Technical Report:

Antimicrobial Effectiveness Testing of Antihistamine and Corticosteroid in LoxaSperse® Dispersion

Abstract: LoxaSperse™ is a powder excipient base used for nebulization and irrigation designed to improve dispersibility and solubility of Active Pharmaceutical Ingredients (APIs). PCCA tested the performance of LoxaSperse formulations containing fluticasone propionate alone and in combination with levocetirizine dihydrochloride, and measured its efficacy against microbial activity when mixed with sterile water. The intent was not to determine clinical efficacy of the API(s) used as antimicrobials but to determine the ability of the dry powder preparation to resist microbial growth. The Antimicrobial Effectiveness Test (AET) was performed at 0.5h, 6h, 28h and 168h – serially diluted, and plated for colony counts. LoxaSperse formulations required 0.5h to significantly reduce and completely eliminate viable *S. aureus* and *P. aeruginosa. The same effect against* viable *E. coli* and *C. albic*ans required 168h. LoxaSperse formulations prevented *A. niger* proliferation over 7 days of testing. The results of this study demonstrate that accidental or intentional contamination of the finished or reconstituted preparation did not result in microbial growth.

Purpose:

The intent of this study was to evaluate results of purposeful inoculation of the formulations with microorganisms specified in USP <51> (The United States Pharmacopeial Convention, 2013a), for nasal and inhalation use with modified Antimicrobial Effectiveness Test (AET) methodology and to quantitatively determine the *in vitro* effectiveness of formulations containing LoxaSperse to prevent microbial proliferation and/or kill the organisms.

Introduction:

LoxaSperse is a powder excipient base used for nebulization and irrigation. LoxaSperse is a blend of specially micronized xylitol with an optimized ratio of micronized poloxamers, designed to improve the dispersibility and solubility of active pharmaceutical ingredients (APIs) (PCCA, 2013). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence of their safety and efficacy (Durairaj *et al.*, 2006; Jagannath *et al.*, 1995; Plataki *et al.*, 2011; Zabner *et al.*, 2000).

Fluticasone propionate is one of the most prescribed inhaled corticosteroids in the United States, being the preferred therapy for persistent asthma by acting directly on the pulmonary airways through topical anti-inflammatory effects (Colice *et al.*, 2013). Levocetirizine dihydrochloride is a second-generation antihistamine for the relief of symptoms associated with allergic rhinitis and uncomplicated skin manifestations of chronic idiopathic urticaria. It is known that current treatment options for allergic rhinitis include antihistamines and corticosteroids (Singh-Franco *et al.*, 2009).

In order to verify the effectiveness of LoxaSperse formulations against microbial activity, capsules containing LoxaSperse with fluticasone propionate alone and in combination with levocetirizine dihydrochloride were mixed with sterile water. The final suspensions designed for nasal administration and local effect were assayed by AET methodology for 7 days.

Methodology:

Materials: Fluticasone Propionate USP Micronized (lot number C145638), and Levocetirizine Dihydrochloride (lot number C150499) were obtained from PCCA (Houston, TX, USA) as well as the excipient LoxaSperse (lot number 5994620). Capsules size #1 were filled with the following formulations and stored at 4°C: *formulation 1*, fluticasone propionate (180 mg) in LoxaSperse (448 mg); *formulation 2*, fluticasone propionate (180 mg) and levocetirizine dihydrochloride (265 mg) in LoxaSperse (448 mg). The test solutions were prepared by EPS by adding the contents of 1 capsule of each formulation to 10 mL of sterile water.

Microorganisms Strains: *E. coli* ATCC 8739, *A. niger* ATCC 16404, *C. albicans* ATCC 13231, *P. aeruginosa* ATCC 9027 and *S. aureus* ATCC 6538 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). All strains were maintained as glycerol stock solutions at -80°C. Working stocks were grown on tryptic soy (bacteria growth) or Sabouraud dextrose (fungi growth) agar media at 35°C.

