Development and validation of a clomifene citrate 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 clomifene citrate using High-Performance Liquid Chromatography and Ultra-Violet detection (HPLC-UV). This method is meant for stability assessment and post-production controls of the capsules.

CONTEXT

The Hospital Group pharmacy of Hospices Civils de Lyon is sought for a clinical trial

→ Need 50 mg clomifene citrate capsules including *verum* et *placebo with an* 18 months stability.

Pharmacy in charge of investigational medical product production, quality control and distribution.

- *Verum* prepared with grinded CLOMID® 50 mg (bulk powder).
- *Placebo* prepared with microcrystalline cellulose.

A clomifene citrate stability indicating quantification method is needed following ICH guidelines.

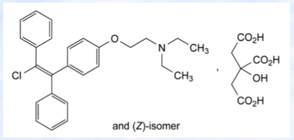

Figure 1: Chemical structure of the two clomifene citrate isomers.

MATERIALS AND METHODS

HPLC – Instrumentation and conditions

Chromatography was performed using a 1290 Infinity Agilent Technologies UHPLC with a UV/visible Diode Array Detector (DAD). 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 : Phosphate buffer (1.8 g/L) pH 8,0 (88:12, v/v)
Stationary phase	Kinetex® 2.6 µm EVO C18 100A 100 x 4.6 mm Liquid Chromatography
Flow rate	1 mL/min
Injection volume	10 µL
Detection wavelenght	290 nm
Column temperature	40°C

Calibration standards

- Prepared with pure CRS (Chemical Reference Substance) clomifene citrate and mobile phase.
- 3 concentration levels (low, medium, high): 20, 50 and 100 µg/mL. Repeated 2 times, 6 calibration points per day.

Validation standards

- Prepared with grinded CLOMID® 50 mg and mobile phase: 30, 60 et 90 µg/mL.
- 3 concentration levels (low, medium, high): 30, 60 and 90 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 clomifene citrate, (ii) grinded CLOMID® 50 mg tablet 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 of clomifene citrate is 2.3 ± 0.2 min.

➢ Specificity and selectivity

- No differences between (i) the pure CRS clomiphene citrate chromatogram and (ii) the CLOMID® 50 mg bulk powder chromatogram.
- Same retention time, same peak area and same baseline signal.
- No co-elution of clomifene citrate 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: 0.94%, 1.67% et 1.04% respectively. Acceptability limit ± 5%.
- The inter-day CV for the low, medium and high validation standards: 1.00%, 1.52% et 0.97% respectively. Acceptability limit ± 8%.
- The relative bias of validation standard determined concentration: 4.14% for day one, 5.00% for day two and 4.74% for day three of validation. Acceptability limit ± 10%.

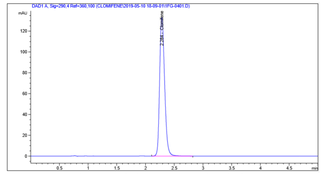

Figure 2: Medium validation standard chromatogram.

➢ Accuracy profile

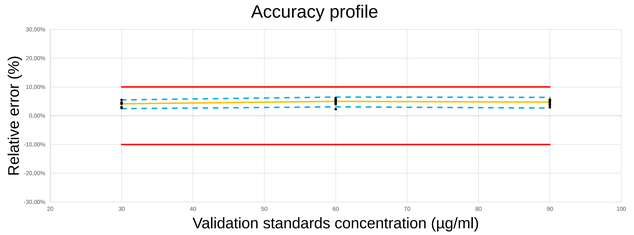

Figure 3: Accuracy profile of the RP-HPLC-UV clomifene citrate quantification method.

Forced degradation study

➢ Acidic hydrolyze (HCl 2 M for 5h)

- Compound degraded up to 10.05%.
- A separation phenomenon of the two clomifene citrate isomers has been noticed.

➢ Alkaline hydrolyze (NaOH 2 M for 5h)

- Compound degraded up to 19.41%.
- A separation phenomenon of the two clomifene citrate isomers has been noticed.

➢ Heat (80°C for 3h)

- Compound degraded up to 1.73%.
- The chromatogram showed 3 elution pics of impurities at 0.73 min, 0.81 min and 1.94 min.

➢ Photo-oxidation (265 nm for 30min)

- Compound degraded up to 38.71%.
- The chromatogram showed the elution pic of an impurity at 1.94 min.

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

- Compound degraded up to 4.57%.
- A separation phenomenon of the two clomifene citrate isomers has been noticed.

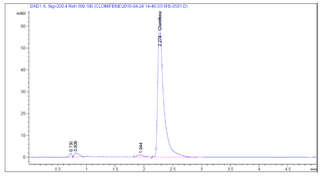

Figure 4: Heat degradation chromatogram.

CONCLUSION

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

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