Development and validation of a fluconazole quantification method for the stability study of an investigational medical product

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OBJECTIF

The main goal of this study was to develop and to validate a stability indicating quantification method for fluconazole using High-Performance Liquid Chromatography and Ultra-Violet detection (HPLC-UV). This method is meant for stability assessment and post-production controls of fluconazole 50 mg capsules.

CONTEXT

The Hospital Group pharmacy of Hospices Civils de Lyon is sought for a clinical trial. Pharmacy in charge of investigational medical product production, quality control and distribution.

- Need 50 mg fluconazole capsules including *verum* and *placebo*.
- *Verum* prepared with the reconditioning of a FLUCONAZOLE ARROW® 50 mg capsules.
- *Placebo* prepared with lactose monohydrate and empty capsules that are the same as the verum.
- Need an analytical tool that allows the stability study of the investigational medical product.

A fluconazole stability indicating quantification method is needed.

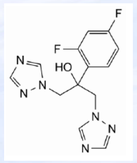

Figure 1: Chemical structure of fluconazole

MATERIALS AND METHODS

HPLC – Instrumentation and conditions

Chromatography was performed using a 1290 Infinity Agilent Technologies HPLC with a UV/visible Detector. Processing and data acquisition were performed using Open Lab Control Panel system software.

Table 1: Analytical conditions of the RP-HPLC method

Chromatographic condition	Parameters
Isocratic mobile phase	Methanol:Acetate buffer pH 5.0 (40:60, v/v)
Stationary phase	Kinetex® 2.6 µm C18 100A 150 x 4.6 mm Liquid Chromatography
Flow rate	1 mL/min
Injection volume	10 µL
Detection wavelenght	261 nm
Column temperature	40°C

Calibration standards

- Prepared with pure CRS (Chemical Reference Substance) fluconazole and mobile phase.
- 3 concentration levels (low, medium, high): 100, 250 and 500 µg/mL. Repeated 2 times, 6 calibration points per day.

Validation standards

- Prepared with FLUCONAZOLE ARROW® 50 mg capsules and mobile phase.
- 3 concentration levels (low, medium, high): 150, 300 and 450 µg/mL. Repeated 3 times, 9 validation points per day.

Method validation

Assay validation procedures were performed according to Société Française des Sciences et Technologies Pharmaceutiques (SFSTP) recommendations and ICH Q2 (R1) guidelines.

➢ Specificity and selectivity

- Chromatograms comparison of (i) pure fluconazole CRS, (ii) FLUCONAZOLE ARROW® 50 mg capsule and (iii) mobile phase.
- Mean variables: retention time, peak area and baseline signal.

➢ Linearity

- 2 sets of calibration standards were prepared and analyzed per day, each time by a different manipulator, on 3 different days.
- The coefficient of determination (R^2) must be at least 0.95 for each calibration standard curve.

➢ Precision and accuracy

- 3 sets of calibration standards were prepared and analyzed per day, on 3 different days.
- The relative bias must be less than ± 10%.
- The intra-day and inter-day Coefficient of Variation (CV) must be less than 5% and 8% respectively.

➢ Accuracy profile

Forced degradation study

Designed according to the "Groupe d'Evaluation et de Recherche sur la Protection en Atmosphère Contrôlée" (GERPAC) guidelines.

- The pharmaceutical substance is tested against 5 different degradation conditions: acidic and alkaline hydrolyze, heat, photo oxidation and oxidation (H_2O_2).
- The degradation condition was gradually increased until either (i) 20% of the compound was degraded or (ii) degradation conditions were judged strong enough to consider the compound resistant.

RESULTS

Method validation

Using this method, the retention time (Rt) of fluconazole is 3.1 ± 0.2 min.

➢ Specificity and selectivity

- No differences between (i) the pure fluconazole CRS chromatogram and (ii) the FLUCONAZOLE ARROW® 50 mg capsule chromatogram.
- Same retention time, same peak area and same baseline signal.
- No co-elution of fluconazole and impurities at their retention times.

➢ Linearity

- Every calibration standard curve had a R^2 higher than 0.95.
- The R^2 of the calibration standard curve obtained from the integration of every calibration standard sample was 0.992.

➢ Precision and accuracy

- The intra-day CV for the low, medium and high validation standards: 3.22%, 3.26% et 3.15% respectively. Acceptability limit ± 5%.
- The inter-day CV for the low, medium and high validation standards: 3.35%, 2.99% et 3.04% respectively. Acceptability limit ± 8%.
- The relative bias of validation standard determined concentration: 0.22% for day one, 1.15% for day two and 1.57% for day three of validation. Acceptability limit ± 10%.

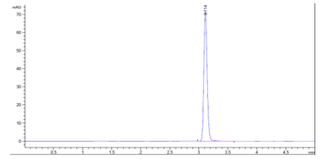

Figure 2: Medium validation standard chromatogram.

➢ Accuracy profile

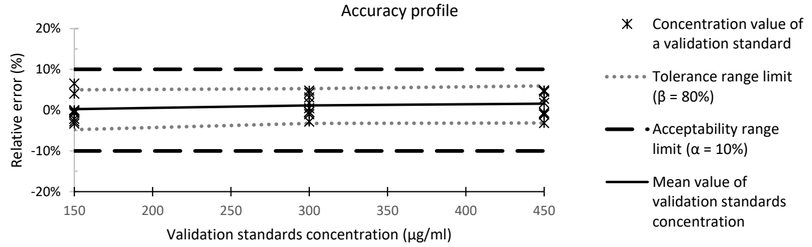

Figure 3: Accuracy profile of the RP-HPLC-UV fluconazole quantification method.

Forced degradation study

➢ Acidic hydrolyze (HCl 1 M for 4h at 80°C)

- No compound degradation is observed.

➢ Alkaline hydrolyze (NaOH 1 M for 4h at 80°C)

- No compound degradation is observed.
- The chromatogram showed 3 elution pics of impurities :
  - Pic n°1: Rt = 1.275 min with an AUC = 132.25 mAU
  - Pic n°2: Rt = 1.435 min with an AUC = 39.35 mAU
  - Pic n°3: Rt = 1.614 min with an AUC = 23.49 mAU
  - Pic n°4: Rt = 1.892 min with an AUC = 51.26 mAU

➢ Heat (60°C for 72h)

- No compound degradation is observed.

➢ Photo-oxidation (265 nm for 3h)

- No compound degradation is observed.

➢ Oxidation (H_2O_2 3% 9:1 (v/v) for 24h)

- The compound is degraded up to 2.88%.

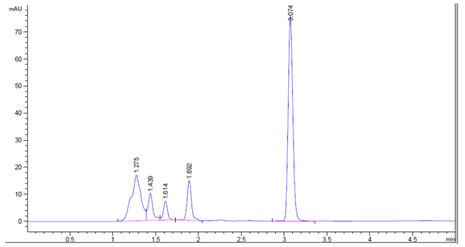

Figure 4: Heat degradation chromatogram.

CONCLUSION

This fluconazole stability indicating quantification method based on the use of HPLC-UV is validated, allowing (i) to perform the stability study of the fluconazole capsules and (ii) to control the capsules' pharmaceutical compound quantity in the context of a clinical trial.

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GERPAC Congress 2020