Testing Specifications for Release and Stability Testing	
BIWG 98 SE tablets **40 mg and placebo**	Number **910-A-01/03** Date **00. 00. 0000** Page **1 of 27**

Responsible:

Analytical Sciences Department
Drug Product Analysis

(Control officer/Clinical trial sample)

Assignment of test attributes

Test attributes	Release testing	Stability testing
Appearance	X	X
Dimensions	X	-
Uniformity of mass	X	-
Loss on drying	X	-
Average mass	-	X
Disintegration time	X	X
Dissolution rate	X	X
Hardness (Resistance to crushing)	X	X
Identification	X	-
Degradation of BIWG 98 SE	X	X
Assay of BIWG 98 SE	X	X
Content uniformity of BIWG 98 SE	X	-
Assessment of packaging material	-	X
Microbial contamination	partly	-

Test attributes and registration acceptance criteria

Test attributes	**Release acceptance criteria**
Appearance	Round, white to off-white tablets
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	$\leq$ 4 %
Disintegration time	$\overline{x}_6$ 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 (Resistance to crushing)	Not less than 40 N
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 38.0 - 42.0 mg per tablet
Content uniformity BIWG 98 SE	Complies with the requirements of USP
Microbial contamination	In accordance with EP, USP The first three batches of BIWG 98 SE SD tablets are investigated, then every 10th batch

Test attributes	**Shelf-life acceptance criteria**
Average mass	$\bar{x}_{20}$ (initial value) + 2.5 %
Disintegration time	$\bar{x}_6$ not more than 15 minutes (each individual value $\leq$ 15 min)
Dissolution rate	Not less than 70 % (Q) after 30 minutes, complies with USP stages S1 and S2
Degradation of BIWG 98 SE	- BIWG 98 D1 not more than 1.0 % $\triangleq$ 1.13 % degraded BIWG 98 SE - any unspecified degradation product not more than 0.2 % - total degradation products not more than 1.3 % $\triangleq$ 1.5 % degraded BIWG 98 SE
Hardness (Resistance to crushing)	$\bar{x}_{10}$ not less than 25 N
Assay of BIWG 98 SE	37.2- 42.0 mg per tablet
Assessment of packaging material	Appearance, dispensing and administration/function must comply.

Test attributes and their Validation parameters

Dissolution rate	Linearity	20 - 120 %
	Accuracy	99.9 %
	Range	40 % - 100 % (Q $\pm$ 30 %)
	Repeatability	RSD 0.38 %
	Robustness	proven, 24 hours
Identification	Specificity	demonstrated separation from degradation product and artificial degradation products
Degradation of BIWG 98 SE	Specificity	demonstrated separation from degradation product and artificial degradation products
	Linearity	0.1 - 2 %
	Reporting threshold	1.6 ng $\triangleq$ 0.1 %
	Accuracy BIWG 98 SE BIWG 98 D1	 98.63 % 99.92 %
	Range	0.1 - 1 %
	Repeatability BIWG 98 SE BIWG 98 D1	 RSD: 3.13 % RSD: 2.49 %
	Robustness	proven, 48 hours
Assay of BIWG 98 SE	Specificity	demonstrated separation of known impurities and forced degradation products
	Linearity	25 - 150 %
	Accuracy	98.86 %
	Range	70 - 130 %
	Intermediate precision	RSD: 0.93 %
	Robustness	proven, 48 hours
Uniformity of content of BIWG 98 SE	Repeatability	RSD: 0.6 %

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 shellow 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 $\pm$ 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 KH_2PO_4 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	=	Absorbance of test solution
AS	=	Absorbance of standard solution
WtRS	=	weight of reference substance used to prepare the standard solution [mg]
SC	=	Stated active ingredient of test sample [mg per tablet]
F	=	Active ingredient content of BIWG 98 SE reference substance used [%]

