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Rapid communication

Stability of micafungin sodium solutions at different concentrations in glass bottles and syringes

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ABSTRACT

Micafungin is a costly treatment and packaging of 50 mg or 100 mg bottles only are available, while doses lower than 5 mg and 20 mg are often necessary in neonates and paediatrics patients, respectively. The stability of micafungin sodium in polypropylene syringes and glass bottles was studied at different concentrations. Solutions of micafungin diluted with NaCl 0.9% were prepared in glass bottles (20 and 10 mg/mL) or syringes (1 and 0.5 mg/mL) and stored at 25 °C, 60% humidity (RH), in the dark (ICH conditions). Solutions were also exposed to heat (70 °C) or alkaline solution (NaOH) in order to force degradation. Samples were analysed at days 1, 5, 8 (for bottles) and also 15 (for syringes) after the preparation and assayed in triplicate. Stability was studied using a stability-indicating high-performance liquid chromatographic method. Syringes stored at 25 °C retained over 90% of their initial concentration over the study period. Temperature and alkaline conditions had significant effect on the stability of micafungin, leading to apparition of degradation products. Moreover, sub visible particles were in the specification of the European Pharmacopeia along 15 days. To conclude, micafungin diluted in NaCl 0.9% and stored in polypropylene syringes was chemically stable for at least 15 days at 25 °C in the dark.

1. Introduction

Candida infection is widely common in neonates and paediatric units. It is the third more common infection in paediatric units in United States and Europe (Cugno and Cesaro, 2012; Raymond and Aujard, 2000; Wisplinghoff et al., 2003). In neonates, the incidence is evaluated to 10% (Benjamin et al., 2010; Cotten et al., 2006). Invasive fungal infection increases hospitalizations and healthcare costs (Cugno and Cesaro, 2012; Raymond and Aujard, 2000), and leads to premature baby mortality with an incidence of approximately 20% while 50% of survivors suffer from severe neurodevelopment damages (Benjamin et al., 2006).

Thus, prevention and treatment of *Candida* infections appear as a critical point. Different antifungal treatments are approved and marketed. The most commonly antifungal monotherapies used in paediatric candidiasis are fluconazole (21%), liposomal amphotericin B (20%) and micafungin (18%), (Steinbach et al., 2012). For neonates, fluconazole (32%), caspofungin (24%) liposomal amphotericin B (16%) and micafungin (8%) are prescribed (Steinbach et al., 2012). In a double-blind clinical trial, treatment success with micafungin (73%) was similar to liposomal amphotericin B success (76%) in paediatric patients treated for candidemia (Queiroz-Telles et al., 2008).

Micafungin is recommended in adults and children including infants to treat or prevent invasive candidemia. Children and infants receive the initial dose of 2 mg/kg/day, increased to 4 mg/kg/day in case of no clinical improvement.

Unfortunately, micafungin (Mycamine®) is a costly drug. As many treatments, only packaging designed for adults are available, e.g. 50 mg and 100 mg bottles, while doses lower than 5 mg and 20 mg are often necessary in neonates and paediatrics patients, respectively. This lack of paediatric form is likely to induce inappropriate dosing (Salunke and Tuleu, 2013). Preparing in a pharmacy a ready-to-use form could provide a solution to reduce mistakes (Berthouzoz et al., 2012) and guarantee the chemical stability and the microbiological quality (Hecq, 2011; Shah et al., 2011). In that way, stability studies are needed and have been published for various formulations (Abdulla et al., 2015; Bazin et al., 2015; Feutry et al., 2015; Jain et al., 2014; Paul et al., 2013; Walker et al., 2014; Zhang and Trissel, 2005). Moreover, in order to reduce costs, it could be interesting to use the same reconstituted vial during several days or to prepare treatment for a week. To our knowledge no stability study of micafungin reconstituted and

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diluted solutions are available for more than four days (supplier data).

The aim of this study was to determine a shelf life of the reconstituted and diluted solutions of micafungin.

2. Material and methods

2.1. Reagents

Micafungin (Mycamine® 50 mg and 100 mg) was provided by Astellas Pharma (Levallois-Perret, France). The drug was reconstituted with sterile sodium chloride (NaCl 0.9%) solution (BBraun, Boulogne, France) in order to obtain 10 mg/mL and 20 mg/mL stock solutions, kept in glass bottle.

The chemicals and reagents were all of analytical grades and included acetonitrile (Hipersolv Chromanorm, VWR, Fontenay sous Bois, France), sodium perchlorate (Sigma–Aldrich, Saint Quentin Fallavier, France), sodium dihydrogen phosphate (VWR, Fontenay sous Bois, France) phosphoric acid (Sigma–Aldrich, Saint Quentin Fallavier, France) and potassium dihydrogen phosphate (VWR, Fontenay sous Bois, France).

Water was obtained from a Prima reverse osmosis system (Elga Labwater, Antony, France).

