Testing Specifications for Release and Stability Testing

<table>
<thead>
<tr>
<th>BIWG 98 SE tablets</th>
<th>Number</th>
<th>910-A-01/03</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 mg and placebo</td>
<td>Date</td>
<td>00.00.0000</td>
</tr>
<tr>
<td></td>
<td>Page</td>
<td>1 of 27</td>
</tr>
</tbody>
</table>

Responsible:

Analytical Sciences Department
Drug Product Analysis

(Control officer/Clinical trial sample)
### Assignment of test attributes

<table>
<thead>
<tr>
<th>Test attributes</th>
<th>Release testing</th>
<th>Stability testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dimensions</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Uniformity of mass</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Average mass</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>Disintegration time</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dissolution rate</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Hardness (Resistance to crushing)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Identification</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Degradation of BIWG 98 SE</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Assay of BIWG 98 SE</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Content uniformity of BIWG 98 SE</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Assessment of packaging material</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>Microbial contamination</td>
<td>partly</td>
<td>-</td>
</tr>
</tbody>
</table>
## Test attributes and registration acceptance criteria

<table>
<thead>
<tr>
<th>Test attributes</th>
<th>Release acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Round, white to off-white tablets</td>
</tr>
</tbody>
</table>
| Dimensions                 | Diameter: about 9 mm  
|                           | height: about 3.1 mm                                                                                                                                                                                                       |
| Uniformity of mass         | Not more than 2 tablets are permitted to deviate from the average mass by more than ± 7.5% and none by more than ± 15%  
|                           | Complies with the requirements of EP                                                                                                                                                                                          |
| Loss on drying             | ≤ 4 %                                                                                                                                                                                                                     |
| Disintegration time        | $\bar{x}$, not more than 8 minutes  
|                           | (each individual value not more than 8 minutes)                                                                                                                                                                             |
| Dissolution rate           | Not less than 75 % (Q) after 30 minutes,  
|                           | complies with USP stages S1 and S2                                                                                                                                                                                          |
| Hardness                   | Not less than 40 N                                                                                                                                                                                                           |
| (Resistance to crushing)   |                                                                                                                                                                                                                             |
| Identification             | Complies with standard                                                                                                                                                                                                     |
| Placebo                    | no signal at about k’ 1.7                                                                                                                                                                                                     |
| Degradation of BIWG 98 SE  | - BIWG 98 D1 not more than 0.2 % ≡ 0.23 %  
|                           |   degraded BIWG 98 SE  
|                           | - any unspecified degradation product not more than 0.2 %                                                                                                                                                                     |
|                           | - total degradation products not more than 0.3 % ≡ 0.34 % degraded BIWG 98 SE                                                                                                                                               |
| Content uniformity BIWG 98 SE | Complies with the requirements of USP                                                                                                                                                                                            |
| Microbial contamination    | In accordance with EP, USP  
<p>|                           | The first three batches of BIWG 98 SE SD tablets are investigated, then every 10th batch                                                                                                                                               |</p>
<table>
<thead>
<tr>
<th>Test attributes</th>
<th>Shelf-life acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average mass</td>
<td>$\bar{x}_{30}$ (initial value) $\pm$ 2.5%</td>
</tr>
<tr>
<td>Disintegration time</td>
<td>$\bar{x}_6$ not more than 15 minutes (each individual value $\leq$ 15 min)</td>
</tr>
<tr>
<td>Dissolution rate</td>
<td>Not less than 70% (Q) after 30 minutes, complies with USP stages S1 and S2</td>
</tr>
<tr>
<td>Degradation of BIWG 98 SE</td>
<td>- BIWG 98 D1 not more than 1.0 % $\pm$ 1.13 %</td>
</tr>
<tr>
<td></td>
<td>- degraded BIWG 98 SE</td>
</tr>
<tr>
<td></td>
<td>- any unspecified degradation product not more than 0.2 %</td>
</tr>
<tr>
<td></td>
<td>- total degradation products not more than 1.3 % $\pm$ 1.5 %</td>
</tr>
<tr>
<td></td>
<td>- degraded BIWG 98 SE</td>
</tr>
<tr>
<td>Hardness (Resistance to crushing)</td>
<td>$\bar{x}_{10}$ not less than 25 N</td>
</tr>
<tr>
<td>Assay of BIWG 98 SE</td>
<td>37.2- 42.0 mg per tablet</td>
</tr>
<tr>
<td>Assessment of packaging material</td>
<td>Appearance, dispensing and administration/function must comply.</td>
</tr>
</tbody>
</table>
# Test attributes and their Validation parameters