Requirements: USP and Europe

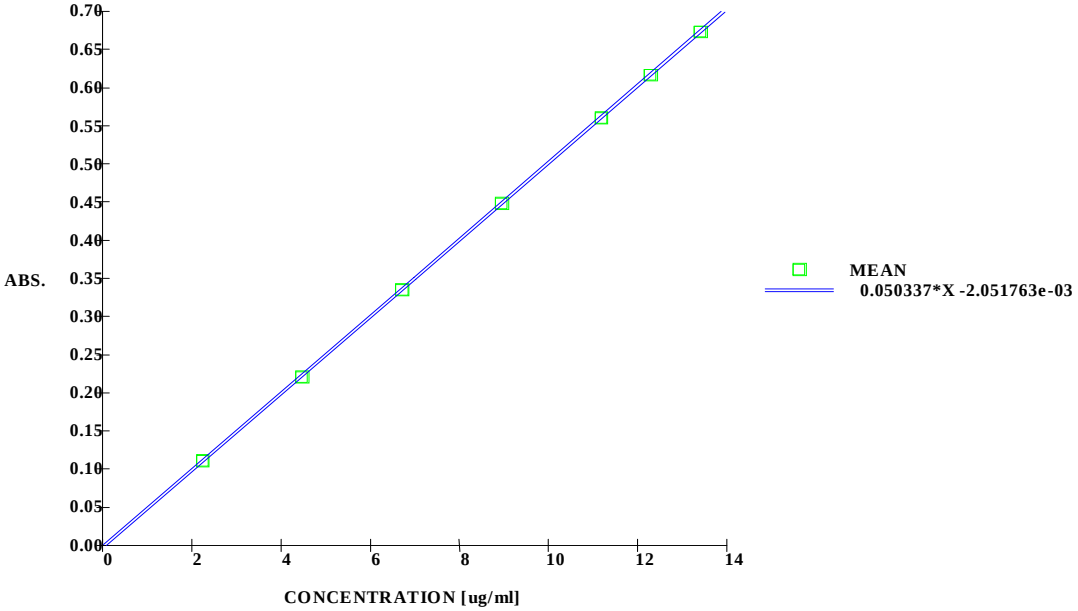
Stage 1 (n = 6): Each individual value $\geq$ Q + 5 %

Stage 2 (n = 12): Mean of 12 $\geq$ 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



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

Retention data:

Substance	t_R	k'	A_s	R_s
BIWG 98 D1	0.74 min	0.48	1.9	3.9
BIWG 98 SE	1.4 min	1.7	1.3	

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 $\geq$ 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)

$$\frac{\text{WtRS1 x IUTS x } F_1}{\text{IUSS x 100}}$$

Degradation product BIWG 98 D1 [%]

$$\frac{\text{WtRS2 x IUDTS x } F_2}{\text{IUDSS x 100 x SC}} \text{ x 10}$$

% degraded BIWG 98 SE

Ratio of relative molecular mass:

