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# Stability study of Fluconazole capsules with Bracketing Design

Aleksandra Chakarjanevska[1]*, Marika Bogoevska Nikolovska[1], Mirsada Dervisevic Andonova[1], Irena Slaveska Spirevska[1], Marija Karapandzova[2], Suzana Trajkovic Jolevska[3], Jasmina Tonic Ribarska[3]

*[1]Replek Farm Ltd., Quality Control Department, Kozle 188, 1000 Skopje, Republic of North Macedonia*
*[2]Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy, Institute of Pharmacognosy, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*
*[3]Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy, Institute of Applied Chemistry and Pharmaceutical Analysis, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*

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## Abstract

International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines provide guidance for stability monitoring of medicines which Food and Drug Administration (FDA) and European Medicines Agency (EMA) refer to. A full stability study design is described as a model in which samples under the influence of appropriate factors are tested at all-time points. Bracketing and matrixing stability study designs represent reduced designs that may be a suitable alternative to a full stability design. Bracketing design refers to the stability such that only samples on the extremes of certain design are tested at all-time points as in a full design, whereas the stability of the intermediate levels is represented by the stability of the tested extremes, which reduces costs and time analysis. The design assumes that the stability of any intermediate levels is represented by the stability of the extremes tested.

Bracketing design of four different dosage strengths of Fluconazole hard capsules is included in this research. The extreme strengths were chosen and parameters average mass of capsule contents, disintegration, dissolution, assay of active and related substances, according to shelf-life specification were tested in accelerated and long-term stability conditions. Based on the obtained results, the stability of any intermediate levels is confirmed by the stability of the tested extremes and show that this reduced design is suitable.

**Key words:** stability study, Bracketing design, quality control methods, capsules, Fluconazole

## Introduction

The stability of an active substance or a finished medicinal product is a very important factor for its design and development, as characteristics that the medicine has immediately after production, remain unchanged until its shelf life (Canefe and Bozkir, 1991).

A stability study includes an examination that provides evidence of how the quality of the active substance or medicinal product varies with time under the influence of various factors such as temperature, humidity and light. The purpose of stability is not only to characterize the degradation of the active substance or medicinal product, but also to determine the shelf life of the medicinal product and the retest period of the active substance. The effect of the temperature, humidity and light can cause degradation of the active substance or pharmaceutical form, reduction

*email: aleksandra_zakovski@yahoo.com

of action and microbiological contamination. Differences in bioavailability may also occur through a change in disintegration and dissolution, as well as a change in organoleptic properties and determination of the content of the active substance (Bozkir et al., 2013; Canefe & Bozkir, 1990).

There are several designs of stability studies. International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) stability guidelines recommend a full or reduced stability study (ICH Q1D, 2002; ICH Q1A, 2003).

A full stability study is a study in which samples for analysis influenced by various factors such as temperature, humidity and light are tested at all-time points. The stability study in which all samples for analysis are not tested at all-time points is called reduced. Such reduced stability designs are bracketing and matrixing designs, based on different principles that provides time and money savings. Bracketing design is a stability design in which only the extremes of the medicine examined are tested at all-time points, assuming that the stability of any intermediate levels is represented by the stability of the tested extremes (ICH Q1D, 2002). The extremes of the tested medicine include the smallest and the biggest dosage strength, the smallest and the biggest packaging (container size) or filling of the preparation.

Bracketing design is used for multiple strengths with identical or closely related formulations. If the relative amounts of active substance and excipients change in a formulation, a justification is needed. In case where different excipients are used in a formulation, this stability design is not applicable. The application of reduced stability designs is risky if there are no data on the stability of the medicinal product and active substance.

Capsules are solid preparations with hard or soft shells of various shapes and capacities, containing a single dose of active substance and are usually intended for oral administration. The contents do not cause deterioration of the shell that is attacked by the digestive fluids which leads to the content release (European Pharmacopoeia 11th edition, 2023).

The aim of the study is reducing the number of analysis, the costs and analysis time, using bracketing design of Fluconazole capsules with different dosage strengths and identical formulation with proportional changedamounts of active substance and excipients accordingly with the strengths.

