Technical Report:

Characterization of the Physical and Microbiological Properties of LoxaSperse®

Abstract: LoxaSperse is a proprietary blend of micronized xylitol and poloxamers, designed to be mixed with active substances in order to improve water solubility, dispersibility, and to prevent microbial growth. The physical and microbiological properties of LoxaSperse were characterized by three laboratory tests performed with LoxaSperse and LoxaSperse with itraconazole.

Introduction:

LoxaSperse is a proprietary blend of micronized xylitol and micronized poloxamers, designed to be mixed with active substances in order to improve their water solubility and dispersibility. The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence for their safety and efficacy (Durairaj *et al*., 2006; Plataki *et al*., 2011). Xylitol and poloxamers exhibit antimicrobial activity and, therefore, LoxaSperse is also expected to prevent microbial growth (Veyries *et al*., 2000; Zabner *et al*., 2000). LoxaSperse mixtures are dry powders, packaged as non-sterile capsules or sachets for dispersion or dissolution in sterile water prior to the administration of compounded medicines for nebulization and irrigation.

Methodology:

The physical and microbiological properties of LoxaSperse were characterized by three types of laboratory tests performed on LoxaSperse and LoxaSperse with itraconazole, a triazole antifungal that is active against a wide spectrum of microorganisms (*Martindale 35*, 2007).

Physical Properties: To determine the particle size distribution of LoxaSperse and LoxaSperse with itraconazole, two different tests were performed respectively: Static Laser Light Scattering and Optical Microscopy.

Microbiological Properties: To characterize the antimicrobial activity of LoxaSperse and LoxaSperse with itraconazole, two different Minimum Inhibitory Concentration (MIC) methods were performed against fungal and bacterial strains by the Broth Microdilution Method and Agar Dilution Method. All strains were obtained from the American Type Culture Collection (ATCC). To estimate microbiological growth in LoxaSperse, water activity of the powder excipient base was determined using the AquaLab Water Activity Meter (AquaLab, 2008; 2013).

Results and Discussion:

The physical and microbiological properties of LoxaSperse are discussed separately below.

Physical Properties of LoxaSperse

Particle Size Distribution: The particles in a sample are not perfectly mono-disperse (i.e., every single particle with exactly the same dimensions) but, instead, they commonly consist of a statistical distribution with particles of differing dimensions. Several tests may be performed in order to characterize this physical property (Malvern, 2012).

Static Laser Light Scattering: This test provides a volume weighed distribution, in which the contribution of each particle in the distribution relates to the volume of that particle (Malvern, 2012). LoxaSperse 6.4% in sterile water exhibits a narrow distribution of particles (**Figure 1**), which demonstrates the optimal physical characteristics and performance of the powder excipient base.

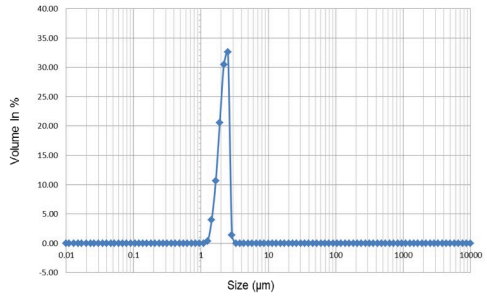

Figure 1. *Particle size distribution of LoxaSperse in sterile water.*

Optical Microscopy: Microscopic examination is suitable to determine the distribution of particles of inhalable size (European Commission JRC, 2002) and, therefore, optical microscopy was performed to characterize the effect of LoxaSperse in the particle size distribution of itraconazole. An AmScope Microscope Digital Camera was used for photographic characterization of itraconazole (1%) in sterile water, with and without LoxaSperse, at 200x magnification (AmScope, 2013). This test was performed in accordance with the respective 'Physical Test' of the US Pharmacopeia (The United States Pharmacopeial Convention, 2013). It was observed that, following the addition of LoxaSperse, large aggregates of itraconazole were converted into small aggregates and single particles (Figure 2). It is therefore concluded that LoxaSperse optimizes the particle size distribution of itraconazole in sterile water.

