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.
- Serum prepared with grated COMID® 50 mg (bulk powder).
- Placebo prepared with microcrystalline cellulose.

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

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 HPLC-UV method

<table>
<thead>
<tr>
<th>Chromatographic condition</th>
<th>Parameters</th>
</tr>
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<tbody>
<tr>
<td>Isocratic mobile phase</td>
<td>Methanol : Phosphate buffer (1.8 g/l) pH 8.0 (88:12), v/v</td>
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<tr>
<td>Stationary phase</td>
<td>Kinetex® 2.6 µm EVO C18 100A 100 x 4.6 mm Liquid Chromatography</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml/min</td>
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<tr>
<td>Injection volume</td>
<td>10 µl</td>
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<tr>
<td>Detection wavelength</td>
<td>290 nm</td>
</tr>
<tr>
<td>Column temperature</td>
<td>40°C</td>
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</tbody>
</table>

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 grated COMID® 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) grated COMID® 50 mg tablet and (ii) mobile phase.
- Mean variables: retention time, peak area and baseline signal.
- Precision and accuracy
- 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 (R²) must be at least 0.95 for each calibration standard curve.
- No co-elution of clomifene citrate and impurities at their retention times.
- Linearly
- 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.

RESULTS

Method validation

Using this method, the retention time of clomifene citrate is 2.3 ± 0.2 min.

- No differences between (i) the pure CRS clomifene citrate chromatogram and (ii) the COMID® 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.
- Linearly
- Every calibration standard curve had a R² higher than 0.95.
- The R² 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%.

Forced degradation study

- Acidic hydrolysis (HCl 2 M for 3h)
  - Compound degraded up to 10.05%.
  - A separation phenomenon of the two clomifene citrate isomers has been noticed.
- Alkaline hydrolysis (NaOH 1 M for 3h)
  - Compound degraded up to 19.43%.
  - A separation phenomenon of the two clomifene citrate isomers has been noticed.
- Heat (80°C for 3h)
  - Compound degraded up to 1.75%.
  - The chromatogram showed 3 elution pics of impurities at 0.73 min, 0.81 min and 1.94 min.
- Photo-oxidation (365 nm for 30mins)
  - Compound degraded up to 38.71%.
  - The chromatogram showed the elution pic of an impurity at 1.94 min.
  - Oxidation (H₂O₂ 3% v/v for 24h)
  - Compound degraded up to 4.57%.
  - A separation phenomenon of the two clomifene citrate isomers has been noticed.

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 content of a clinical trial.