Responsible:

Analytical Sciences Department
Drug Product Analysis

(Control officer/Clinical trial sample)

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Introduction

The validation report describes the validation procedure for the Testing Specifications for Release and Stability Testing of BIWG 98 SE tablets 40 mg and placebo No. 910-A-01/03 dated 00. 00. 0000

The validation has been performed according to:

ICH Harmonised Tripartite Guideline

- Validation of Analytical Methods, Definitions and Terminology ICH Q2A
- Validation of Analytical Procedures and Methodology, ICH Q2B

Test Attributes and their Validation Parameters

Validation parameters	Test attributes				
	Dissolution rate	**Identification**	**Degradation of BIWG 98 SE**	**Assay of BIWG 98 SE**	**Content uniformity**
Specificity		X	X	X	X
Linearity	X		X	X	X
Reporting Threshold			X		
Accuracy	X		X	X	X
Range	X		X	X	X
Repeatability	X		X		X
Intermediate Precision				X	
Robustness	X		X	X	X

Summary of Validation results

Dissolution rate	Linearity	20 - 120 %
	Accuracy	99.9 %
	Range	40 % - 100 % (Q ± 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 ≙ 0.1 %
	Accuracy	
	BIWG 98 SE	98.63 %
	BIWG 98 D1	99.92 %
	Range	0.1 - 1 %
	Repeatability	
	BIWG 98 SE	RSD: 3.13 %
	BIWG 98 D1	RSD:2.49 %
	Robustness	proven, 48 hours
Assay of BIWG 98 SE	Specificity	demonstrated separation from degradation product and artificial 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 %

Documentation of Validation

Test attributes and their Validation parameters		Drug substance	40 mg tablets	placebo	Technician
Dissolution rate	Linearity				
	Accuracy				
	Range				
	Repeatability				
	Robustness				
Identification	Specificity BIWG 98 SE BIWG 98 D1				
Degradation of BIWG 98 SE	Specificity				
	Linearity BIWG 98 SE BIWG 98 D1				
	Reporting threshold BIWG 98 SE BIWG 98 D1				
	Accuracy BIWG 98 SE BIWG 98 D1				
	Range				
	Repeatability BIWG 98 SE BIWG 98 D1				
	Robustness				
Assay of BIWG 98 SE	Linearity				
	Accuracy				
	Range				
	Intermediate precision				
	Robustness				
Content uniformity	Repeatability				

Documentation: Corresponding BIWG 98 SE – Laboratory A 1, AZ

Validation Test attributes

1. Dissolution rate

The following validation parameters are included:

Linearity, accuracy, range, repeatability, robustness.

Linearity

The linearity of the drug substance BIWG 98 SE was investigated in the range of 20 to 120 % by dilution of a standard stock solution. Each concentration was measured tree times. The mean out of three was used for linearity testing as shown in figure 4.

The data are summarised the following table:

Concentration [µg/ml]	Absorption x_1	x_2	x_3	$\tilde{x}$	RSD [%]	Theoretical value	deviation [%]
2.234	0.111	0.111	0.112	0.11	0.52	0.11	0.0
4.468	0.221	0.221	0.223	0.22	0.52	0.22	0.0
6.702	0.335	0.335	0.336	0.34	0.17	0.34	0.0
8.936	0.447	0.448	0.449	0.45	0.22	0.45	0.0
11.170	0.560	0.560	0.560	0.56	0.00	0.56	0.0
12.287	0.614	0.616	0.618	0.62	0.32	0.62	0.0
13.404	0.673	0.673	0.674	0.67	0.09	0.67	0.0

Equation: $y = 0.050337 x - 0.002051763$
Correlation coefficient: 0.999994

The calibration curve is linear. The regression line is not significantly different from a curve passing through the origin $y = ax$. The analysis can be performed using a test sample and a reference substance.

Accuracy

To verify the accuracy of the procedure the recovery was determined in the range of 40 % (Q – 30 %), 70 % (Q), 100 % (Q + 30 %) of the stated content by adding 16 mg, 28 mg, 40 mg BIWG 98 SE to corresponding placebo tablets. For further procedure as under dissolution. The determination was performed three times with 3 measurements.

