Stability of frozen 1% voriconazole ophthalmic solution

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Ocular fungal infections are a major cause of blindness. Among these infections, fungal keratitis, a fungal infection in the cornea, remains one of the most difficult to treat. Although surgical procedures are efficacious in patients with acute corneal perforation, the primary treatment option for patients with fungal keratitis is antifungal therapy. First-line therapy includes a topical antifungal agent alone or in combination with systemic antifungal medications. Topical natamycin, amphotericin B, and voriconazole are commonly used to treat fungal keratitis.

Voriconazole, a triazole antifungal agent, inhibits the synthesis of ergosterol in the fungal membranes, thereby inhibiting growth of the microorganisms. Voriconazole is highly effective against Candida, Aspergillus, Fusarium, Paecilomyces, and Scedosporium species. Although oral and i.v. preparations are available, the majority of patients with fungal keratitis require further topical treatment to achieve a successful outcome. Moreover, the use of ocular topical formulations may be associated with fewer adverse effects and interactions with concomitant medications; thus, off-label use of extemporaneously prepared voriconazole ophthalmic solution has increased.

Previous studies have revealed promising results when topical 1% voriconazole, compounded as an extemporaneous formulation from the commercially available i.v. product, was used as monotherapy.

Purpose. The physicochemical stability of frozen 1% voriconazole ophthalmic solution was evaluated.

Methods. Multiple batches of voriconazole 10-mg/mL eye drops were aseptically prepared in a laminar-airflow cabinet. One batch was analyzed immediately after preparation, and the rest were stored at –20 °C and analyzed using high-performance liquid chromatography at 30, 60, and 90 days to test their physicochemical stability and sterility. All samples were analyzed in triplicate. Additional analyses were performed to determine the solution’s in vitro activity once thawed. The sterility of the 1% voriconazole solution was evaluated using blood–agar media and thioglycolate broth. Samples were incubated for 14 days and checked daily for signs of growth. Stability was defined as the absence of particles, color variation, or changes in pH and a remaining antifungal concentration of 90–110% of the initial concentration.

Results. All solutions remained clear and colorless throughout the study, and no precipitation or turbidity was observed in any of the batches, regardless of solution temperature. The pH and osmolality of all batches remained essentially unchanged during storage at –20 °C and after thawing. No significant differences in concentration were observed during the storage at –20 or 5 °C. The voriconazole concentration remained within 10% of the initial concentration during the 90-day period of storage at –20 °C. The percentage of recovery was also optimal after thawing.

Conclusion. Voriconazole 1% solution prepared for ophthalmic use was stable and retained antifungal activity when stored at –20 °C for 90 days. After thawing, this extemporaneously prepared formulation was stable at 5 °C for 14 days.

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and administered over at least 6–10 weeks. Based on the information available, 1% voriconazole ophthalmic solution prepared in sterile water for injection appears to be stable for up to 30 days when stored at 4 °C. Freezing could provide long-term stability and preserve the sterility of some formulations. For this reason, freezing could be an efficient way of preserving formulations, especially high-cost drugs such as voriconazole 1% ophthalmic solution.

A previous study suggested that though the physicochemical properties are not altered during preservation, antifungal activity might be affected, compromising the efficacy of the formulation. Isla et al. found that this solution was physicochemically stable for four weeks, while its in vitro activity was lower after the third week of the study.

The objective of this study was to analyze the physicochemical and microbiological stability of a compounded 1% voriconazole ophthalmic solution after a 90-day freezing period and to evaluate the formulation’s in vitro antifungal activity after thawing.

Methods

Sample preparation. Voriconazole lyophilized powder for injection (200 mg) was reconstituted with 19 mL of sterile water for injection and 3200 mg of sulfobutyl ether beta-cyclodextrin sodium (to enhance solubility) to obtain 20 mL of a 10-mg/mL 1% voriconazole solution. Four batches of four bottles each containing 1% voriconazole ophthalmic solution were prepared under aseptic conditions in a laminar-airflow cabinet in accordance with the manufacturer’s instructions. Ten milliliters of the 1% solution was filtered through 0.22-μm hydrophilic PUDF membrane filters into sterile high-density polyethylene eye drop bottles. One batch was analyzed immediately after preparation, and the rest were stored at −20 °C and analyzed using high-performance liquid chromatography (HPLC) at 30, 60, and 90 days to test their physicochemical stability and sterility. All samples were analyzed in triplicate.

