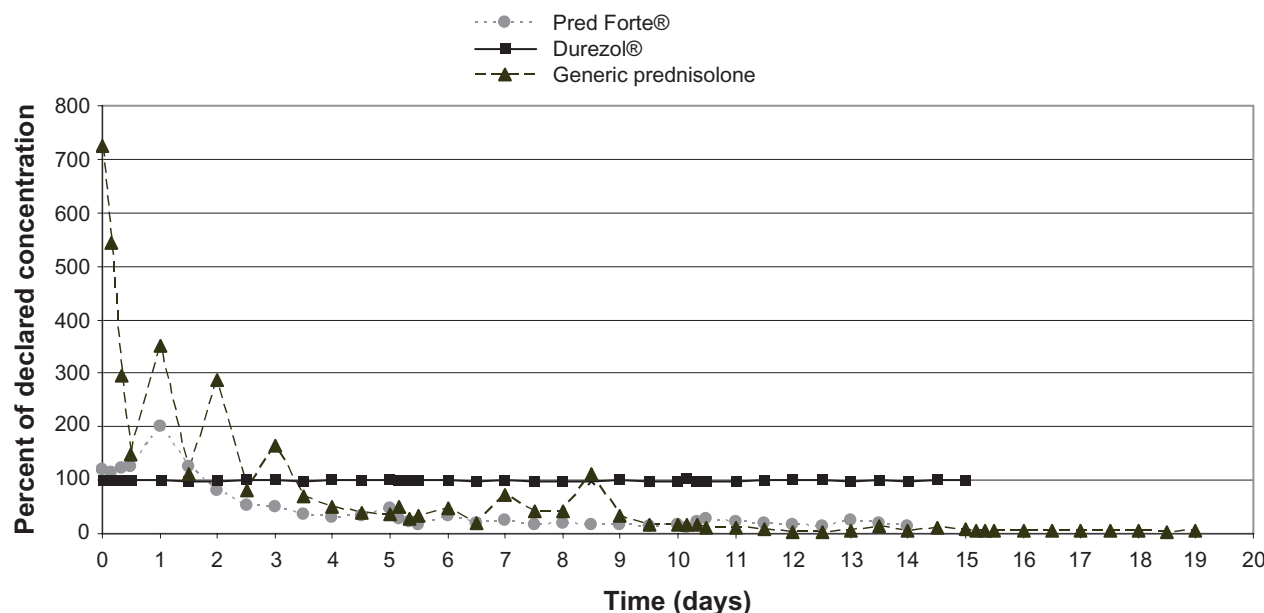


Figure 3 Dose uniformity of difluprednate emulsion and prednisolone acetate suspensions with inverted storage orientation and no shaking of contents.

Table 2 Percentage of data points within 15% of declared concentration

Usage condition	Pred Forte®	Durezol®	Generic prednisolone
Upright, not shaken	54%	100%	13%
Upright, shaken	40%	100%	6%
Inverted, not shaken	0%	100%	4%

Branded prednisolone acetate concentrations within 10% of declared concentration were 34% for the upright and not shaken bottle, 23% for the upright and shaken bottle, and 0% for the inverted bottle. Generic prednisolone acetate concentrations within 10% of declared concentration were 4% for the upright and not shaken bottle, 6% for the upright and shaken bottle, and 0% for the inverted bottle.

Discussion

This study compared two corticosteroid suspensions with a corticosteroid formulated in an emulsion. Suspensions have a well characterized tendency to settle, agglomerate, and recrystallize over time, all of which reduce dispersion homogeneity and allow potential dosing inconsistencies. One approach to reduce these dosing errors is to shake the contents prior to use in an attempt to redisperse the drug; another approach is to formulate the drug as an emulsion rather than a suspension. Emulsions are produced by dissolving the active drug molecule within the oil phase of the formulation. Emulsions also contain a surfactant, which acts as a stabilizer, preventing the oil droplets from coalescing and allowing aqueous compatibility.⁶ Thus, emulsions create homogeneous dispersions that are not subject to sedimentation or aggregation. Empirically, emulsions have been shown to produce greater ocular bioavailability than suspensions, as demonstrated by a pharmacokinetic study conducted in rabbits, in which a corticosteroid emulsion showed a 4.1-fold higher area under the curve (AUC) in aqueous humor and a 4.6-fold higher AUC in cornea than the same corticosteroid formulated as a suspension.⁷ A similar relationship between emulsion and suspension formulations was reported by Inoue et al in another pharmacokinetic animal study, in which the corticosteroid emulsion produced an aqueous humor concentration that was 40% higher than the corticosteroid suspension.⁸