<table>
<thead>
<tr>
<th>Test attribute</th>
<th>Validation parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dissolution rate</strong></td>
<td>Linearity</td>
<td>20 - 120 %</td>
</tr>
<tr>
<td></td>
<td>Accuracy</td>
<td>99.9 %</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>40 % - 100 % (Q ± 30 %)</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>RSD 0.38 %</td>
</tr>
<tr>
<td></td>
<td>Robustness</td>
<td>proven, 24 hours</td>
</tr>
<tr>
<td><strong>Identification</strong></td>
<td>Specificity</td>
<td>demonstrated separation from degradation product and artificial degradation products</td>
</tr>
<tr>
<td><strong>Degradation of BIWG 98 SE</strong></td>
<td>Specificity</td>
<td>demonstrated separation from degradation product and artificial degradation products</td>
</tr>
<tr>
<td></td>
<td>Linearity</td>
<td>0.1 - 2 %</td>
</tr>
<tr>
<td></td>
<td>Accuracy</td>
<td>98.63 %</td>
</tr>
<tr>
<td></td>
<td>BIWG 98 SE</td>
<td>99.92 %</td>
</tr>
<tr>
<td></td>
<td>Reporting threshold</td>
<td>1.6 ng ± 0.1 %</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.1 - 1 %</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>RSD: 3.13 %</td>
</tr>
<tr>
<td></td>
<td>BIWG 98 SE</td>
<td>RSD: 2.49 %</td>
</tr>
<tr>
<td></td>
<td>Robustness</td>
<td>proven, 48 hours</td>
</tr>
<tr>
<td><strong>Assay of BIWG 98 SE</strong></td>
<td>Specificity</td>
<td>demonstrated separation of known impurities and forced degradation products</td>
</tr>
<tr>
<td></td>
<td>Linearity</td>
<td>25 - 150 %</td>
</tr>
<tr>
<td></td>
<td>Accuracy</td>
<td>98.86 %</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>70 - 130 %</td>
</tr>
<tr>
<td></td>
<td>Intermediate precision</td>
<td>RSD: 0.93 %</td>
</tr>
<tr>
<td></td>
<td>Robustness</td>
<td>proven, 48 hours</td>
</tr>
<tr>
<td><strong>Uniformity of content of BIWG 98 SE</strong></td>
<td>Repeatability</td>
<td>RSD: 0.6 %</td>
</tr>
</tbody>
</table>
Analytical Procedures

Appearance

Assess by visual examination.

Stability testing:

Freshly manufactured product may be used as a reference sample.

Alternatively the appearance may be assessed by visual examination using the Taschenlexikon der Farben (Kornerup A., Wanscher J. H., Taschenlexikon der Farben, Musterschmidt Verlag Zürich, Göttingen).

Colour shade, colour intensity and depth of shade are reported.

Dimensions

Determine on 10 tablets.

Uniformity of mass

Weigh 20 tablets separately. Determine the average mass from the sum of these values and compare the individual values with the average.
Loss on drying

Determine in a shallow glass weighing dish 2.0 g of grounded tablets which are dried at 105°C for 3 hours and accurately weighed.

Average mass

Determine on 20 tablets.

Disintegration time

Determine the disintegration time in minutes of 6 tablets using the EP/USP disintegration testing apparatus with discs.

Immersion fluid: Distilled water at 37°C ± 1°C
Calculate the mean and the RSD.