Additional analyses were performed to determine the solution’s in vitro activity once thawed. For this purpose, three additional batches of four bottles each containing 1% voriconazole ophthalmic solution were prepared using the same procedure mentioned above and stored at −20 °C for 90 days. One batch was analyzed immediately after thawing (90 days), and the remaining three batches were stored at 5 °C and analyzed after 97 and 104 days to check for changes in antifungal activity. Physicochemical stability and sterility also were analyzed at 90, 97, and 104 days. All samples were analyzed in triplicate.

Sterility testing. The sterility of the 1% voriconazole solution was evaluated using blood–agar media and thioglycollate broth. Samples were incubated for 14 days and checked daily for signs of growth.

Stability testing. Stability was defined as the absence of particles, color variation, or changes in pH and a remaining antifungal concentration of 90–110% of the initial concentration, according to United States Pharmacopeia chapter 797’s recommendations for sterile compounded preparations.

Samples were visually examined by two different observers under a white-light at each time point for particulate matter particles and color changes. A nephelometric quantitative method was used to evaluate turbidity. Osmolarity was determined with an ultraprecipitation electrode using 200-μL portions. Changes in pH were monitored with a calibrated pH electrode.

HPLC analysis. The stability of voriconazole samples was analyzed using HPLC with ultraviolet-light detection using a C18 column, based on the methodology described by Khetre et al. The method was validated and applied for drug quantification in ultrapure water at the pharmacy research laboratory. The mobile phase was water:acetonitrile (60:40, v/v) delivered at a flow rate of 1 mL/min; the ultraviolet-light absorbance detector was set at 254 nm. Validation was assessed in accordance with the Food and Drug Administration’s Guidance for Industry Bioanalytical Method Validation and the European Medicines Agency’s Guideline on Validation of Analytical Procedures. The method showed a good linearity: 3.13–100 μg/mL (goodness of fit [r] > 0.99). Intra- and interassay precision were 0.19–3.63% and 4.28–9.59%, respectively. The limit of detection and lower limit of quantification were 1.56 and 3.13 μg/mL, respectively.

The stability-indicating nature of the HPLC analytical method was also validated using forced degradation. Stock solutions were exposed to heat (1 hour in a boiling water bath and heated at 70 °C for 48 hours), a strong acidic solution, a strong basic solution, and hydrogen peroxide to accelerate decomposition. There was no interference of degradation product peaks with the peak of voriconazole. The retention time of voriconazole standard solution was 3.76 minutes.

In vitro sensitivity test. An in vitro sensitivity test was performed, following the standardized methods of the Clinical Laboratory Standard Institute for antifungal susceptibility testing, including M27-A3 for macrobroth and microtiter yeast testing and M38-A2 for microtiter mold testing. To determine voriconazole’s minimum inhibitory concentration (MIC), dilutions of the ophthalmic solution between 0.03 and 16 μg/mL were prepared and tested with Candida parapsilosis and Aspergillus fumigatus, both of which are frequently implicated in fungal keratitis. Manufactured vori-
conazole powder was reconstituted per manufacturer instructions and used as a control solution, assuming its full antifungal activity.

**Statistical analysis.** Results were expressed as a range or mean ± S.D. Student’s *t* test was performed to detect significant concentration differences.

**Results and discussion**

All solutions remained clear and colorless throughout the study, and no precipitation or turbidity was observed in any of the batches, regardless of solution temperature (Tables 1 and 2).

The pH and osmolarity of all batches remained essentially unchanged during storage at −20 °C and after thawing (pH, 6.28 ± 0.06; osmolarity, 563.67 ± 42.10 mOsm/L). Both values were in accordance with acceptable values for ophthalmic solutions, since tears have a wide buffering capacity, and a desirable pH value for ophthalmic solutions might be between 5.5 and 11.4. The slight hyperosmolarity could be attributed to the adjuvant cyclodextrin, an excipient in the i.v. product. Nevertheless, this hyperosmolarity is common with several antimicrobial ophthalmic preparations and is considered safe for and compatible with ocular administration.

Regarding HPLC analysis, no significant differences in concentration were observed during the storage at −20 °C (*p = 0.14*) or 5 °C (*p = 0.20*). The voriconazole concentration remained within 10% of the initial concentration during the 90-day period of storage at −20 °C. The percentage of recovery was also optimal after thawing (Table 2). The small increase in concentration was likely due to the semipermeable nature of the eye drop container.

The stability-indicating nature of the assay was demonstrated, as no additional peaks corresponding to degradation products were observed on the chromatograms.

