Stability of Extemporaneously Prepared 0.5-Percent Caspofungin Eye Drops: a Potential Cost-Savings Exercise

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While the successful use of topical caspofungin for patients has been reported, topical caspofungin is not commercially available and its stability is unknown, limiting its usefulness in treating fungal keratitis. Caspofungin (0.5%) eye drops were aseptically prepared, and the concentrations were measured using a validated high-performance liquid chromatography (HPLC) analysis. The preparations remained stable for 28 days under refrigerated condition but not at 25.0°C. Our study supports the cost-saving use of caspofungin eye drops in the clinical setting.

Fungal keratitis is difficult to treat, with *Candida*, *Aspergillus*, and *Fusarium* species (1, 16) as the common causative pathogens. Current treatment options for keratomycosis are inadequate (3) given the restricted range of effective topical preparations (e.g., natamycin is the only commercially available antifungal eye drop). Caspofungin is active against a broad spectrum of fungi (MIC₉₀, 60 to 2,000 ng/ml) (8, 20). After topical delivery in animals, caspofungin has good penetration into eyes with inflammation or corneal abrasion (4, 20), and it has been shown previously to be effective in treating fungal keratitis in rabbits (5, 12). While penetration into intact human eyes may be limited (10), successful application of topical caspofungin alone (7) or in combination with voriconazole (11, 18) for patients with fungal keratitis has been reported. However, the stability of extemporaneously prepared caspofungin eye drops is unknown and the eye drops have to be freshly prepared and used within 24 h (7), making their use in clinical settings uneconomical. This study investigated the stability of 0.5% caspofungin eye drops to support the clinical use of topical caspofungin.

The eye drops were prepared aseptically; 10.5 ml of water for injection was added to a 50-mg vial of caspofungin acetate (Candidas) (2). Aliquots (0.5 ml) of the reconstituted 0.5% solution were then transferred into presterilized, lightproof, low-density polyethylene eye dropper bottles (Sik Hong Holding Pty. Ltd.). The bottles were kept sealed until the day of analysis, stored either (i) under refrigeration (at 4.0 ± 1.0°C; *n* = 18) or (ii) at room temperature (25.0 ± 1.0°C; *n* = 18). On days 0, 3, 7, 14, 28, and 56, 50 μl of 0.5% caspofungin from each bottle was diluted with 450 μl of water to obtain a concentration of 0.5 mg/ml; 10-μl aliquots were injected into a high-performance liquid chromatograph (HPLC). Three bottles were analyzed in triplicate for each condition at the designated time points.

The HPLC method of Spriet et al. (15) was adapted. A liquid chromatography (LC) system (Shimadzu, Japan) comprised an LC-10AD pump, a DGU-14A degasser, and a CTO-10AC column oven. A PhenoSphere-NEXT C₁₈ column (pore size, 120 Å; dimensions, 150 by 4.6 mm; and particle size, 5 μm; Phenomenex) and a guard column (dimensions, 4 by 2 mm; Phenomenex) were used. The column temperature was 35.0°C, and the flow rate was 1.5 ml/min. The mobile phase comprised Milli-Q water–trifluoroacetic acid (mobile phase A, 100:0.1 [vol/vol]) adjusted to pH 3

with diethylamine and 100% methanol (mobile phase B). A gradient elution program from 30% mobile phase B to 90% mobile phase B over 8.0 min was used. The UV absorbance detector wavelength was set at 215 nm. Calibration standard and quality control (QC) stock solutions of caspofungin (10 mg/ml) were prepared in water independently. Working solutions of caspofungin (0.5 to 8 mg/ml) were prepared by serial dilution of stock solutions with water, and these working solutions were further diluted 1:10 with water to prepare calibration standards (0.05, 0.1, 0.2, 0.4, and 0.8 mg/ml) and QC samples (0.075, 0.3, and 0.6 mg/ml).

Caspofungin eluted at 5.5 min (Fig. 1A). Good linearity over a range of concentrations from 0.05 to 0.8 mg/ml was observed (*n* = 5; *r*² = 0.9997 ± 0.0002). The intraday (*n* = 6) and interday (*n* = 5) accuracies (percent bias) were within ±7.0%, and precisions (coefficients of variation [CV]) were within 6.0%. The lower limit of quantification was 0.05 mg/ml, with an accuracy of 93.3% and a CV of 4.4% (*n* = 6). Forced degradations (at 60.0°C and pH 12) were performed. Two peaks (most likely caspofungin degradation products) at retention times of 6.2 and 6.6 min were well separated from the intact drug, with caspofungin concentrations reduced by 48.9 to 78.1% compared to the initial concentration (Fig. 1B and C), indicating the suitability of the assay to determine the stability of caspofungin. Samples were monitored for pH by using a Cyber-Scan pH 1500 bench meter (Eutech Instruments, USA) with a calibrated micro-pH sensor (TPS, Australia) and for osmolality by using a Fiske 110 osmometer (Fiske Associates, USA).

