

Evaluation of Physical-Chemical and Microbiological Stability of Fluconazole Oral Suspensions for Hospital Use

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Fluconazole is an important drug in the treatment of cutaneous and systemic mycoses. The *Hospital de Clínicas de Porto Alegre* performs a derivation of fluconazole capsules to obtain an oral liquid formulation that is easily administered and whose dose can be adjusted. In order to replace the derivation for a formulation produced from an active pharmaceutical ingredient, this study sought to develop a liquid oral formulation, evaluate its physical chemical and microbiological stability and demonstrate suitability of the analytical method for the formulation assay. Seven different formulations of pharmaceutical suspension form were produced and evaluated for pH, viscosity, sedimentation volume and assay. The analytical method by High Performance Liquid Chromatography was demonstrated. Two most promising formulations were manipulated in the *Farmácia Semi-Industrial do Hospital de Clínicas de Porto Alegre* and stored in amber PET bottles under three different conditions: room temperature, under refrigeration (2 to 8 °C) and in an oven (40 °C). Samples were collected after 0, 7 and 14 days to evaluate physical-chemical stability, assay, pH and macroscopic aspects. Samples were collected after 0 and 21 days to evaluate microbiological stability. It was possible to demonstrate stability for one of the formulations for a 14-day period. Throughout the study, the chosen formulation presented adequate quantification of fluconazole, constant pH, no organoleptic changes and no microbial growth. The results suggest the incorporation of a new formulation for fluconazole to the *Farmácia Semi-Industrial portfolio*.

Keywords: Fluconazole. Analytical Method Suitability. Formulation Development. Formulation Stability.
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Introduction

Fluconazole is a triazole antifungal drug, one of the most important drug classes used in cutaneous and systemic mycoses treatment¹. Triazoles act by inhibiting ergosterol synthesis, an important fungal cell membrane component. Fluconazole has a broad spectrum of activity and is used to treat infections like candidiasis and cryptococcal meningitis. It is especially useful in transplanted patients' prophylactic treatment and to fight infections in immunosuppressed patients^{2, 3, 4, 5, 6}.

The *Hospital de Clínicas de Porto Alegre* has many patients with needs for specific medications, such as pediatric patients, transplanted patients and patients using catheters, drains and tubes. To treat those cases, the *Farmácia Semi-Industrial* (FSI) performs manipulations or pharmaceutical derivations. Formulation stability studies are necessary to look at the best way of preparation, the package to be used, storage conditions and expiration date.^{7, 8}. Fluconazole oral suspension is among the FSI's products.

Most of the medications are available in solid dosage form, which is not the most appropriate form to be considered for patients with specific administration and dosage needs (pediatric and transplanted patients, for example)^{9, 10}. One alternative is the oral administration of parenteral formulations, but their concentrations demand dilution and manipulation, which can make the final volume unfeasible for oral administration^{11, 12}. This can be solved by preparing oral solutions or suspensions from solid forms or even from the active pharmaceutical ingredient itself¹³. In order to facilitate the administration of fluconazole to patients admitted to the hospital, the present study sought to prepare a fluconazole liquid oral formulation, in the pharmaceutical suspension form. We also attempted to verify the suitability of a

compendial analytical method for the quantitative determination of fluconazole in the developed suspension and to evaluate its physical-chemical and microbiological stability to propose a safe time interval for its use.

Materials and methods

Reagents: Purified water, Direct-Q3UV, Millipore®; Potassium phosphate monobasic, Merck®; Methanol for liquid chromatography, LiChrosolv®; Acetonitrile for liquid chromatography, Vetec®; Fluconazole standard substance, declared content of 100.9% (*LAPPS-Faculdade de Farmácia/UFRGS*)

Pharmaceutical ingredients: Fluconazole active pharmaceutical ingredient, Delaware®, declared content of 99.68%, Batch 1007/17; Carboxymethylcellulose, Delaware®, batch 1006/16; Simple Syrup, *FSI/HCPA*, Batch 002/18; Glycerin, Rioquímica®, Batch 1701655; Propyleneglycol, Rioquímica®, Batch 1512043.