Antimicrobial Effectiveness Test (AET):
Growth, harvesting, and enumeration of *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans* and *A. niger* were performed according to universal AET methodology (Moser and Meyer, 2011) with minor modifications. 1 mL aliquots of the test solutions (formulations) were prepared in 15 mL polycarbonate test tubes. 10 µL of cell culture (from 10^4 to 10^5 CFU/mL stock, diluted in phosphate buffered saline, PBS, Sigma Aldrich®) was added to each 1 mL aliquot to initiate the AET assay. 10 µL of cell culture was also added to 1 mL PBS for initial colony counts at the start of the AET assay. During the AET assay carried out at 20-25°C (room temperature), 100 µL of each challenged contaminated test solution was removed at intervals of 0.5h, 6h, 24h, and 7d (168h), serially diluted, and plated for colony counts on specific growth media. The results are presented as final colony counts, reported in CFU/mL and Log_{10} reductions in viable cell numbers at defined time intervals, being compared to the time zero performed on the PBS control inoculum levels.

Results and Discussion:

Initial colony counts of *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. niger* indicated that a 10^2 to 10^3 CFU/mL product challenge was performed for these organisms (Table 1).

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Over the course of the AET, viable cell/spore counts changed according to the test solution (formulation) and organism tested. Formulation 1 eliminated the viable cells of *S. aureus* and *P. aeruginosa* in 0.5h and kept the solution free of bacteria for 7 days. *E. coli* was progressively (1-Log reduction/time interval from 0.5h) eliminated in 7 days, while *C. albicans* had the cell counts reduced only after 24h of incubation, being killed at 7 days. Formulation 2 induced the death of *S. aureus* in 0.5h, maintaining the solution free of bacteria for 7 days. A 2-Log reduction was achieved for *P. aeruginosa* in 0.5h exposure, with the solution completely cleared of bacteria by 6h and lasting through 7 days. This formulation showed a similar profile as formulation 1 against *E. coli* and *C. albicans*. The cell counts of *A. niger* did not change significantly over time for both formulations.

Table 1. Initial colony counts from adjusted cultures.

Organism	CFU/mL
Control	≤10*
E. coli	2.04 x 10^3
A. niger	2.4 x 10^2
C. albicans	1.5 x 10^2
P. aeruginosa	1.07 x 10^3
S. aureus	4.7 x 10^2

Table 2. Recovered colony counts from AET (CFU/mL).

CFU/mL at time (h):					
Organism	**F**	**0.5**	**6**	**24**	**168**
Control		≤10*	≤10*	≤10*	≤10*
E. coli	*1* *2*	2.6 x 10^3 2.3 x 10^3	7.9 x 10^2 5.9 x 10^2	50 60	≤10* ≤10*
A. niger	*1* *2*	6.0 x 10^2 2.6 x 10^2	5.4 x 10^2 3.2 x 10^2	50 7.8 x 10^2	2.4 x 10^2 1.7 x 10^2
C. albicans	*1* *2*	1.5 x 10^2 2.1 x 10^2	4.4 x 10^2 1.5 x 10^2	2.7 x 10^2 1.3 x 10^2	≤10* ≤10*
P. aeruginosa	*1* *2*	≤10* 10	≤10* ≤10*	≤10* ≤10*	≤10* ≤10*
S. aureus	*1* *2*	≤10* ≤10*	≤10* ≤10*	≤10* ≤10*	≤10* ≤10*

*≤10 denotes below detection limits USP <1227> (The United States Pharmacopeia Convention, 2013b); F = formulation

Conclusions:

Both formulations containing LoxaSperse required 0.5h to significantly reduce and completely eliminate viable *S. aureus* and *P. aeruginosa* and no bacterial growth was observed in the solutions up to 7 days. This behavior characterizes a 2-Log to 3-Log reduction in viable bacterial cells. *E. coli* counts were reduced over time and completely killed in 7 days while *C. albicans* was killed at 7 days. *A. niger* remained viable throughout the test. The chosen formulas when intentionally contaminated with microorganisms specified in USP <51> resisted microbial growth. Further, this study demonstrated these formulations after reconstitution were not at risk or did not support microbial growth.

Financial Disclosure:

PCCA contracted Emeryville Pharmaceutical Services (EPS, Emeryville, CA) to conduct this study. EPS has no proprietary or financial interests in the test products, or equity interest in PCCA, the sponsor of the study.

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