## Material and methods

*Chemicals, reagents and reference materials*

Hydrochloric acid 37 %, potassium dihydrogen phosphate, methanol (HPLC grade), acetonitrile (HPLC grade) and ammonium formate were purchased from Carlo Erba, Italy. Demineralized (ultrapure) water, *in-house* prepared with Simplicity system. Valid batches of reference standards: Fluconazole RS was purchased from Sigma Aldrich, Germany; Fluconazole for peak identification CRS (containing impurity A), Fluconazole impurity B CRS and Fluconazole impurity C CRS were supplied by European Directorate for the quality of Medicines (EDQM, Strasbourg France). Fluconazole capsules (50 mg and 200 mg) and placebo were from Replek Farm, North Macedonia, Skopje.

*Equipment*

Average mass of capsule contents was determined on analytical balance Mettler Toledo AG285. The disintegration test was done on apparatus for disintegration Vankel VK 100. For dissolution test was used apparatus for dissolution Sotax AT7 Smart and UV-spectrophotometer Analytik Jena Specord. For determination the assay of active substance and related substances were used analytical balance Mettler Toledo AG285 and HPLC system Shimadzu Nexera XRwith low pressure gradient quaternary pump with degasser, autosampler, controller and PDA detector. Signals were monitored and processed using the Lab solutions software.

Table 1. Applied bracketing design

| Stability | Medicine | 50 mg | | | 100 mg | 150 mg | 200 mg | | |
|---|---|---|---|---|---|---|---|---|---|
| | Batch | F50 batch 1 | F50 batch 2 | F50 batch 3 | | | F200 batch 1 | F200 batch 2 | F200 batch 3 |
| Long-term 25°C ± 2°C/60 % ± 5 % RH | | 0, 3, 6, 9, 12, 18, 24, 36 months | 0, 3, 6, 9, 12, 18, 24, 36 months | 0, 3, 6, 9, 12, 18, 24, 36 months | x | x | 0, 3, 6, 9, 12, 18, 24, 36 months | 0, 3, 6, 9, 12, 18, 24, 36 months | 0, 3, 6, 9, 12, 18, 24, 36 months |
| Accelerated 40°C ± 2°C/75 % ± 5 % RH | | 0, 3, 6 months | 0, 3, 6 months | 0, 3, 6 months | x | x | 0, 3, 6 months | 0, 3, 6 months | 0, 3, 6 months |

*Methods*

Fluconazole hard capsules, a medicine that is present on the market in four different strengths (Fluconazole capsules 50 mg, 100 mg, 150 mg and 200 mg), where used in this study. Extreme strengths, Fluconazole capsules 50 mg (F50) and Fluconazole capsules 200 mg (F200), were tested in accelerated and long-term stability conditions. Bracketing design is shown in Table 1.

1) Appearance

The appearance of Fluconazole capsules 50 mg and Fluconazole capsules 200 mg was done with visual determination. Fluconazole capsules 50 mg should be uniform in size Nº2, dark green, hard, gelatinous and filled with white to almost white homogenous powder with possible agglomerate particles which are easily cracked. Fluconazole capsules 200 mg should be uniform in size Nº0, white, hard, gelatinous and filled with white to almost white homogenous powder with possible agglomerate particles which are easily cracked.

2) Average mass of capsule contents

Average mass of capsule contents was determined according to European Pharmacopoeia (Ph.Eur.) 11th edition, Volume I, 2.9.5. Uniformity of mass of single-dose preparations. An analytical balance Mettler Toledo AG285 was used to determine this parameter.

3) Disintegration

The disintegration test was performed according to Ph.Eur. 11th edition, Volume I, 2.9.1. Disintegration of tablets and capsules. Disintegration Test A was used, with purified water as a medium in total duration of 30 minutes and was done on apparatus for disintegration Vankel VK 100.