Formulation Development

Seven fluconazole suspensions of 50 mg/mL were manipulated and evaluated for pH, viscosity and sedimentation volume. The composition for each formulation is shown in Table 1. For all formulations, fluconazole was previously ground in a mortar and levigated with glycerin or propyleneglycol. HANNA Instruments pH21 model pH meter was used to determine the pH of the formulations. Viscosity was measured using a model RDVD-1 + Brookfield Viscosimeter with a n° 4 spindle at 100 rpm. The sedimentation volume was calculated (Equation 1) and the volume of formed sediment was measured in a graduated cylinder 1, 2, 24, 48, 72 and 168 hours after resuspension.

$$\text{Sedimentation Volume} = \frac{\text{Sediment Volume (mL)}}{\text{Total Volume (mL)}} \quad \text{Eq (1)}$$

Table 1 – Fluconazole suspensions content.

Formulation	Fluconazole	Glycerin	PPG ¹	CMC ²	Simple Syrup
1	5 g	-	-	-	qs 100 mL
2	5 g	10 mL	-	-	qs 100 mL
3	5 g	-	10 mL	-	qs 100 mL
4	5 g	10 mL	-	qs 100 mL	-
5	5 g	10 mL	-	40 mL	qs 100 mL
6	5 g	10 mL	-	25 mL	qs 100 mL
7	5 g	10 mL	-	10 mL	qs 100 mL

¹Propyleneglycol, ²Carboxymethylcellulose, 1% aqueous solution.

Analytical Method Suitability

The compendial method for fluconazole assay in suspension recommended by the United States Pharmacopeia 41th edition¹⁴ was used to evaluate the quantitative content of the formulations. Samples were analyzed on an Agilent High Efficiency Liquid Chromatograph, LC 1200 series, equipped with quaternary pump (G1311A), autoinjector (G1329A) and diode array detector (1315B), using a Zorbax Eclipse Plus C18 Agilent® chromatographic column (150 mm x 4.6 mm x 5 µm). ChemStation software version B03.02 was used for data acquisition and analysis of results. The suitability of the method was demonstrated by evaluating the following parameters.

Selectivity: The chromatograms of Formulation 6 (which contains all evaluated excipients) were compared to those of a placebo formulation with no active ingredient.

Linearity: 1 mg/mL fluconazole standard stock solution was prepared and diluted to reach fluconazole final concentrations of 80, 90, 100, 110 and 120 µg/mL. This procedure was repeated three times independently.

Precision: Six fluconazole sample solutions at 100 µg/mL concentration were prepared. DPR of the six samples was used to determine Repeatability. DPR among twelve samples prepared on different days by different analysts was used to determine intermediate precision.

Accuracy: 100, 110 and 120 µg/mL fluconazole solutions were obtained by adding different amounts of 1 mg/mL fluconazole standard solution in 1 mg/mL fluconazole sample solution. Accuracy was assessed by the recovery in each concentration in triplicate.

Stability Study

The formulations that showed previous results within limits established internally were produced on a semi-industrial scale and placed in amber PET bottles for the stability study. A sample was taken to perform a microbiological test. Five bottles of each formulation were stored at room temperature, in a refrigerator (2 to 8 °C) and in an oven (40 °C). Three of

these bottles were used for the HPLC assay, one bottle was used for pH and macroscopic analyses and the last one for the microbiological assay.

Microbiological Assay

Samples were sent to an outsourced lab (*GRAM - Laboratório de Análises Microbiológicas Ltda*) at 0 and 21 days of storage. An assay for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. was performed by the Cultural Method described in the Bacteriological Analytical Manual (2001) - Food and Drug Administration. Counts of total aerobic bacteria and total molds and yeasts were performed using the Pour Plate method described in *Farmacopeia Brasileira*, 5th Edition.

Results and Discussion

Formulation Development

Formulation 1, which consisted of fluconazole incorporated into syrup, developed aggregates after manipulation. Levigating the drug with glycerin prior to the incorporation in Formulation 2 solved that problem. When using propylene glycol to replace glycerin in Formulation 3, levigation proved to be difficult and inefficient. The use of a 1% aqueous solution of carboxymethylcellulose (CMC) as a vehicle instead of syrup (Formulation 4) impaired fluconazole incorporation due to high viscosity, which led to the development of lumps in the formulation. Different quantities of 1% CMC solution were then tested (Formulations 5, 6 and 7). All of them were easily prepared and demonstrated good appearance, free of lumps or air bubbles.