2.2. Chromatographic conditions

A high pressure liquid chromatography (HPLC) method, previously developed and validated by Zhu et al. (2013) was adapted to our conditions. The system was characterized by a PerkinElmer Series 200 pump, injector and oven. A diode array detector (Flexar PDA detector, Perkin Elmer, Walthman, USA) operating between 190 and 700 nm was used and managed by the Chromera software (v4.1.0) (PerkinElmer, Walthman, USA). The mobile phase consisted of a mixture of sodium dihydrogen phosphate (1.20 g), sodium perchlorate (6.15 g) in water (1000 mL), which pH was adjusted to pH 2.9 (with phosphoric acid) and acetonitrile (62:38 v:v). The flow rate was set to 1 mL/min. The column used was a C18 ODS Hypersil (250 mm × 4 mm, 5 μm) (Thermo-Scientific, Villebon sur Yvette, France), maintained at 45 °C. The sample injection volume was 10 μL and the analysis time was 20 min. Micafungin detection and quantification were processed at 210 nm. Micafungin spectra (190–700 nm) were extracted at the apex of the chromatogram peak of the micafungin and along the chromatogram to detect potential degradation products.

2.3. Method validation

The validation of the method was performed according to the ICH Q2R1, (International Conference on Harmonisation, 2015). The standard curve was established with five different concentrations prepared with an appropriate amount of a 10 mg/mL stock solution in order to obtain 300, 400, 500, 600 and 700 μg/mL micafungin solutions, by diluting stock solution with a mixture of phosphate buffer and acetonitrile 1:1 (pH adjusted with phosphoric acid to 6.5). The linearity of the method was evaluated on three different standard curves. The repeatability of the method was evaluated by six samples prepared in order to obtain a 0.5 mg/mL solution. The accuracy of the method was established using three concentration levels (0.4, 0.5 and 0.6 mg/mL) in three replicates.

2.4. Preparation of micafungin syringes

Three different polypropylene (BBraun, Boulogne, France) syringes containing a 0.5 mg/mL micafungin solution and three others syringes containing a 1 mg/mL micafungin solution were prepared. The 0.5 mg/mL and 1 mg/mL solutions were prepared by dissolving respectively an appropriate amount of micafungin 10 and 20 mg/mL stock solution in NaCl 0.9%. All syringes and glass bottles containing 10 and 20 mg/mL stock solutions were conserved in a qualified climatic chamber which temperature was defined at 25 °C ± 2 °C and 60% RH ± 5% RH, in the dark.

2.5. Samples preparation for chromatographic analysis

For each studied concentration (20, 10, 1 and 0.5 mg/mL), three samples analysis per day were prepared at a concentration of 500 μg/mL by diluting an appropriate amount of solution in water. Chromatographic analyses were performed at day 0 just after the reconstitution for the bottles and dilution for the syringes. Relative concentration were next measured at days 1, 5 and 8 for syringes and bottles and moreover at day 15 for syringes only. For each analysis time, the mean percentage was expressed with the 95% confidence interval of the mean. The mean and confidence interval were considered acceptable if superior to 90% of the initial concentration.

2.6. Preparation of sample for forced degradation

A solution of 1 mg/mL was placed in a dark chamber maintained at 70 °C during 30 min (heat degradation) and 1.25 mL of a 2 mg/mL micafungin solution was mixed with 100 μL of a 0.1 M NaOH solution for 30 min (alkaline degradation), 100 μL 0.1 M HCl was then added and the solution was finally diluted to 5 mL with the mobile phase of HPLC. Degradation product peaks were observed at 210 nm.

2.7. pH study

For each storage conditions, pH was measured on days 0, 1, 5, 8 and 15 with an InLab® Ultra-Micro pH electrode (Mettler Toledo, Viroflay, France).

2.8. Sub visible particle determination

For syringes, at days 0 (just after the dilution of the stock solution), 8 and 15 a determination of the sub-visible particles formation has been established according to the 2.9.19 section of the pharmacopoeia and more specifically the first method, which is the light-obscuration-particle count rate. Ten syringes of the two concentrations tested were mixed in order to obtain a volume of 25 mL and the solutions were analyzed with a HIAC 9703+ system (Beckman Coulter, Roissy, France) and managed by the PharmSpec v3.3.0.30040 software (Beckman Coulter, Roissy, France). For each day (0, 8 and 15) and concentration, 4 runs of 5 mL were processed. According to the pharmacopoeia, the preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10 μm and does not exceed 600 per container equal to or greater than 25 μm

2.9. Visual examination

A visual examination of the tested samples was conducted along the study in order to detect color changes, precipitates or visible particles

3. Results and discussion

3.1. HPLC method

The retention time of the micafungin was found to be 14.70 min (Fig. 1A). The method allowed to obtain a well-defined and symmetric peak, and no impurities were found. The three standard