4) Dissolution test

The dissolution test was performed according to Ph.Eur. 11th edition, Volume I, 2.9.3. Dissolution test for solid dosage forms. Dissolution testing was carried out under the following conditions: Apparatus 1 (Basket apparatus, Sotax AT7 Smart); medium 0.1 M hydrochloric acid, 500 mL for Fluconazole capsules 50 mg and 900 mL for Fluconazole capsules 200 mg; temperature of the medium 37ºC ± 0.5 ºC; testing time 30 minutes and stirring speed 100 rotations per minute (rpm). After 30 minutes, a specimen of about 50 mL was taken and filtered. The filtrate was used as a test solution for Fluconazole capsules 50 mg. For Fluconazole capsules 200 mg, 5 mL of the filtrate (filtered through filter paper Sartorius, grade 391, diameter 125 mm) was diluted to 10 mL with 0.1 M hydrochloric acid and this solution was used as a test solution. The whole procedure was performed on six capsules individually.

Correction solution was prepared on the same way as a test solution, but an empty capsule was treated. The blank was 0.1 M hydrochloric acid.

A standard solution was also prepared. 10 mg Fluconazole Reference Standard (RS) was transferred into a 100 mL volumetric flask and 70 mL 0.1 M hydrochloric acid was added. The standard solution was shaken until complete dissolution was obtained and filled with the same solvent.

The absorbencies of the test solutions, correction solution and standard solution were measured on a suitable spectrophotometer (UV-spectrophotometer Analytik Jena Specord) at a maximum absorbance wavelength of 261 ± 2 nm. Each capsule was dissolved not less than 80 %, (Q + 5 %), expressed as a percentage of the labeled content. The quantity Q is the specified amount of dissolved active substance, expressed as a percentage of the labeled content.

5) Assay of active substance

After random choice, at least 20 capsules were taken and the contents of each were emptied, ensuring that the capsules were not damaged. The content was homogenized and a quantity of the powder corresponding to about an average mass of capsule contents (accurately weighed) was transferred into a 50 mL and 200 mL volumetric flask for Fluconazole capsules 50 mg and for Fluconazole capsules 200 mg, respectively. About 30 mL mobile phase for Fluconazole capsules 50 mg and about 150 mL mobile phase for Fluconazole capsules 200 mg were added, then sonicated for 15 minutes, mechanically shaken for further 15 minutes and diluted to volume with the same solvent. The mixture was filtered through filter paper (Sartorius, grade 389, diameter 125 mm), the clear filtrate was filtered through 0.45 µm PTFE filter and thanused for high pressure liquid chromatography (HPLC) determination (HPLC instrument Shimadzu Nexera XR Labsolution).

A standard solution was also prepared. For this purpose, 25 mg Fluconazole RS was transferred into a 25 mL volumetric flask and 20 mL mobile phase was added. The standard solution was shaken until complete dissolution was obtained and diluted to volume with the same solvent.

Chromatographic conditions were: chromatographic column RP Select B 125 x 4.0 mm, 5 µm; mobile phase 0.025 M potassium dihydrogen phosphate and methanol in a ratio 55:45; flow rate 1.0 mL/min.; UV-detection at 254 nm; injection volume 10 µL and column temperature 30ºC.

6) Related substances

This parameter was performed according to Ph.Eur. 11th edition, Volume II, 01/2017:2287, monograph of Fluconazole.

Chromatographic conditions were: chromatographic column Purospher RP18, 125 x 4.0 mm, 5 µm; mobile phase mixture of acetonitrile and 0.63 g/L solution of ammonium formate in volume ratio 14:86 (%, *v/v*); flow rate 1.2 mL/min.; UV-detection at 260 nm; injection volume 20 µL, column temperature 40ºC and this determination was done on HPLC instrument Shimadzu Nexera XR Labsolution.

## Results and discussion

In this study, bracketing stability design was used, in which the extremes of Fluconazole capsules were tested in accelerated (40°C ± 2°C/75 % ± 5 % RH) and long-term (25°C ± 2°C/60 % ± 5 % RH) stability conditions (Table 2 to Table 9).