The current study illustrates the predictability in dose uniformity of difluprednate ophthalmic emulsion 0.05% compared with prednisolone acetate suspension 1%. Regardless of the simulated patient usage conditions (upright versus inverted storage, shaking prior to use versus no shaking),

difluprednate remained near 100% of declared concentration with little variability. Only one datum point was not within 10% of declared concentration, and this was likely an outlier, as evidenced by the fact that the other 40 data points from this testing condition were within 4.2% of declared concentration. These results were reproduced in multiple additional runs conducted under similar conditions using other difluprednate lots (data not shown). In contrast, both branded and generic prednisolone acetate suspensions varied substantially in all three simulated patient usage conditions, with none of them showing consistent approximation of declared concentration. Concentrations from the branded prednisolone acetate bottle stored upright with no shaking prior to use varied considerably throughout the study, alternating between very high and low concentrations. Even prednisolone acetate drops dispensed on the same day differed dramatically, with the largest variability exhibited on day 5, ie, 20.5% at the first time point compared with 181.4% at the last time point. Concentrations from the generic prednisolone acetate bottles demonstrated the largest variability, ranging from 224.5% for the upright and not shaken bottle to 720.8% for the inverted bottle.

The dose uniformity of difluprednate can be attributed to its emulsion formulation. Because the concentration of drug throughout the bottle is consistent, it is not subject to bottle storage orientation. Moreover, difluprednate emulsion does not require shaking of the contents in order to resuspend the drug within its vehicle. In contrast, prednisolone acetate is required to be shaken prior to use because of its poor homogeneity upon storage.⁵ In the current study, however, shaking the prednisolone acetate bottles, either branded or generic, prior to dispensing did little to improve the dose uniformity, as shown in Figure 2. In this data set, only 40% of the time points from the branded prednisolone and 6% from the generic prednisolone had concentrations within 15% of declared concentration. Of interest is the difference in the effect of shaking between the branded prednisolone and generic prednisolone products. Overall, very low concentrations in the generic prednisolone bottle were observed through day 15, while the branded product exhibited overall higher concentrations with much less variability. In most cases, a lower dispensed drug concentration of generic prednisolone was observed on the first dose of each day compared with the final dose of each day. This suggests that with enough agitation, the generic prednisolone will eventually disperse uniformly in the bottle. It is possible that the type, intensity, or duration of the mechanical shaking performed in this study was not adequate to resuspend the drug fully, but this cannot

be evaluated because no studies have been published that determine optimal shaking parameters. Nonetheless, these results suggest that the effect of shaking on the resuspension of prednisolone acetate should be further evaluated.

The inverted bottles of prednisolone acetate, which were not shaken prior to use, demonstrated the greatest concentration variability of all suspension bottles tested. Initially, both the branded and generic prednisolone acetate concentrations from the inverted bottles were greater than the declared concentration. However, by day 3, concentrations rapidly dropped below declared concentration, not reaching above 50% of declared concentration for the final 10 days of either suspension. This pattern of dose variability (increased concentrations at early time points followed by low concentrations later) is consistent with settling occurring within the tip of the bottle, allowing for highly concentrated drops to be dispensed initially until the majority of the drug has been depleted from the bottle, resulting in little drug dispensed at later time points. The discrepancy between the dose variability of prednisolone acetate suspension in this study and the dose uniformity exhibited by difluprednate emulsion suggests that the clinical use of difluprednate may produce more predictable efficacy and safety.

Conclusion

The dose uniformity of difluprednate ophthalmic emulsion 0.05% was highly predictable in all simulated patient usage conditions, confirming that this product is not dependent on bottle shaking. The drop concentration of both branded and generic prednisolone acetate suspension 1% was highly variable over the duration of the study and across patient usage conditions. Shaking did not improve the dose uniformity of these products.

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Disclosure

WS was an employee of Sirion Therapeutics, Inc., during the generation of these data. Sirion Therapeutics sold the rights to market difluprednate ophthalmic emulsion 0.05% to Alcon Laboratories in March 2010. Roy Bryant is COO of Encompass Pharmaceutical Services, Inc, who was paid a consulting fee by Sirion Therapeutics, Inc. to perform this study. No other conflicts of interest existed for either author.

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