Release: minimum, maximum, $\bar{x}_6$, RSD

Stability: $\bar{x}_6$, RSD
Dissolution rate

Method: UV spectrophotometry
Apparatus: EP/USP paddle method

Solvent and reagents

- BIWG 98 SE reference substance
- Methanol
- 0.1 M NaOH
- Dissolution medium: phosphate buffer solution pH 7.5:

  In a 1000 ml volumetric flask dissolve 13.61 g monobasic potassium phosphate KH₂PO₄ in 800 ml water, adjust to pH 7.5 with 2 M NaOH solution and make up to the mark with water.
Dissolution rate

Procedure

Test solution: Place 900 ml of the dissolution medium in a test vessel and then place one tablet in the vessel.

Operate the apparatus at 75 rpm.

The samples are withdrawn after 30 minutes through an immersion filter (glass frit G 2). Finally they are filtered through a suitable membrane filter e.g. polyamide 0.45 µm. Discard the first 4.0 ml of the filtrate.

Dilution: 1 : 4 with buffer pH 7.5

Standard solution: Accurately weigh about 44.4 mg BIWG 98 SE reference substance into a 100 ml volumetric flask, add 1 ml 0.1 M NaOH, dissolve in methanol and make up to the mark with methanol.

Dilution: 10 : 100 with buffer pH 7.5

Further dilution in analogy to the test solution (1 : 4 with buffer pH 7.5)

Measurement: With the aid of a suitable spectrophotometer, record the test solution and standard solution in 1 cm cells against phosphate buffer pH 7.5.

Measure the absorbance at the wavelength of maximum absorption 298 nm.
Dissolution rate

Evaluation

Calculation

Dissolution of BIWG 98 SE [%]

\[
\frac{WtRS \times AT \times F}{AS \times SC \times 100}
\]

Report the mean value of the corresponding step and the relative standard deviation RSD.

\[
AT = \text{Absorbance of test solution}
\]
\[
AS = \text{Absorbance of standard solution}
\]
\[
WtRS = \text{weight of reference substance used to prepare the standard solution [mg]}
\]
\[
SC = \text{Stated active ingredient of test sample [mg per tablet]}
\]
\[
F = \text{Active ingredient content of BIWG 98 SE reference substance used [%]}
\]

Requirements: USP and Europe

Stage 1 (n = 6): Each individual value ≥ Q + 5 %

Stage 2 (n = 12): Mean of 12 ≥ Q; no tablet < Q - 15 %

The dissolution rate can be determined also with an automated equipment SOTAX AT 700
Dissolution rate

Calibration curve of BIWG 98 SE

**ABS.**

**CONCENTRATION [ug/ml]**

**MEAN**

\[0.050337 \times X - 2.051763 \times 10^{-3}\]

**CORRELATION COEFFICIENT R = 0.999994**
Hardness (Resistance to crushing)

Determine the resistance to crushing on 10 tablets with the aid of a suitable apparatus. Record the results in Newtons (N).

Recorded data for release:
- minimum, maximum
- mean value $\bar{x}$
- relative standard deviation (RSD)

(the individual values must comply with the specification)

Recorded data for stability testing:
- mean value $\bar{x}$
- relative standard deviation (RSD)

(the mean value must comply with the specification)
Identification

Method: HPLC determination

The identification is performed together with the BIWG 98 SE assay. Identification is assured, if both the retention time and the k' value of the test solution correspond to those of the standard solution.

An UV-spectrum of the BIWG 98 SE peak recorded with the aid of a diode array detector must comply with the UV-spectrum of the standard.
Degradation and assay of BIWG 98 SE

Method: HPLC determination

Solvents and reagents

- BIWG 98 SE reference substance
- BIWG 98 D1 reference substance
- Methanol (HPLC grade)
- Solvent: Methanol
- Buffer solution:
  Dissolve 2.0 g Ammonium dihydrogen phosphate in 1 litre of water and adjust to pH 3.0 with diluted phosphoric acid
- Eluent solution:
  Methanol : Buffer solution pH 3.0
  650 / 350 (V/V)
- Column:
  Nucleosil 100 C18, particle size 5 µm, length 4.0 cm, internal diameter 4 mm
Degradation and assay of BIWG 98 SE

Procedure

Test solution: Place 4 tablets in a 100 ml volumetric flask, add about 80 ml solvent and ultrasonicate for 10 minutes. Make the flask up to the mark with the solvent at room temperature. Dilute with the eluent in the ratio 1 : 15.