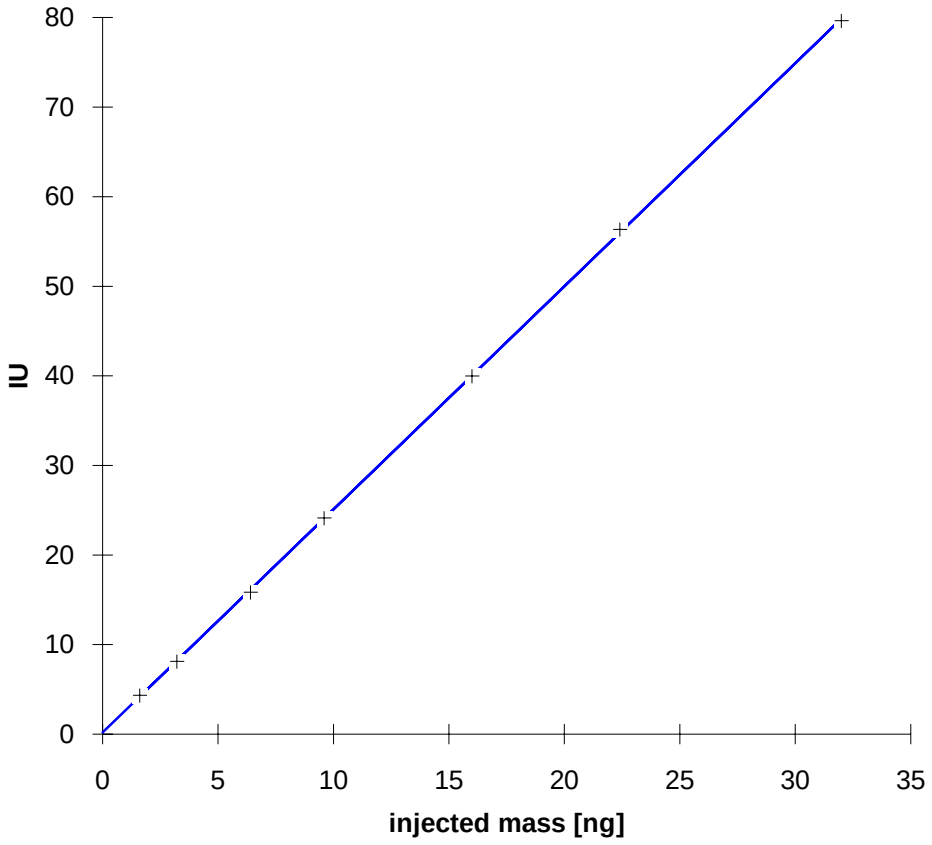
$$\frac{\text{BIWG 98 SE}}{\text{BIWG 98 D1}} = \frac{545}{481} = 1.13$$

% BIWG 98 D1 x 1.13

WtRS1	=	Weight of BIWG 98 SE used to prepare standard solution 1 [mg]
IUTS	=	Integrator units of BIWG 98 SE in test solution
F_1	=	Active ingredient content of BIWG 98 SE reference substance used [%]
IUSS	=	Integrator units of BIWG 98 SE in standard solution 1
WtRS2	=	Weight of BIWG 98 D1 used to prepare standard solution 1 and 2 [mg]
F_2	=	Degradation content of BIWG 98 D1 reference substance used [%]
IUDTS	=	Integrator units of BIWG 98.D1 in test solution
IUDSS	=	Integrator units of BIWG 98 D1 in standard solution 2
SC	=	Stated content of drug substance [mg per tablet]

Degradation and assay of BIWG 98 SE

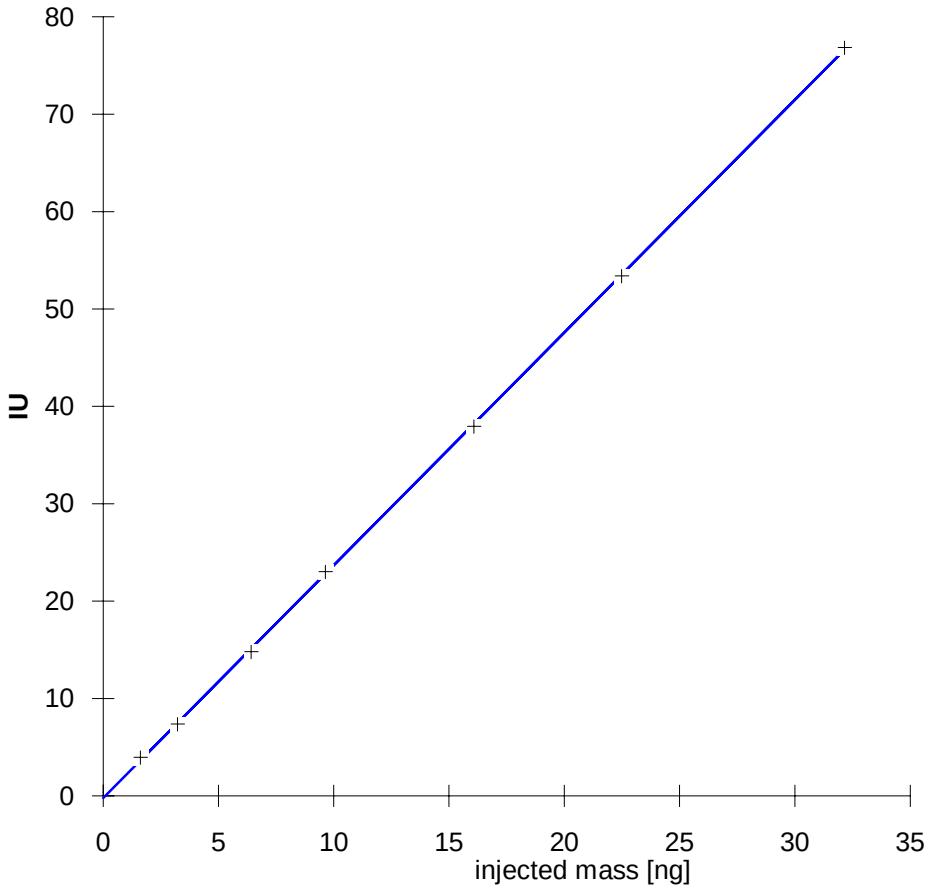
Calibration curve of BIWG 98 SE (range 1.6 - 30 ng)