Table 2. Accelerated stability test results of F50 of three different batches

| **Batch** | **F50 batch 1** | | | **F50 batch 2** | | | **F50 batch 3** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** |
| **Average net mass of capsule contents:** 117 mg ± 10 % | 115.6 mg | 123.3 mg | 118.2 mg | 117.1 mg | 124.9 mg | 122.5 mg | 122.7 mg | 126.6 mg | 118.3 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 7 minutes | 4 minutes | 4 minutes | 6 minutes | 4 minutes | 4 minutes | 5 minutes | 4 minutes | 4 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 94.81% | 94.14% | 93.22% | 96.70% | 95.67% | 94.76% | 97.64% | 97.31% | 96.33% |
| **Assay of active substance:** 45.0-55.0 mg Fluconazole/capsule or 90-110 % of declared content | 97.04% | 96.44% | 95.90% | 98.60% | 98.81% | 98.40% | 100.23 % | 99.44% | 98.63% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | imp. A 0.19% imp. B, imp. C, unspecified imp. are not detected Total: 0.19% | imp. A 0.19% imp. B, imp. C, unspecified imp. are not detected Total: 0.19% | imp. A 0.19% imp. B, imp. C, unspecified imp. are not detected Total: 0.19% | imp. A 0.12% imp. B, imp. C, unspecified imp. are not detected Total: 0.12% | imp. A 0.16% imp. B, imp. C, unspecified imp. are not detected Total: 0.16% | imp. A 0.13% imp. B, imp. C, unspecified imp. are not detected Total: 0.13% | imp. A 0.10% imp. B, imp. C, unspecified imp. are not detected Total: 0.10% | imp. A 0.12% imp. B, imp. C, unspecified imp. are not detected Total: 0.12% | imp. A 0.11% imp. B, imp. C, unspecified imp. are not detected Total: 0.11% |

Table 3. Accelerated stability test results of F200 of three different batches

| **Batch** | **F200 batch 1** | | | **F200 batch 2** | | | **F200 batch 3** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** |
| **Average net mass of capsule contents:** 468 mg ± 7.5 % | 465.8 mg | 469.5 mg | 464.3 mg | 472.0 mg | 490.4 mg | 485.6 mg | 469.7 mg | 476.0 mg | 473.0 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 3 minutes | 4 minutes | 6 minutes | 8 minutes | 8 minutes | 4 minutes | 9 minutes | 7 minutes | 6 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 95.97% | 94.65% | 94.31% | 95.34% | 95.58% | 96.10% | 101.59 % | 100.55 % | 100.01 % |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Assay of active substance:** 180.0-220.0 mg Fluconazole/capsule or 90-110 % of declared content | 96.67% | 96.13% | 96.88% | 95.80% | 96.82% | 94.43% | 99.87% | 98.70% | 98.70% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | not detected | imp. A 0.19% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.19% | imp. A 0.19% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.19% | imp. A 0.13% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.13% | imp. A 0.14% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.14% | imp. A 0.14% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.14% | imp. A 0.11% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.11% | imp. A 0.12% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.12% | imp. A 0.11% imp. B, imp. C, unspecif ied imp. are not detected Total: 0.11% |

Table 4. Long-term stability test results of F50 of batch 1

| **Batch** | **F50 batch 1** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 9 months** | **Time: 12 months** | **Time: 18 months** | **Time: 24 months** | **Time: 36 months** |
| **Average net mass of capsule contents:** 117 mg ± 10 % | 115.6 mg | 115.9 mg | 117.9 mg | 117.1 mg | 117.5 mg | 118.1 mg | 118.3 mg | 118.8 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 7 minutes | 4 minutes | 4 minutes | 4 minutes | 4 minutes | 4 minutes | 4 minutes | 4 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 94.81% | 94.79% | 94.57% | 94.33% | 94.56% | 94.66% | 94.12% | 93.58% |
| **Assay of active substance:** 45.0-55.0 mg Fluconazole/ capsule or 90-110 % of declared content | 97.04% | 96.90% | 97.20% | 96.56% | 96.93% | 96.24% | 96.36% | 95.54% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | imp. A 0.19% imp. B, imp. C, unspec. imp. are not detected Total: 0.19% | imp. A 0.19% imp. B, imp. C, unspec. imp. are not detected Total: 0.19% | imp. A 0.19% imp. B, imp. C, unspec. imp. are not detected Total: 0.19% | imp. A 0.19% imp. B, imp. C, unspec. imp. are not detected Total: 0.19% | imp. A 0.18% imp. B, imp. C, unspec. imp. are not detected Total: 0.18% | imp. A 0.19% imp. B, imp. C, unspec. imp. are not detected Total: 0.19% | imp. A 0.20% imp. B, imp. C, unspec. imp. are not detected Total: 0.20% | imp. A 0.20% imp. B, imp. C, unspec. imp. are not detected Total: 0.20% |