Standard solution 1: Accurately weigh about 40.0 mg BIWG 98 SE reference substance and about 5 mg BIWG 98 D1 reference substance into a 50 ml volumetric flask, dissolve with solvent and make up to the mark with the same solvent. Dilute with eluent in the ratio 2 : 15.

Standard solution 2: Dilute standard solution 1 with eluent in the ratio 1 : 10.

Standard solution 3: Dilute standard solution 2 with eluent in the ratio 1 : 10.

About 1.5 ml of the test- and the standard solutions are filtered through a suitable membrane filter (e.g. polyamide 0.45 µm) into a sample vial.

Inject 5 µl of the test- and standard solution 1 equivalent to 0.53 µg BIWG 98 SE for assay and 15 µl of the test solution and standard solution 2 equivalent to 1.6 µg BIWG 98 SE and 0.016 µg BIWG 98 D1 for degradation into a suitable liquid chromatograph (for example a Hewlett Packard 1100 M with DAD).

Chromatographic conditions:

- Injection volume:
  - for assay: 5 µl
  - for degradation: 15 µl

- Flow rate: 0.7 ml/min

- Wavelength: 298 nm

- Column temperature: 40°C
Degradation and assay of BIWG 98 SE

Procedure

<table>
<thead>
<tr>
<th>Retention data:</th>
<th>Substance</th>
<th>t_R</th>
<th>k'</th>
<th>A_s</th>
<th>R_s</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIWG 98 D1</td>
<td>0.74 min</td>
<td>0.48</td>
<td>1.9</td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td>BIWG 98 SE</td>
<td>1.4 min</td>
<td>1.7</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

System suitability:

Before starting the sample sequence

• 5 ml of standard solution 1 are injected 6 times to verify the chromatographic system,

• 15 µl of standard solution 3 are injected once to verify the reporting limit

System suitability is assured under the following conditions:

• the k’ range of the relevant peak remains approximately constant (if necessary, after correction of the elutionic strength) and Rs ≥ 1.5, elution order unchanged

• the asymmetry factor remains within a range allowing quantification (0.5 < A_s < 2.0)

Confirmation of accuracy of injection:

• the relative standard deviation of the peak area obtained from six injections of the standard solution is < 2 %.

According to chromatographic theory, no significant influence on precision and accuracy is to be expected during quantitative determination under these conditions.

The reporting limit is assured under the following conditions:

• Integration is possible according to visual verifications.
Degradation and assay of BIWG 98 SE

Evaluation

BIWG 98 SE content (mg per tablet)

\[
\frac{\text{WtRS}_1 \times \text{IUTS} \times F_1}{\text{IUSS} \times 100}
\]

Degradation product BIWG 98 D1 [%]

\[
\frac{\text{WtRS}_2 \times \text{IUDTS} \times F_2}{\text{IUDSS} \times 100 \times SC} \times 10
\]

% degraded BIWG 98 SE

Ratio of relative molecular mass:

\[
\frac{\text{BIWG 98 SE}}{\text{BIWG 98 D1}} = \frac{545}{481} = 1.13
\]

% BIWG 98 D1 x 1.13

\[
\text{WtRS}_1 = \text{Weight of BIWG 98 SE used to prepare standard solution 1 [mg]}
\]
\[
\text{IUTS} = \text{Integrator units of BIWG 98 SE in test solution}
\]
\[
F_1 = \text{Active ingredient content of BIWG 98 SE reference substance used [%]}
\]
\[
\text{IUSS} = \text{Integrator units of BIWG 98 SE in standard solution 1}
\]
\[
\text{WtRS}_2 = \text{Weight of BIWG 98 D1 used to prepare standard solution 1 and 2 [mg]}
\]
\[
F_2 = \text{Degradation content of BIWG 98 D1 reference substance used [%]}
\]
\[
\text{IUDTS} = \text{Integrator units of BIWG 98.D1 in test solution}
\]
\[
\text{IUDSS} = \text{Integrator units of BIWG 98 D1 in standard solution 2}
\]
\[
SC = \text{Stated content of drug substance [mg per tablet]}
\]
Degradation and assay of BIWG 98 SE