pH and Viscosity

Table 2 describes the pH and viscosity results obtained for the seven formulations tested. Formulation 2 had the lowest pH value of 5.78, and the highest recorded value was 6.82 for Formulation 4. The viscosity analysis indicated that formulations containing only simple syrup as a vehicle were the most fluid. The viscosity and torque values increase as the amount of CMC in the formulation increases. It is desirable that suspensions have a suitable viscosity to keep solid particles dispersed, ensuring homogeneity and dose uniformity. On the other hand, very viscous formulations should be avoided when it comes to the administration of the drug, as drug dispersion becomes difficult and the product can be partially retained in measuring cups, syringes and tubes¹⁵.

Table 2 - pH, viscosity and torque values for the formulations manipulated.

Formulation	pH ¹	Viscosity (cP)	Torque (%)
1	6.24 (±0.04)	259	13.0
2	5.78 (±0.02)	356	13.9
4	6.82 (±0.07)	1437	71.8
5	6.36 (±0.03)	1024	51.2
6	6.18 (±0.03)	906	45.5
7	6.30 (±0.13)	422	19.3

¹Mean and standard deviation for triplicates.

Volume of Sedimentation

Table 3 describes the results obtained for the sedimentation volume for the seven formulations tested. Formulations 1 and 2, which only had simple syrup as a vehicle, presented the highest sediment volumes and the highest sedimentation speeds. The inclusion of CMC in the formulations delayed this process. The use of a mixture of simple syrup and CMC as a vehicle proved to be more promising in Formulations 5, 6 and 7, with minimal sedimentation over 7 days. The volume of sedimentation obtained was proportionally higher to the quantity of CMC in the formulation.

Stokes' Law dictates that a particle rate of sedimentation is directly proportional to its diameter and inversely proportional to the medium viscosity¹⁴. Grinding the drug prior to manipulation aims to reduce and normalize its particle size, leaving the medium viscosity as the only variable that affects sedimentation. The results obtained from the viscosity corroborate those from sedimentation volume, since the volumes tend to be proportionally higher to the viscosity of the formulations.

Table 3 - Sedimentation volume calculated through the sediment volumes formed in each formulation over a 7-day follow-up.

Time	Volume of Sedimentation					
	Formulation					
	1	2	4	5	6	7
0h	1.00	1.00	1.00	1.00	1.00	1.00
1h	1.00	1.00	1.00	1.00	1.00	1.00
2h	1.00	1.00	0.98	1.00	1.00	1.00
24h	0.29	0.78	0.94	0.97	0.97	0.97
48h	0.29	0.66	0.89	0.97	0.95	0.94
72h	0.29	0.58	0.84	0.97	0.94	0.91
168h	0.29	0.50	0.60	0.94	0.87	0.85

Stability Study

A pH close to neutrality was sought in formulations tested since this is more desirable for oral administration and because fluconazole degradation products were previously detected at extreme pH values⁶. A high viscosity value was not desired because it would make the administration with syringes and tubes difficult. The formulation also needs to keep its particles in suspension for as long as possible, ensuring the particles would not tend to compact, making the redispersion process easier. Thus, Formulations 6 and 7 were selected to continue the stability study.

The formulation quantification assay results performed over 14 days under different storage conditions are shown in Table 4. The profiles can be analyzed in Figure 1. Fluconazole content was depleted by 8.5%, 12.5% and 11.5% in Formulation 6 at the end of 14 days when stored at room temperature, under refrigeration and in an oven at 40 °C, respectively. In Formulation 7, the reduction of content was 3.4% for room temperature storage, 5.6% under refrigeration and 6.5% for oven storage. Both formulations remained above 90% of content during the stability study. After 14 days, Formulation 6 presented equivalent results when stored under refrigeration and in an oven, since there is no statistically significant difference between these conditions

($P > 0.05$). When stored at room temperature, a smaller loss of content was observed, indicating greater stability. When Formulation 7 is stored in an oven, an increase in content is noticed between days 7 and 14. This may be due to loss of water by evaporation, resulting in formulation concentration.

The pH of the formulations was also evaluated along with the assay. Table 5 shows the pH values over time. Although both formulations have differences in composition and have been subjected to three storage temperatures, the pH presented little variation in all samples, remaining stable throughout the study. Both formulations showed the same profile in all cases, with a small drop in pH on the 7th day, returning to values close to the original on the 14th day.