Table 5. Long-term stability test results of F50 of batch 2

| **Batch** | **F50 batch 2** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 9 months** | **Time: 12 months** | **Time: 18 months** | **Time: 24 months** | **Time: 36 months** |
| **Average net mass of capsule contents:** 117 mg ± 10 % | 117.1 mg | 121.1 mg | 122.5 mg | 119.3 mg | 119.6 mg | 119.9 mg | 120.2 mg | 121.9 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 6 minutes | 4 minutes | 4 minutes | 4 minutes | 3 minutes | 5 minutes | 4 minutes | 4 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 96.70% | 96.37% | 96.66% | 96.41% | 95.77% | 95.12% | 95.85% | 95.07% |
| **Assay of active substance:** 45.0-55.0 mg Fluconazole/ capsule or 90-110 % of declared content | 98.60% | 98.71% | 98.53% | 98.22% | 98.53% | 97.12% | 97.14% | 96.99% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.17% imp. B, imp. C, unspec. imp. are not detected Total: 0.17% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.14% imp. B, imp. C, unspec. imp. are not detected Total: 0.14% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% | imp. A 0.14% imp. B, imp. C, unspec. imp. are not detected Total: 0.14% |

Table 6. Long-term stability test results of F50 of batch 3

| **Batch** | **F50 batch 3** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 9 months** | **Time: 12 months** | **Time: 18 months** | **Time: 24 months** | **Time: 36 months** |
| **Average net mass of capsule contents:** 117 mg ± 10 % | 122.7 mg | 123.2 mg | 120.1 mg | 121.4 mg | 122.0 mg | 122.8 mg | 123.2 mg | 123.9 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 5 minutes | 4 minutes | 4 minutes | 4 minutes | 4 minutes | 4 minutes | 5 minutes | 6 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 97.64% | 97.55% | 97.32% | 97.01% | 97.32% | 96.90% | 96.58% | 96.16% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Assay of active substance:** 45.0-55.0 mg Fluconazole/ capsule or 90-110 % of declared content | 100.23% | 99.93% | 100.14% | 99.81% | 99.73% | 99.45% | 99.06% | 98.61% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | imp. A 0.10% imp. B, imp. C, unspec. imp. are not detected Total: 0.10% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% |

Table 7. Long-term stability test results of F200 of batch 1

| **Batch** | **F200 batch 1** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 9 months** | **Time: 12 months** | **Time: 18 months** | **Time: 24 months** | **Time: 36 months** |
| **Average net mass of capsule contents:** 468 mg ± 7.5 % | 465.8 mg | 474.6 mg | 467.4 mg | 469.9 Mg | 464.1 mg | 470.0 mg | 465.0 mg | 469.2 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 3 minutes | 4 minutes | 5 minutes | 6 minutes | 7 minutes | 4 minutes | 4 minutes | 5 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 95.97% | 95.02% | 95.71% | 95.36% | 96.24% | 96.20% | 95.58% | 94.80% |
| **Assay of active substance:** 180.0-220.0 mg Fluconazole/capsule or 90-110 % of declared content | 96.67% | 97.03% | 96.91% | 96.58% | 95.84% | 96.10% | 95.30% | 95.80% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | not detected | imp. A 0.20% imp. B, imp. C, unspec. imp. are not detected Total: 0.20% | imp. A 0.18% imp. B, imp. C, unspec. imp. are not detected Total: 0.18% | imp. A 0.19% imp. B, imp. C, unspec. imp. are not detected Total: 0.19% | imp. A 0.19% imp. B, imp. C, unspec. imp. are not detected Total: 0.19% | imp. A 0.20% imp. B, imp. C, unspec. imp. are not detected Total: 0.20% | imp. A 0.22% imp. B, imp. C, unspec. imp. are not detected Total: 0.22% | imp. A 0.22% imp. B, imp. C, unspec. imp. are not detected Total: 0.22% |