\+ = $\bar{x}_3$
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)



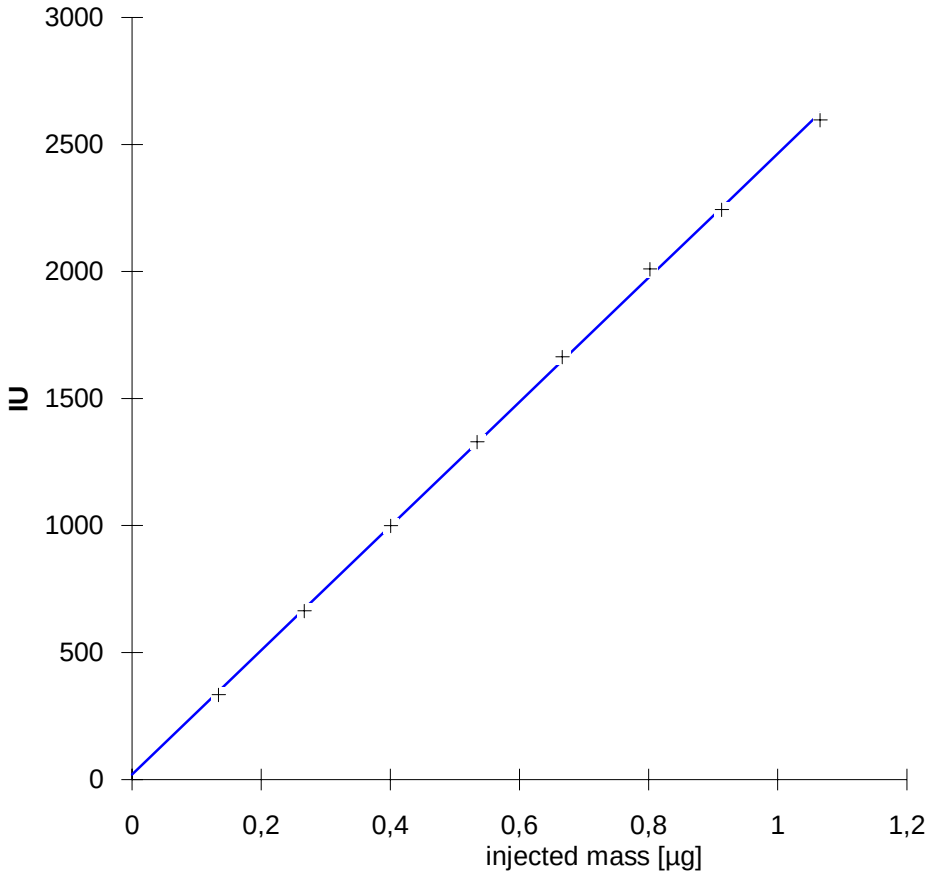
\+ = $\bar{x}_3$

y = 2.39 x - 0.23

Correlation coefficient: 0.99996

Degradation and assay of BIWG 98 SE

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



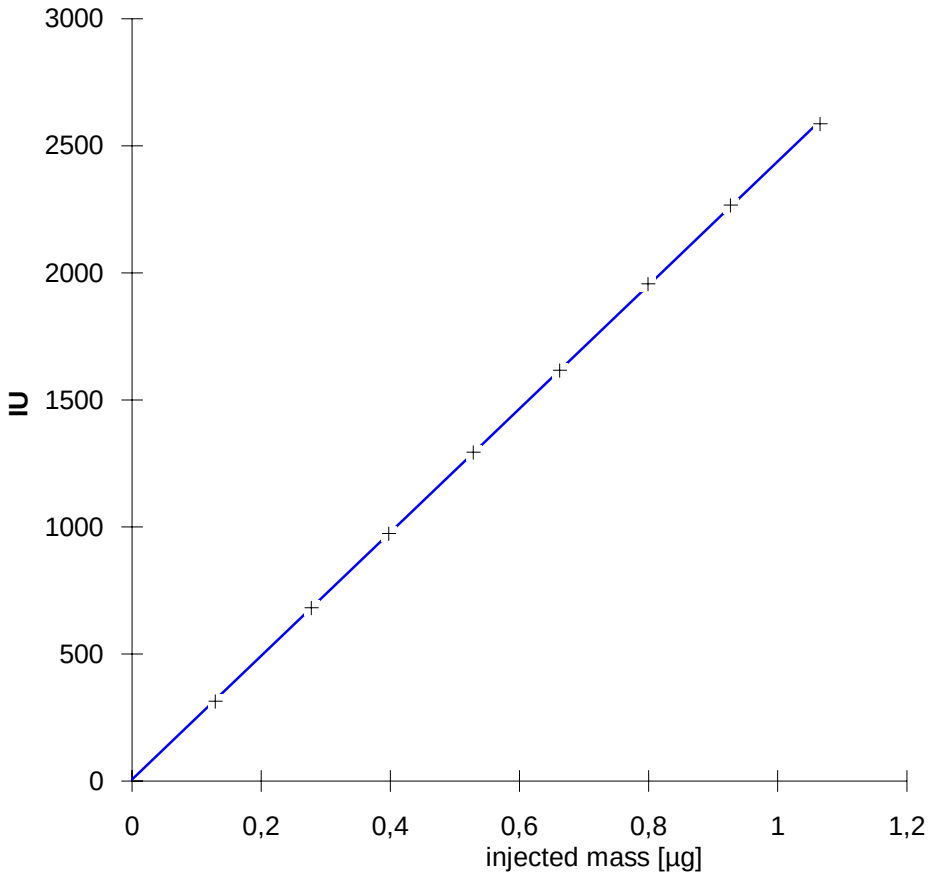
\+ = $\bar{x}_3$

y = 2443.74 x + 20.43

Correlation coefficient: 0.99976

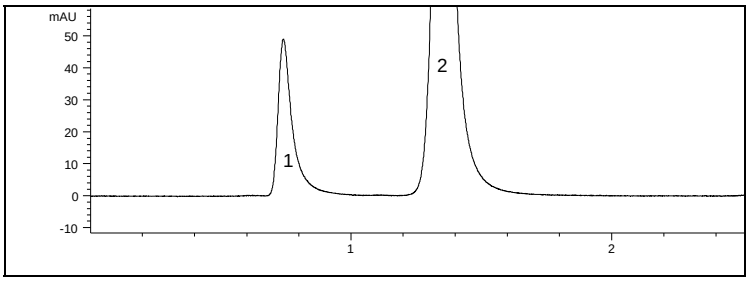
Degradation and assay of BIWG 98 SE

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



\+ = $\bar{x}_3$
y = 2432.92 x + 6.16
Correlation coefficient: 0.9996

Chromatogram of drug substance and degradation product



Peak No	Substance
1	BIWG 98 D1
2	BIWG 98 SE

Chromatographic Conditions

Eluent solution

Methanol	(65 Vol %)
Buffer solution	(35 Vol%)