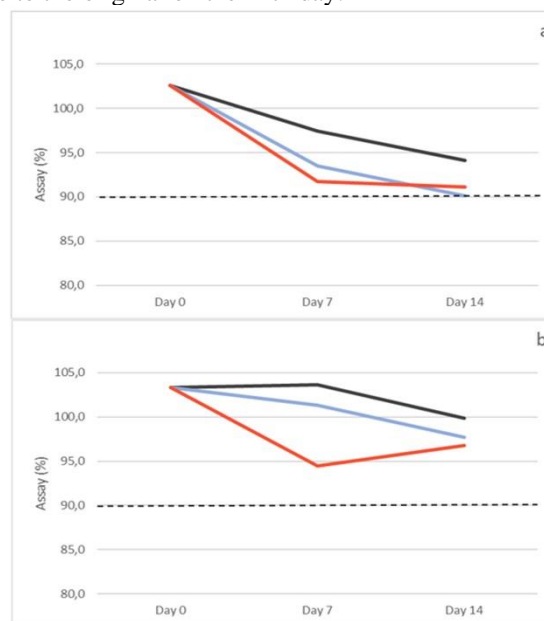


Figure 1 – Graphical representation of assay results in the stability study for Formulations 6 (a) and 7 (b). In black, bottles stored at room temperature. In blue, bottles stored under refrigeration (2 - 8 °C). In red, bottles stored in an oven (40 °C).

Throughout the study, Formulation 6 showed no change in organoleptic properties. However, Formulation 7 showed a slightly darker color and phase separation under all storage conditions on the 14th day of stability (Figure 2). Since the only difference between the formulations is CMC proportion, it is assumed that the quantity used in Formulation 7 was insufficient to keep fluconazole in suspension, causing phase separation. Therefore, formulation 7 is not recommended for use due to the results obtained during the stability study.

Both formulations presented absence of pathogens used in control and low aerobic bacteria, molds and total yeasts counts under all storage conditions after 0 and 21 days of manipulation. The evaluation of microbiological stability is used to determine a safe interval for product consumption, demonstrating storage conditions and product ability to avoid harmful effects from microbial contamination⁹

Table 4 – Formulation assay results throughout 14 days of stability study when stored at room temperature, under refrigeration (2 °C – 8 °C) and in an oven (40 °C).

	Day	Room Temperature		Refrigeration		Oven	
		Assay (%)	RSD (%)	Assay (%)	RSD (%)	Assay (%)	RSD (%)
Formulation 6	0	102.6	1.9	102.6	1.9	102.6	1.9
	7	97.4	3.8	93.5	0.8	91.7	3.7
	14	94.1	2.8	90.1	3.1	91.1	1.7
Formulation 7	0	103.3	5.9	103.3	5.9	103.3	5.9
	7	103.6	2.1	101.3	1.0	94.5	4.8
	14	99.9	6.0	97.7	8.4	96.8	5.4

Table 5 – Formulation pH results throughout 14 days of stability study when stored at room temperature, under refrigeration (2 °C – 8 °C) and in an oven (40 °C).

	Day	pH		
		Room Temperature	Refrigeration	Oven
Formulation 6	0	5.42	5.47	5.45
	7	4.55	4.57	4.34
	14	5.42	5.70	5.30
Formulation 7	0	5.45	5.47	5.42
	7	4.58	4.54	4.28
	14	5.66	5.84	5.42

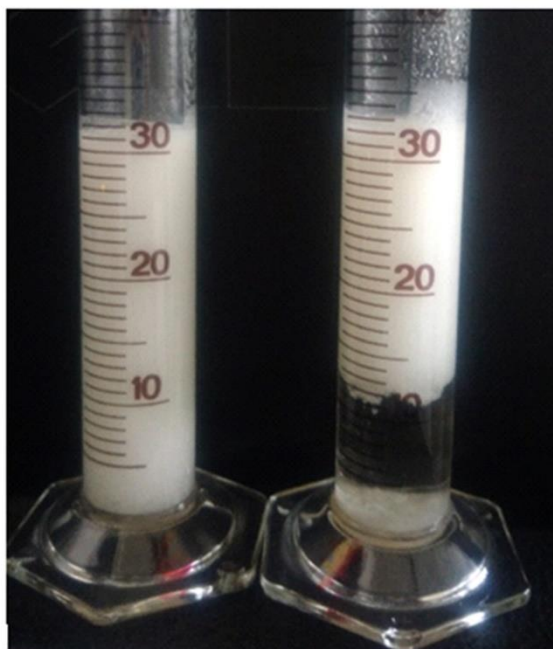


Figure 2 – Phase separation observed after 14 days of stability in Formulation 7 (right graduated cylinder). Formulation 6 after 14 days of stability for comparison (left graduated cylinder).

