Technical Report:

Antimicrobial Effectiveness Testing of a Gentamicin 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 PCCA Formula #10337 (gentamicin 80 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 gentamicin as an antimicrobial. The Antimicrobial Effectiveness Test (AET) was conducted at 0.5h, 6h, 28h and 168h – serially diluted, and plated for colony counts. Gentamicin LoxaSperse dispersions reduced the number of viable bacteria (*E. coli, S. aureus* and *P. aeruginosa*) within 0.5h of exposure and no bacterial growth was observed in the test article up to 128h after exposure. A 3-Log to 4-Log reduction in viable bacterial cells was observed within 0.5h. 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). Gentamicin is an aminoglycoside antibiotic and has bactericidal action against many gram-negative aerobes and against strains of staphylococci (*Martindale 35*, 2007). PCCA tested the performance of Formula #10337 which is gentamicin (80 mg) in a LoxaSperse mixture and measured efficacy against microbial activity when mixed with sterile water.

Methodology:

The efficacy of gentamicin 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* in the intervals of 0.5h, 6h, 28h and 168h.

Materials and Methods:

A Gentamicin Sulfate USP (lot number C150822) capsule was prepared by PCCA (Houston, TX, USA) following the instructions on PCCA Formula #10337 (gentamicin 80 mg/LoxaSperse). The final solutions were prepared by an outside lab at time of testing by adding one capsule of Gentamicin Sulfate USP (PCCA Formula #10337) 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 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.

No viable cells of *E. coli, S. aureus* or *P. aeruginosa* were recovered after 0.5h exposure.

C. albicans: a 1-Log reduction observed after 0.5h and no viable cells were observed after 24h.

A. niger: colony forming spores were recovered up to 128h in solutions.

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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	≤10*	≤10*	≤10*	≤10*
A. niger	5.0 x 10^1	4.0 x 10^1	2.0 x 10^1	1.0 x 10^1
C. albicans	2 x 10^1	2 x 10^1	≤10*	≤10*
P. aeruginosa	≤10*	≤10*	≤10*	≤10*
S. aureus	≤10*	≤10*	≤10*	≤10*

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

Conclusions:

The Test Article containing Gentamicin Sulfate USP and LoxaSperse reduced the number of viable bacteria (*E. coli, S. aureus* and *P. aeruginosa*) within 0.5h of exposure and no bacterial growth was observed up to 168 h after exposure. A 3-Log to 4-Log reduction in viable bacteria was observed within 0.5h (**Tables 1-2**). A 1-Log reduction in the number of viable *C. albicans* cells was observed within 6h and no *C. albicans* cells were recovered after 24h (a 2-Log reduction). The gentamicin and LoxaSperse formulation continued to reduce the number of viable *A. niger* spores throughout testing. Additionally, the low number of spores introduced at the initiation of the AET and the subsequent low limit of detection prevented the observation of a significant 1-Log reduction of viable spores. 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.

References:

Durairaj, L., Launspach, J., Watt, J.L., Mohamad, Z., Kline, J. and Zabner, J. (2007) 'Safety assessment of inhaled xylitol in subjects with cystic fibrosis', *Journal of Cystic Fibrosis*, 6 (1), p.31-34.

Jagannath, C., Allaudeen, H. and Hunter, R. (1995) 'Activities of Poloxamer CRL8131 against *Mycobacterium tuberculosis in vitro* and *in vivo*', *Antimicrobial agents and chemotherapy*, 39 (6), p.1349-1354.

Martindale 35 (2007) [CD-ROM]. RPSGB and Sweetman, S. (ed.). Available: Pharmaceutical Press.

Moser, C. and Meyer, B. (2011) 'Comparison of compendial antimicrobial effectiveness tests: a review', *AAPS PharmSciTech*, 12 (1), p.222-226.

PCCA (2013) *Loxasperse*. Available at: http://www.pccarx.com/pcca-products/pcca-exclusives/bases/loxasperse (Accessed: 10 October 2013).

Plataki, M., Lee, Y., Rasmussen, D. and Hubmayr, R. (2011) 'Poloxamer 188 facilitates the repair of alveolus resident cells in ventilator-injured lungs', *American Journal of Respiratory and Cri tical Care Medicine*, 184, p.939-947.

The United States Pharmacopeial Convention (2013a) 'Microbiological Tests / <51> Antimicrobial Effectiveness Testing'. *USP 36-NF 31*. Rockville: USP, p.54-55.

The United States Pharmacopeial Convention (2013b) 'General Information / <1227> Validation of Microbial Recovery from Pharmacopeial articles. *USP 36 -NF 31*. Rockville: USP, p.989-991.

Zabner, J., Seiler, M., Launspach, J., Karp, P., Kearney, W., Look, D., Smith, J. and Welsh, M. (2000) 'The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing', *Proceedings of the National Academy of Sciences of the United States of America*, 97 (21), p.11614-11619.

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