Column

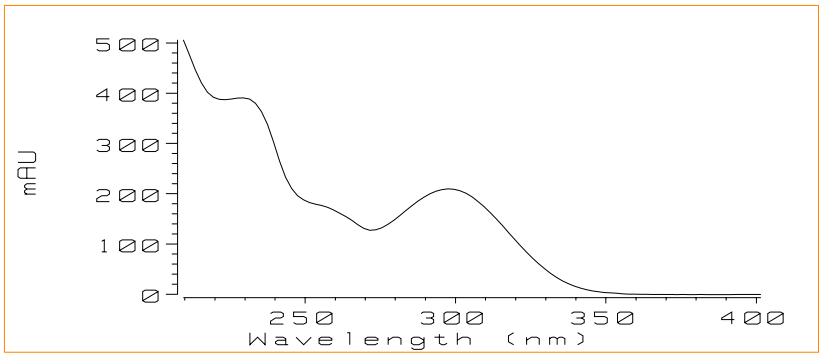
Material : Nucleosil 100, C 18, 5 µm
Length : 4.0 cm
Diameter : 4.0 mm

Flow rate **:** 0.7 ml/min

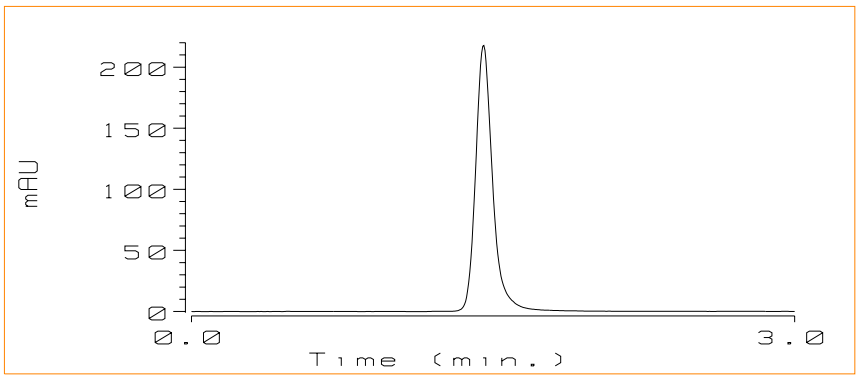
Column temperature **:** 40°C

Wavelength **:** 298 nm

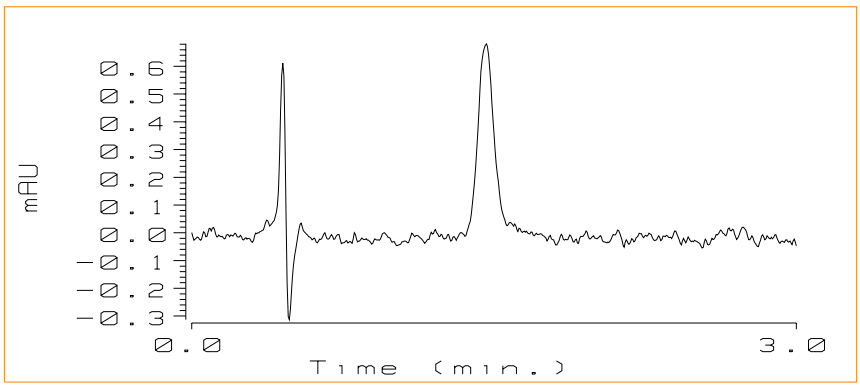
Degradation and assay of BIWG 98 SE



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

mg BIWG 98 SE / tablet

$$\frac{WtRS \times IUTS \times F}{IUSS \times 100}$$

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.

Structural formulae

BIWG 98 SE

BIWG 98 D1