Characterization of the physical and microbiological properties of a powder excipient base (Loxasperse)

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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 evidence-base for their safety and efficacy (Durairaj *et al.*, 2007; Plataki *et al.*, 2011). *Xylitol and poloxamers exhibit antimicrobial activity and, therefore, Loxasperse was also designed to prevent microbial growth* (Veyries *et al.*, 2000; Zabner *et al.*, 2000). *Loxasperse mixtures are thus dry powders, formulated as non-sterile capsules or sachets, indicated to be dissolved in purified water or saline prior to the administration of compounded medicines for nebulization and irrigation (Figure 1).*

Figure 1. Powder excipient base (Loxasperse)

Methodology

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 a suite of fungal and bacterial strains, as follows: broth microdilution method and agar dilution method. All strains were obtained from the American Type Culture Collection (ATCC). To estimate the microbiological growth in Loxasperse, the water activity of the powder excipient base was determined using the AquaLab Water Activity Meter (AquaLab, 2008; 2013).

Results & Discussion

Particle size distribution: The particles in a sample are not perfectly mono-disperse (i.e. every single particle has exactly the same dimensions) but, instead, these commonly consist of a statistical distribution of particles of different 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 purified water exhibits a narrow distribution of particles (Figure 2), which demonstrates the optimal physical characteristics and performance of the powder excipient base.

Optical microscopy: The microscopy 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 purified water, with and without Loxasperse, at 400x magnification (AmScope, 2013). This test was performed in accordance with the respective 'Physical Test' of the USP Pharmacopoea (The United States Pharmacopeial Convention, 2013). It was observed that, following the addition of Loxasperse, large aggregates of itraconazole were converted in small aggregates and single particles (Figure 3). It is therefore concluded that Loxasperse optimizes the particle size distribution of itraconazole in purified water.

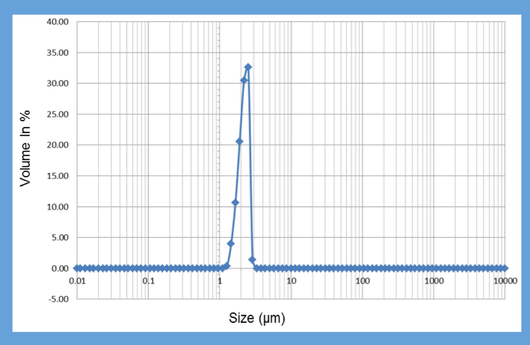

Figure 2. Particle size distribution of Loxasperse 6.4% in purified water

Minimum inhibitory concentration: The MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MIC is the gold standard research tool to determine the *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 4 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 itraconazol itself (Table 1). It is concluded that the Loxasperse mixture has improved antifungal activity against all fungal strains tested.

Agar dilution method: The *in vitro* antimicrobial activity of Loxasperse was determined against 7 microbial strains. A MIC of 17% of Loxasperse was achieved for the majority of the microbial strains tested (Table 2). No antimicrobials were added.

Water activity: The a_w is defined as the amount of free or available water in a system and it is a measure of how efficiently the 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 for microbial growth than the water content (Blandamer *et al.*, 2005; AquaLab, 2008; 2013). The a_w of Loxasperse was determined after 90 days of storage at three different temperatures, with and without desiccant, and an average of a_w=0.321 and a_w=0.456 was obtained (Table 3). It is concluded that no microbiological growth is possible in Loxasperse, after 90 days of storage at T<45°C, because of its low a_w (<0.60).

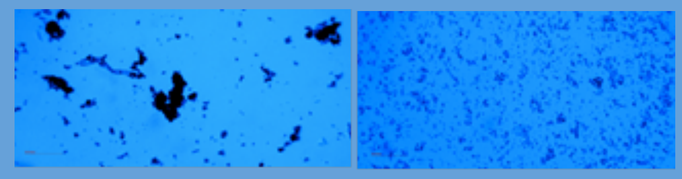

Figure 3. (left) Itraconazole 1% in purified water (right) Itraconazole 1% and Loxasperse in purified water, both at 400x magnification and blue coloured

Table 1. MIC (µg/mL) of itraconazole and itraconazole with Loxasperse (9:1) against 4 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

Table 2. MIC (%) of Loxasperse against 7 microbial strains

Microbial strains	Concentration of Loxasperse			
	15%	16%	17%	18%
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**

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

Loxasperse with itraconazole has improved particle size distribution in water and also improved antifungal activity in comparison to itraconazole itself. It is concluded that Loxasperse improves the water dispersibility and antifungal activity of itraconazole. Considering the MIC and a_w of Loxasperse alone, it is also concluded that, as expected, Loxasperse prevents microbial growth.

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