Analytical Method Suitability

Selectivity: Figure 3 shows Formulation 6 and placebo chromatograms. No interfering peak is observed in fluconazole retention time (3.491 min).

Linearity: Linear regression obtained provided the equation $y = 4.2113x - 26.105$ and a 0.9972 coefficient of determination (r^2). Residue analysis demonstrated homoscedasticity, absence of outlier points and absence of trend in their variances, demonstrating a random distribution.

Precision: Based on criteria from the Association of Official Analytical Chemists (AOAC), the minimum relative standard deviation (RSD) required is 2.7% for repeatability and 4% for intermediate precision. In the present study, the RSD obtained were 0.54% and 1.46% respectively.

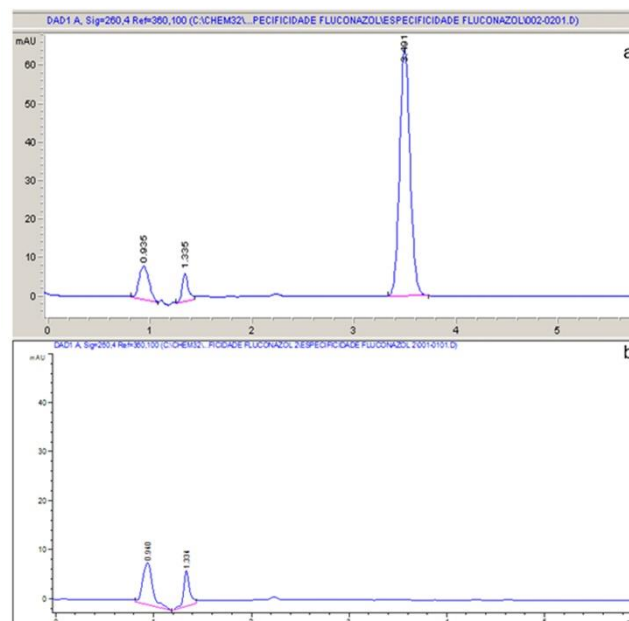


Figure 3 – Formulation 6 (a) and Placebo (b) chromatograms. Mobile phase composed of acetonitrile and monobasic potassium phosphate buffer 20 mM pH 2.5 (80:20) at isocratic flow rate of 1.5 mL/min, injection volume of 50 μ L, room temperature, UV detector at 260 nm, Zorbax Eclipse column Plus C18 Agilent® (150 mm x 4.6 mm x 5 μ m).

Accuracy: Based on criteria of the Association of Official Analytical Chemists (AOAC), recovery should be in the range of 97% to 103%. Table 6 shows recovery obtained and demonstrates method accuracy.

Table 6 – Standard solution recoveries from samples obtained during analytical method accuracy.

Level (%)	Fluconazole Concentration (μ g/mL)	Accuracy	
		Standard Added (μ g)	Mean Recovery (%)
100	100	200	99.77
110	110	300	102.64
120	120	400	100.20

Conclusion

It was possible to develop and characterize a liquid formulation for oral use in pharmaceutical suspension form containing fluconazole suitable for hospital use. It was also possible to demonstrate the suitability for a compendial assay method for the developed formulation.

Formulation 6 was the most appropriate for hospital use and its results suggest incorporation of the suspension into the *Farmacia Semi-Industrial* portfolio, with a recommended shelf life of 14 days, stored in amber PET bottles and at room temperature.

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To the *Farmácia Semi-Industrial* at *Hospital de Clínicas de Porto Alegre*.

To the *Laboratório de Controle de Qualidade Farmacêutico* at *Universidade Federal do Rio Grande do Sul*.

Conflict of interest

None to declare.

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