Under refrigerated condition (4.0 ± 1.0°C), 0.5% caspofungin eye drops were stable for 4 weeks, with a concentration (mean ± standard deviation [SD]) of 4.42 ± 0.15 mg/ml on day 28 (Table 1 and Fig. 2); this outcome meets the minimum requirement of the U.S. Pharmacopeia (≥90% of initial concentration) (19). At room temperature (25.0 ± 1.0°C), they were stable for only 3 days (Ta-

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FIG 1 Typical chromatograms for caspofungin (A), caspofungin exposed to 60°C (B), and caspofungin exposed to an alkaline pH of 12 (C). mAU, milli-absorbance units.

ble 1 and Fig. 2). The solution remained clear and colorless throughout the study.

Negligible changes in pH (mean ± SD, 6.43 ± 0.14) and osmolality (mean ± SD, 45.1 ± 3.94 mosmol/kg) were observed at all time points for both storage conditions (Table 1). The measured pH of topical caspofungin was within the range tolerated by human eyes (6). Ideally, ophthalmic solutions and lachrymal secretions should be isotonic; however, in practice, eye drops with an osmolality of 240 to 514 mosmol/kg, which is equivalent to 0.7 to 1.5% sodium chloride (13), are acceptable for use. The low osmolality may suggest that 0.5% caspofungin eye drops are not suitable for topical administration; however, this finding is due to the choice of diluent used in the present study. The manufacturer recommends either water for injection or 0.9% saline (Candidas package insert; Merck & Co. Inc., Whitehouse Station, NJ). It is envisaged that using 0.9% saline as the diluent will afford osmolality of the reconstituted solution that is within the acceptable range (17).

To minimize the risk of microbial contamination, a 1-week

expiry after the bottle is opened is recommended (14). By following an intensive regime of one drop (0.05 ml) administered every 1 or 2 h in clinical practice (11, 18), a total of 0.6 to 1.2 ml of eye drops would be applied daily. A single 50-mg caspofungin vial provides two 5-ml bottles of eye drops, each sufficient for 4 to 8 days of treatment. Data from this study facilitate batch manufacturing, reducing overall waste and production time (9). Importantly, patients could access a 4-week supply at each dispensing, enabling outpatient treatment and minimizing frequent visits to a hospital for resupply.

Discarding caspofungin eye drops within 24 h after manufacture is extremely costly, given that fungal keratitis often requires weeks to months of antifungal eye drop therapy. A 50-mg caspofungin vial costs 714.28 Australian dollars (<http://www.hpv.org.au/>). We estimated that the monthly drug acquisition cost per patient using 0.5% caspofungin eye drops is 1,429 Australian dollars based on 1-month stability data, 93% less expensive than the cost of 21,428 Australian dollars for drops freshly prepared on a daily basis.

TABLE 1 Stability, pH, and osmolality of 0.5% caspofungin eye drops^a

No. of days of storage	Value at 4 ± 1°C for:			Value at 25 ± 1°C for:		
	Concn (mg/ml)	pH	Osmolality (mosmol/kg)	Concn (mg/ml)	pH	Osmolality (mosmol/kg)
0	4.81 ± 0.17	6.59 ± 0.04	44.3 ± 2.74	4.81 ± 0.17	6.59 ± 0.04	44.3 ± 2.74
3	4.76 ± 0.08	6.53 ± 0.05	42.9 ± 2.47	4.41 ± 0.07	6.55 ± 0.02	42.0 ± 1.66
7	4.62 ± 0.05	6.50 ± 0.02	45.4 ± 2.46	4.09 ± 0.08	6.48 ± 0.04	43.8 ± 1.79
14	4.59 ± 0.05	6.36 ± 0.04	46.8 ± 2.17	3.37 ± 0.10	6.33 ± 0.02	45.1 ± 2.62
28	4.42 ± 0.15	6.32 ± 0.03	45.3 ± 4.36	2.53 ± 0.04	6.19 ± 0.02	50.6 ± 5.55
56	4.16 ± 0.34	6.50 ± 0.14	41.2 ± 3.99	0.94 ± 0.02	6.27 ± 0.06	48.8 ± 3.03

^a All data are presented as means ± SD (n = 9).

FIG 2 Stability of 0.5% caspofungin eye drops.

In summary, the present study provides critical data to support the economical use of caspofungin eye drops as an alternative treatment for fungal keratitis in an atmosphere of financial constraints.

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