Table 8. Long-term stability test results of F200 of batch 2

| **Batch** | **F200 batch 2** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 9 months** | **Time: 12 months** | **Time: 18 months** | **Time: 24 months** | **Time: 36 months** |
| **Average net mass of capsule contents:** 468 mg ± 7.5 % | 472.0 mg | 473.2 mg | 462.9 mg | 471.8 mg | 476.9 mg | 462.9 mg | 473.5 mg | 477.6 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 8 minutes | 8 minutes | 4 minutes | 6 minutes | 4 minutes | 6 minutes | 8 minutes | 8 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 95.34% | 94.75% | 95.00% | 95.99% | 96.27% | 94.60% | 94.30% | 94.23% |
| **Assay of active substance:** 180.0-220.0 mg Fluconazole/capsule or 90-110 % of declared content | 95.80% | 95.76% | 95.28% | 95.31% | 95.22% | 95.25% | 94.34% | 94.85% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% | imp. A 0.15% imp. B, imp. C, unspec. imp. are not detected Total: 0.15% | imp. A 0.15% imp. B, imp. C, unspec. imp. are not detected Total: 0.15% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% |

Table 9. Long-term stability test results of F200 of batch 3

| **Batch** | **F200 batch 3** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Specification limits** | **Time: 0 months** | **Time: 3 months** | **Time: 6 months** | **Time: 9 months** | **Time: 12 months** | **Time: 18 months** | **Time: 24 months** | **Time: 36 months** |
| **Average net mass of capsule contents:** 468 mg ± 7.5 % | 469.7 mg | 468.2 mg | 463.4 mg | 465.3 mg | 466.2 mg | 468.4 mg | 470.1 mg | 471.7 mg |
| **Disintegration:** - maximum 30 minutes, water purified, 37ºC ± 2ºC | 9 minutes | 8 minutes | 9 minutes | 8 minutes | 6 minutes | 7 minutes | 8 minutes | 9 minutes |
| **Dissolution:** - not less than 80 % in 30 minutes | 101.59% | 100.85% | 101.24% | 101.03% | 100.85% | 100.51% | 100.20% | 99.63% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Assay of active substance:** 180.0-220.0 mg Fluconazole/capsule or 90-110 % of declared content | 99.87% | 100.09% | 99.73% | 99.55% | 99.09% | 98.63% | 98.44% | 98.01% |
| **Related substances:** - impurity A max.0.4% - impurity B max.0.3% - impurity C max.0.1% - unspecified impurities max.0.1% - total impurities max.0.6% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.11% imp. B, imp. C, unspec. imp. are not detected Total: 0.11% | imp. A 0.12% imp. B, imp. C, unspec. imp. are not detected Total: 0.12% | imp. A 0.13% imp. B, imp. C, unspec. imp. are not detected Total: 0.13% |

Extreme strengths (Fluconazole capsules 50 mg and Fluconazole capsules 200 mg both packed in PVC/PVDC/Al blister x 7 capsules/cardboard box), were tested at all-time points in accelerated and long-term stability conditions. The tested medicinal product has four different strengths (50 mg, 100 mg, 150 mg and 200 mg) with identical formulation and proportional changed amounts of active substance and excipients accordingly with the strengths. Therefore, this reduced design was used (ICH Q1D, 2002).

Extreme strengths F50 and F200 were tested in accelerated and long-term stability conditions on three different batches according to quality control methods in the pharmacopoeia. Several quality parameters such as appearance, average mass of capsule contents, disintegration, dissolution, assay of active substance and related substances were tested.

No significant changes to appearance and color were observed during the stability testing.

All the pharmacopoeia limits were met during testing the average mass of capsule contents of the medicines coded F50 and F200 at all-time points in accelerated and long-term stability conditions (European Pharmacopoeia 11$^{th}$ edition, 2023).