**MIC values of voriconazole for** *C. parapsilosis* and *A. fumigatus* were 0.25 µg/mL or lower at each time point (Table 3). Although MIC values obtained for voriconazole eye drops were slightly greater than those of the control, these values were within the MIC range for these microorganisms (<0.0078–2.0 and <0.03–8.0 µg/mL for *C. parapsilosis* and *A. fumigatus*, respectively). These findings indicate that voriconazole’s fungal activity was not affected by exposure to low temperatures.

No microbiological contamination was noted during storage, regardless of temperature.

**Conclusion**

Voriconazole 1% solution pre-

| Table 1. **Stability of 1% Voriconazole Ophthalmic Solution Stored at −20 °C**a |
|---------------------------|---------|---------|---------|---------|
| Variable                  | Day 0   | Day 30  | Day 60  | Day 90  |
| Mean ± S.D. osmolarity    | 542.67 ± 12.42 | 548.67 ± 9.07 | 625.33 ± 15.95 | 531.00 ± 2.00 |
| Mean ± S.D. turbidity (FNU)| 0.15 ± 0.09 | 0.34 ± 0.17 | 0.12 ± 0.02 | 0.14 ± 0.04 |
| Mean ± S.D. pH            | 6.17 ± 0.03 | 6.20 ± 0.01 | 6.18 ± 0.06 | 6.20 ± 0.06 |
| % Initial concentration remaining | 100     | 106.16  | 104.45  | 105.95  |

a *n* = 12 (3 bottles each time point). FNU = formazin nephelometric units.

| Table 2. **Stability of Voriconazole 1% Ophthalmic Solution Stored at 5 °C After Thawing** |
|-----------------|---------|---------|---------|---------|
| Variable        | Day 90  | Day 97  | Day 104 |
| Mean ± S.D. osmolarity (mOsm/L) | 544.67 ± 8.93 | 535.33 ± 2.08 | 612.33 ± 68.65 |
| Mean ± S.D. turbidity (FNU)       | 0.12 ± 0.04 | 0.10 ± 0.01 | 0.12 ± 0.02 |
| Mean ± S.D. pH                  | 6.21 ± 0.04 | 6.31 ± 0.06 | 6.33 ± 0.02 |
| % Initial concentration remaining | 100     | 100.23  | 101.91  |

* *n* = 9 (3 bottles each time point). FNU = formazin nephelometric units.

| Table 3. **MIC Values of Voriconazole in the Presence of Candida parapsilosis and Aspergillus fumigatus After Thawing** |
|-----------------|---------|---------|---------|---------|
| Organism and Treatment | Day 90 | Day 97 | Day 104 |
| C. parapsilosis  |
| Voriconazole    | 0.06    | 0.12    | 0.12    |
| Control         | 0.06    | 0.12    | 0.12    |
| A. fumigatus    |
| Voriconazole    | 0.12    | 0.12/0.25 | 0.25    |
| Control         | 0.12    | 0.12    | 0.12    |

*MIC = minimum inhibitory concentration.*

*C. parapsilosis* and *A. fumigatus* MIC values were <0.0078–2.0 and <0.03–8.0 µg/mL, respectively.
pared for ophthalmic use was stable and retained antifungal activity when stored at −20 °C for 90 days. After thawing, this extemporaneously prepared formulation was stable at 5 °C for 14 days.

1Voriconazole for injection (Vfend i.v., Pfizer, Sandwich (Kent), United Kingdom, lot Z241006.
2Water for injection, 10-mL bottles, B.Braun, Barcelona, Spain.
3Nillex GV syringe filter unit, Merck Millipore, Cork, Ireland.
4Photoprotective drop bottle, 10 mL, Bexen Mediccal, Guipuzcoa, Spain.
5Columbia agar with 5% sheep blood, Becton Dickinson, Heidelberg, Germany.
6Thioglycollate + vitamin K + hemin, Rain SL, Barcelona.
7HI 98713 portable turbidimeter, Hanna Instruments, Elbar (Guipúzcoa), Spain.
8ON6050 Osmo Station, A. Menarini Diagnostics, Badalona, Spain.
9pH Basic 20, Crison Instruments, S.A., Alella (Barcelona), Spain.
10Agilent 1100, Agilent Technologies Spain, SL, Madrid, Spain.
11Hyperlite C18, 250 × 4.6 mm, Thermo Fischer Scientific Inc., Waltham, MA.

References