Calibration curve of BIWG 98 SE (range 1.6 - 30 ng)

\[ + = \bar{x}_3 \]
\[ y = 2.49 x + 0.19 \]
Correlation coefficient: 0.99997
Degradation and assay of BIWG 98 SE

Calibration curve of BIWG 98 D1 (range 1.6 - 30 ng)

\[ y = 2.39x - 0.23 \]

Correlation coefficient: 0.99996

\[ + = \bar{x}_3 \]
Degradation and assay of BIWG 98 SE

Calibration curve of BIWG 98 SE (range 0.13 - 1 µg)

\[ y = 2443.74x + 20.43 \]

Correlation coefficient: 0.99976
Degrudation and assay of BIWG 98 SE

Calibration curve of BIWG 98 SE with placebo (range 0.13 – 1 µg)

\[ + = \bar{x}_3 \]

\[ y = 2432.92 \times + 6.16 \]

Correlation coefficient: 0.9996
Chromatogram of drug substance and degradation product

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BIWG 98 D1</td>
</tr>
<tr>
<td>2</td>
<td>BIWG 98 SE</td>
</tr>
</tbody>
</table>

**Chromatographic Conditions**

**Eluent solution**
- Methanol: (65 Vol %)
- Buffer solution: (35 Vol%)

**Column**
- Material: Nucleosil 100, C 18, 5 µm
- Length: 4.0 cm
- Diameter: 4.0 mm

**Flow rate**: 0.7 ml/min

**Column temperature**: 40°C

**Wavelength**: 298 nm
Degradation and assay of BIWG 98 SE

UV spectrum to derive the wavelength 298 nm

Chromatogram: assay of BIWG 98 SE, 0.53 µg

Chromatogram: degradation of BIWG 98 SE, 1.6 ng
Content uniformity of BIWG 98 SE

Method: HPLC determination, corresponding assay

Test solution: Place 1 tablet in a 50 ml volumetric flask, add about 40 ml solvent and ultrasonicate for 10 minutes. Make the flask up to the mark with the solvent at room temperature. Then dilute with eluent 1 : 10.

Standard solution: Accurately weigh about 40 mg BIWG 98 SE reference substance into a 50 ml volumetric flask, dissolve with solvent and make up to the mark with the same solvent. Then dilute with eluent 1 : 10.

Continue corresponding assay procedure.

Evaluation

\[
\text{mg BIWG 98 SE / tablet} = \frac{\text{WtRS} \times \text{IUTS} \times F}{\text{IUSS} \times 100}
\]

- IUTS = Integrator units of BIWG 98 SE in test solution
- IUSS = Integrator units of BIWG 98 SE in standard solution
- WtRS = Weight of BIWG 98 SE used to prepare standard solution [mg]
- F = Active ingredient content of BIWG 98 SE reference substance used [%]
Uniformity of content of BIWG 98 SE

Requirements

**EP:**
A (n = 10): All tablets are within 85 – 115 % of average content.

B (n = 30): 1 of 30 tablets is outside 85 – 115 %, no tablet is outside 75 – 125 % of average content.

**USP:**
A (n = 10): All tablets are within 85 – 115 % of label claim and RSD ≤ 6.0 %.

B (n = 30): 1 of 30 tablets is outside 85 – 115 %, no tablet is outside 75 – 125 % of label claim and RSD ≤ 7.8 %.

The assay of BIWG 98 SE is determined with the data of content uniformity if the mean \( \bar{x} \) is within release specification 38.0 – 42.0 mg. Otherwise it is determined separately.
Assessment of packaging material

Inspect for the presence of any visually detectable changes and check that the functional characteristics are unimpaired.

Microbial contamination

Test in conformity with the current Ph. Eur., USP.
<table>
<thead>
<tr>
<th>Structural formulae</th>
</tr>
</thead>
</table>

BIWG 98 SE

BIWG 98 D1