All the pharmacopoeia limits were met during disintegration test of medicine coded F50 at all-time points in accelerated and long-term stability conditions. Based on the obtained results, disintegration time data show that medicine coded F200 meets the pharmacopoeia limits (European Pharmacopoeia 11$^{th}$ edition, 2023). Fluconazole capsules used in this study should be dissolved not less than 80 % of labeled amount of Fluconazole in period of 30 minutes. This limit was met during dissolution test of the medicines coded F50 and F200 at all-time points in accelerated and long-term stability conditions (European Pharmacopoeia 11$^{th}$edition, 2023). The dissolution of medicine coded F50 and F200 was slightly decreased at all-time points in accelerated and long-term stability conditions. Release specification limit of assay of active substance for Fluconazole hard capsules is 95.0 – 105.0% of the declared content. This limit is narrower to guarantee us that the medicine during the shelf-life will meet the prescribed limit for testing the assay of active substance in the shelf -life specification with acceptance limit 90.0 – 110.0 % of the declared content and also according to the results obtained in stability trials. The shelf-life specification limit was met during testing the assay of active substance of the medicines coded F50 and F200 at all-time points in accelerated and long-term stability conditions, as well.

Related substances were tested according to Ph.Eur. 11$^{th}$ edition. All the pharmacopoeia limits were met during testing the related substances for both studies. No significant changes to related substances were observed during the stability testing at all-time points in accelerated and long-term stability conditions during the shelf-life period.

## Conclusion

This study used bracketing stability design of Fluconazole capsules. Since this medicine is present on the market in four different dosage strengths, the lowest and the highest strength of the medicinal product were chosen and several quality parameters were tested in accelerated and long-term stability conditions. Based on the obtained results, it can be concluded that it is not necessary to test the extremes under intermediate stability conditions, because there are no significant differences in the results obtained under accelerated stability conditions for the tested medicines coded F50 and F200 (ICH Q1A, 2003). This reduced stability study design is convenient not only because of the costs reduction, but also because of the

shortening of the time required for analyses, as the full stability study increases the number of tested samples as well as the number of tested parameters compared to a bracketing design.

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**Резиме**

# Испитување на стабилност на Флуконазол капсули со дизајн на бракетирање

Александра Чакарјаневска[1]*, Марика Богоевска Николовска[1], Мирсада Дервишевиќ Андонова[1], Ирена Славеска Спиревска[1], Марија Карапанџова[2], Сузана Трајковиќ Јолевска[3], Јасмина Тониќ Рибарска[3]

*[1]Реплек Фарм, Сектор за контрола на квалитет, Козле 188, 1000 Скопје, Република Северна Македонија*
*[2]Универзитет „Св. Кирил и Методиј", Фармацевтски факултет, Институт за фармакогнозија, Мајка Тереза 47, 1000 Скопје, Република Северна Македонија*
*[3]Универзитет „Св. Кирил и Методиј", Фармацевтски факултет, Институт за применета хемија и фармацевтски анализи, Мајка Тереза 47, 1000 Скопје, Република Северна Македонија*

**Клучни зборови:** стабилност, дизајн на бракетирање, методи за контрола на квалитет, капсули, Флуконазол

Меѓународната конференција за хармонизација на техничките барања за регистрација на фармацевтските производи за човечка употреба (ICH) дава насоки за следење на стабилност на фармацевтските препарати, на кои се повикуваат Агенцијата за храна и лекови (FDA) и Европската агенција за лекови (EMA). Комплетната стабилност е опишана како модел во кој примероците за анализа под влијание на соодветни фактори, се тестираат во сите временски точки. Дизајни на бракетирање и матриксирање претставуваат редуцирани дизајни на стабилност кои можат да бидат погодна алтернатива на комплетната стабилност. Дизајн на бракетирање е дизајн во кој стабилноста на било кој интермедиер е претставена преку стабилноста на тестираните екстреми, со кој се намалува трошокот и времето за анализа.

Во ова истражување е употребен дизајн на бракетирање на Флуконазол тврди капсули, препарат кој е присутен на пазарот со четири различни јачини. Избрани се екстремните јачини и параметрите просечно полнење, распадливост, растворливост, содржина на активна супстанца и сродни супстанци се тестирани во забрзани и долгорочни услови на стабилност во согласност со спецификацијата во рок на употреба.

Врз основа на добиените резултати, стабилноста на било кој интермедиер се потврдува преку стабилноста на тестираните екстреми и се покажува дека овој редуциран дизајн на стабилност е погоден и соодветен.