Preparation and stability of voriconazole eye drop solution.

Running title: VORICONAZOLE EYE DROP

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ABSTRACT

Combined systemic and topical administration of voriconazole have been successfully used to treat keratomycosis infection. No voriconazole eye drop product being commercially available, we prepared a sterile eye drop solution (10 mg/ml). Voriconazole remains stable over 30 days providing an eye drop solution suitable for topical treatment of fungal keratitis.
Voriconazole is a recent triazole antifungal agent highly effective against *Aspergillus*, *fusarium* and *Candida* species. Furthermore, voriconazole is one of the few drugs active against *Scedopsorium apiospermum* (10). These fungi may cause severe keratomycosis, a rare but sight-threatening infection of the cornea (4).

Conventional management of keratomycosis infection includes local application with or without systemic administration of antifungal drugs. Voriconazole has been proposed for treatment of visually devastating disease due to *Scedopsorium apiospermum* (7,14). The treatment combines systemic and topical administration of voriconazole (6). More recently, voriconazole have been successfully used in a variety of fungal keratitis caused for the most common by *Aspergillus* species, *Fusarium* species, *Candida* species (2,8,9,11).

For voriconazole, as for many antimicrobial agents, no eye drop solution is commercially available. Whereas several authors report the potential of voriconazole for topical ophthalmic administration, none of them focus on stability and storage of voriconazole. This paper describes how we prepared a voriconazole ophthalmic solution. In addition we performed a study in order to assess the stability of this solution over one month. Finally sterility of the preparation have been evaluated.

Based on the available literature (8,9,11), a 1% (10 mg/ml) voriconazole eye drop solution was prepared using branded product : powder for solution for intravenous infusion (Vfend®, Pfizer). Voriconazole powder (200 mg) was reconstituted with 19 ml of water for injections in order to obtain 20 ml of a 10 mg/ml voriconazole solution. Then a sterile filtration was performed through a 0.20 μm filter. Finally voriconazole solution was packaged in a specific sterile vial for eye drop solution. In order to keep the solution sterile, reconstitution was performed in respect of aseptic preparation recommendations.
In order to cure keratomycosis, antifungal eye drop has to be administrated over a long period, at least 6 weeks. Whereas eye drop needs to be kept during several days after preparation before or during its use in patient, its stability was unknown. Therefore, in order to assess stability of voriconazole eye drop solution, three batches of the formulation were stored up to 30 days at different projected conditions suitable in routine use.

Condition A: solutions (n=6) were kept at room temperature (24±3°C), not protected from light (colorless type I glass vial)

Condition B: solutions (n=6) were kept at room temperature (24±3°C), protected from light (colored type I glass vial)

Condition C: solutions (n=6) were kept refrigerated (4±2°C), protected from light (colored type I glass vial)

After storage in different appropriate conditions, solutions were inspected visually. Osmolarity assay was performed and the pH of the solutions was determined. Finally, voriconazole content of solutions was determined by high performance liquid chromatography (HPLC) at day 0, 1, 2, 3, 7, 10, 15 and 30. A previously described HPLC assay (5) was used with minor modifications. Briefly, separation was performed with a Kromasil® C\textsubscript{18} (5 µm, 150 x 3 mm) column. Mobile phase consisted of 0.04 M aqueous phosphate buffer pH 6.0 containing 50% (v/v) methanol and the flow rate was 1 ml min\textsuperscript{-1}. Retention time of voriconazole was equal to 7.5 min. Wavelength of the u.v. absorbance detector was set at 225 nm. The method was linear over the range 0-20 µg ml\textsuperscript{-1} (r\textsuperscript{2} ≥ 0.9997) and the limit of quantitation of voriconazole was equal to 1.25 µg ml\textsuperscript{-1}. Intra-day and inter-day coefficients of variation calculated at two concentrations were equal to or less than 5.7%. To ensure that the method can be regarded as suitably stability-indicating, we checked that decomposition products obtained from voriconazole solution subjected to severe stress (100°C, pH 1) do not interfere (12).
Sterility tests were performed using a closed membrane filtration method (Steritest®, Millipore). Samples were incubated for 14 days and read daily. Bacterial growth was determined by visual examination. Test sterility was performed at day 0 and 30 and requirements for sterility were met only when no growth was observed.

Mean recovery of voriconazole content in eye drop preparation was more than 99%, demonstrating that almost no drug is lost during preparation. The solution was clear and no color have been underlined just after preparation neither during storage. In addition, sterility of the sealed preparation was preserved throughout the storage period. Whatever the storage condition is, no significant degradation of voriconazole occurred (Figure 1). Indeed, during the period tested, the percentage of initial voriconazole concentration remaining was >90%, limit set by the U.S. Pharmacopeia (13). Voriconazole is stable for regular storage temperature conditions (+4°C and room temperature). Furthermore, exposure to light does not affect voriconazole degradation.

During storage, the pH of voriconazole solution remained stable (7.0 ± 0.1) and no significant variation of the osmolarity occurred (562 ± 10 mosmol/l). These results are in accordance with a chemical stability of voriconazole for at least 30 days and the amount of intact drug remaining is consistent with preservation of its microbiological activity.

The parenteral formulation of the commercial product (Vfend®) contains a new excipient sulphobutylether-beta-cyclodextrin sodium (SBEβCD) enhancing voriconazole solubility. Cyclodextrin facilitate eye drop formulation while improving clinical properties (1). SBEβCD have already been used with success for ocular drug delivery, at a final concentration ranging from 5 to 15% (1). SBEβCD concentration in our eye drop solution is equal to 160 mg/ml, leading to hyperosmolar ophthalmic solution compared to physiological tear osmolarity. Nevertheless, several anti-infectious eye drop preparations commonly used to treat infectious
keratitis have similar high osmolarity (3) without providing significant side effects. Therefore, osmolarity of our preparation was considered safely compatible with ocular administration. Finally, voriconazole preparation provided a neutral pH solution close to the physiological pH of tear (around 7.4) ; tear having a wide buffer capacity, no pH adjustment was made.

Recent increasing use of topical voriconazole in patients with severe keratomycosis raise the issue regarding eye drop stability and storage. Several authors recommend that topical voriconazole solution be kept refrigerated for no longer than 48 hours, as recommended by the manufacturer (2,9). These storage conditions are highly constraining in particular for outpatient treatment. Therefore, according to this work, stability of voriconazole eye drop solution prepared in our conditions could be extended up to 30 days.

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REFERENCES


Figure 1

- Condition A
- Condition B
- Condition C

Voriconazole concentration (mg/ml)

Days
Figure legends

Fig. 1: Stability of voriconazole eye drop solution under various storage conditions. Condition A: 24 ± 3°C not protected from light. Condition B: 24 ± 3°C protected from light. Condition C: 4 ± 2°C protected from light. All values are the mean of six independent determinations ± standard deviations (symbols without bars correspond to values for which the standard deviation is smaller than the symbol size).