Technical Report:

Antimicrobial Effectiveness Testing of a Budesonide LoxaSperse® Dispersion

Abstract: LoxaSperse is a powder excipient base used for nebulization and irrigation designed to improve dispersability and solubility of Active Pharmaceutical Ingredients (APIs). PCCA tested the performance of PCCA Formula #10341 (budesonide 0.5 mg in a LoxaSperse mixture) and measured its efficacy against microbial activity when mixed with sterile water. The intent is not to determine efficacy of budesonide as an antimicrobial. The Antimicrobial Effectiveness Test (AET) was conducted at 0.5h, 6h, 28h and 168h – serially diluted, and plated for colony counts. Budesonide LoxaSperse dispersions required 0.5h to 28h to significantly reduce the number of viable bacterial cells (*E. coli, S. aureus* and *P. aeruginosa*). 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 formulation 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 determine the *in vitro* efficacy of formulas containing LoxaSperse to reduce microbial counts or inhibit viable cell growth.

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 dispersability and solubility of 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). Budesonide is a corticoid with mainly glucocorticoid activity (*Martindale 35*, 2007). PCCA tested the performance of Formula #10341, budesonide 0.5 mg in a LoxaSperse mixture, and measured its efficacy against microbial activity when mixed with sterile water.

Methodology:

The efficacy of budesonide LoxaSperse dilutions were evaluated by serially diluting the formula in sterile water and plating for colony counts with *S. aureus, P. aeruginosa, E. coli*, *C. albicans* and *A. niger* at intervals of 0.5h, 6h, 28h and 168h.

Materials and Methods:

A Budesonide Micronized USP (lot number C158080) capsule was prepared by PCCA (Houston, TX, USA) following the instructions on PCCA Formula #10341 (budesonide 0.5 mg in a LoxaSperse mixture). The final solutions were subsequently prepared by an outside laboratory at time of testing by adding one budesonide capsule (PCCA Formula #10341) to 10 mL of sterile water.

Bacterial Strains:

The strains were 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 or Sabouraud 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 procedures (Moser and Meyer, 2011). 1 mL aliquots of the test articles were prepared in 15 mL polycarbonate test tubes. 10 µL of cell culture (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, 100 µL of the mixture was removed at intervals of 0.5h, 6h, 28h, and 7d (168h), serially diluted, and plated for colony counts. Final colony counts, reported in CFU/mL and Log_{10} reductions in viable cell numbers, are discussed in this report.

Results and Discussion:

Initial colony counts of *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* indicated that a 10^2 to 10^4 CFU/mL product challenge was performed for these organisms (**Table 1**). *A. niger* colonies were not obtained from these initial plates (≤10 CFU/mL, **Table 1**), but counts from subsequent plates indicated that 10^1 to 10^2 spores were present at the start of the AET (**Table 2**).

Over the course of the AET, viable cell/spore counts varied depending upon the test article, where it was prepared, and the test organism.

E. coli: a 1-Log reduction after 6h and no viable cells after 28h.
S. aureus: little change in cell viability was observed over 168h, when no viable cells were recovered.

C. albicans: viable cells were recovered and continued to increase in number over the course of the AET.

A. niger: little change in the number of viable cells was observed.

P. aeruginosa: a 2-Log reduction after 0.5h. No viable cells were recovered after 6h.

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Table 1. Initial colony counts from adjusted cultures.

Organism	CFU/mL
Control	≤10*
E. coli	9.7 x 10^3
A. niger	≤10*
C. albicans	3.2 x 10^2
P. aeruginosa	5.9 x 10^3
S. aureus	1.0 x 10^4

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

	CFU/mL at time (h):			
Organism	**0.5**	**6**	**28**	**168**
Control	≤10*	≤10*	≤10*	≤10*
E. coli	2.6 x 10^3	4.6 x 10^2	≤10*	≤10*
A. niger	1.0 x 10^1	1.0 x 10^1	1.0 x 10^1	≤10*
C. albicans	2.5 x 10^2	6.7 x 10^2	≤10*	3.0 x 10^3
P. aeruginosa	4.0 x 10^1	≤10*	≤10*	≤10*
S. aureus	7.98 x 10^3	6.36 x 10^3	8.50 x10^3	≤10*

*≤10 denotes below detection limits USP <1227> (The United States Pharmacopeial Convention, 2013b).

Conclusions:

The test article containing budesonide and LoxaSperse required 0.5h to 28h to significantly reduce the number of viable bacteria (*E. coli, S. Aureus* and *P. aeruginosa*). *A. niger* showed a decrease in the number of viable cells up to 168h. The chosen formula when intentionally contaminated with microorganisms specified in USP 51 resisted microbial growth. Further, this study demonstrated this formulation after reconstituted was not at risk or did not support microbial growth.

Financial Disclosure:

For this study, PCCA contracted a third party laboratory with no proprietary or financial interests in the test products, or equity interest in PCCA.

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