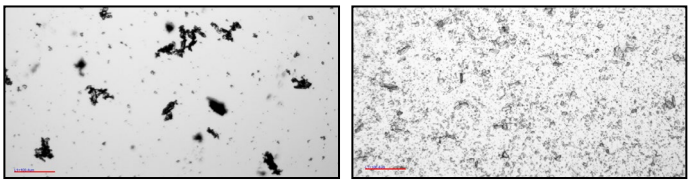

Figure 2. *(Left) Itraconazole 1% in sterile water. (Right) Itraconazole 1% and LoxaSperse in sterile water. Both at 200x magnification.*

Microbiological Properties of LoxaSperse

Minimum Inhibitory Concentration: MIC is the lowest concentration of an antimicrobial that will inhibit visible growth of a microorganism after overnight incubation. MIC is the gold standard research tool to determine *in vitro* activity of antimicrobials (Andrews, 2001). A lower MIC is indicative of a better antimicrobial agent.

Broth Microdilution Method: The *in vitro* antifungal activity of itraconazole and LoxaSperse with itraconazole (9:1) was determined against four fungal strains using the National Committee for Clinical Laboratory Standards (NCCLS) reference methods for yeast and filamentous fungi (Espinel-Ingroff, 2002; NCCLS, 2002a; 2002b). A lower MIC was found

in LoxaSperse with itraconazole than in itraconazole itself (**Table 1**). It is concluded that the LoxaSperse mixture has improved antifungal activity against all fungal strains tested.

Table 1. *MIC (µg/mL) of itraconazole and itraconazole with LoxaSperse (9:1) against four fungal strains (filamentous and yeast).*

Fungal strains	***A.fumigatus*** ATCC 204305	***A.niger*** ATCC 16404	***C.albicans*** ATCC 90028	***R.oryzae*** ATCC 9363
Itraconazole	0.5	0.5	≤0.125	0.25
Itraconazole +LoxaSperse	0.2	0.2	0.025	0.20

Agar Dilution Method: The *in vitro* antimicrobial activity of LoxaSperse was determined against eight microbial strains. An MIC of 17% LoxaSperse was achieved for the majority of the microbial strains tested (**Table 2**). No antimicrobials were added to this test.

Table 2. *MIC (%) of LoxaSperse against eight microbial strains.*

Microbial strains	Concentration of LoxaSperse			
	15%	**16%**	**17%**	**18%**
E.coli *ATCC 8739*	Growth	Growth	**No growth**	**No growth**
E.coli *ATCC 8739*	Growth	Growth	**No growth**	**No growth**
S.aureus *ATCC 6538*	Growth	Growth	**No growth**	**No growth**
P.aeruginosa *ATCC 9027*	Growth	Growth	**No growth**	**No growth**
C.albicans *ATCC 10231*	Growth	Growth	**No growth**	**No growth**
A.niger *ATCC 16404*	Growth	Growth	**No growth**	**No growth**
S.typhimurium *ATCC 14028*	Growth	**No growth**	**No growth**	**No growth**
S.aureus MRSA *ATCC 33591*	Growth	**No growth**	**No growth**	**No growth**

Water Activity (a_w): is defined as the amount of available, or free, water in a system and is a measure of how efficiently water can take part in a chemical reaction. Reducing the a_w minimizes undesirable chemical reactions and microbiological growth. Most bacteria do not grow at a_w<0.91 and no microbiological growth is possible at a_w<0.60. The a_w is a better index of microbial growth than total water content (Blandamer *et al*., 2005; AquaLab, 2008; 2013). The a_w of LoxaSperse was measured after 90 days storage at three different temperatures. An average a_w of 0.321 (with desiccant) and a_w of 0.456 (without desiccant) was measured (Table 3).

Table 3. *Water activity of LoxaSperse, with and without desiccant, after 90 days of storage at three different temperatures.*

Temperature	Water Activity (a_w) (with desiccant)	Water Activity (a_w) (without desiccant)
T=4°C (±1°C)	0.297	0.409
T=25°C (±1°C)	0.321	0.471
T=45°C (±1°C)	0.344	0.489

It is concluded that no microbiological growth is possible in LoxaSperse, after 90 days storage at T<45°C, due to its low a_w (<0.60).

Conclusions:

LoxaSperse with itraconazole has improved particle size distribution in sterile water and also improved antifungal activity compared to itraconazole alone. Considering the MIC and a_w of LoxaSperse, it is also concluded that LoxaSperse prevents microbial growth as expected.

References:

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