Table of recovery:

Content adjusted		Content determined				% of	RSD	Deviation
mg/tablet	% stated content	X_1	X_2	X_3	$\tilde{x}_3$	adjusted content x_3	tion [%]	[%]
16.14	40.35	16.04	16.20	16.16	16.13	99.96	0.52	- 0.04
16.31	40.78	16.28	16.57	16.38	16.41	100.06	0.89	+ 0.06
15.92	39.80	16.09	16.01	15.95	16.02	100.60	0.44	+ 0.60
28.30	70.75	28.25	28.31	28.15	28.24	99.78	0.29	- 0.22
27.14	67.85	27.14	27.19	27.06	27.13	99.96	0.24	- 0.04
27.73	69.33	27.50	27.60	26.45	27.52	99.24	0.28	- 0.76
39.55	98.88	39.81	39.64	39.45	39.63	100.21	0.45	+ 0.21
39.73	99.33	39.50	39.65	39.85	39.67	99.84	0.44	– 0.16
40.12	100.30	40.04	40.24	40.14	40.14	100.05	0.25	+ 0.05
						99.97	0.42	-0.30

The recovery of 9 determinations in the range of 40 % to 100 % is 99.97 % thereby the accuracy is verified.

Range

Together with the accuracy the range was validated for 40 to100 %.

Repeatability

The repeatability is derived from the 9 determinations of the accuracy.

Total mean: $\bar{x}_9$ 99.9 %
RSD repeatability: 0.42 %

Confidence interval of the mean at the 95 % level is $\pm$ 0.42 % for all values between 40 % (Q - 30 %) and 100 % (Q + 30 %) for six samples.

Robustness

Stability of solution:

The test and standard solutions were kept for 24 hours at room temperature without undergoing any changes.

Standard and test solution after preparation				Standard and test solution after storage for 24 hours			
Standard solution		dissolution $\bar{x}_6$	RSD	Standard solution		dissolution $\bar{x}_6$	RSD
absorption	[%]	[%]	[%]	absorption	[%]	[%]	[%]
0.526	101.6	80.3	6.61	0.526	101.6	80.6	6.59

There is no difference between the data after preparation and after storage for 24 hours.

2. Identification

The following validation parameter is included:

Specificity

The HPLC method of assay is applied. The method separates the drug substance BIWG 98 SE, the degradation product BIWG 98 D1 and the two artificial degradation products BIWG 98 O and BIWG 98 L. Thereby the specificity is confirmed, BIWG 98 SE can be determined unambiguously. See figure 1 HPLC chromatogram

Furthermore the BIWG 98 SE peak was investigated for peak purity. The three UV spectra half peak heights and maximum are superimposable.

Thereby the peak purity is verified (see figure 2).
298 nm has bee selected as the wavelength (see figure 3).

3. Degradation of BIWG 98 SE

The following validation parameters are included:

Specificity, linearity of BIWG 98 SE, BIWG 98 D1, reporting limit of BIWG 98 SE, BIWG 98 D1, accuracy of BIWG 98 SE, BIWG 98 D1, range, repeatability of BIWG 98 SE, BIWG 98 D1, robustness.

Specificity

The applied HPLC method differentiates between the drug substance BIWG 98 SE, the degradation products BIWG 98 D1 and the two artificial degradations products BIWG 98 O (oxidation), BIWG 98 L (light) (see figure 1).

The chromatographic data are as follows:

Substance	retention time [min]	k'	As	Rs
BIWG 98 D1	0.7	0.5	1.6	
				3.9
BIWG 98 SE	1.4	1.7	1.2	
				5.4
BIWG 98 O	2.7	4.3	1.2	
				4.8
BIWG 98 L	4.4	7.7	1.0	

With a resolution of > 1.5 the separation of the adjustment peaks is successful. Thereby the selectivity is verified.

Linearity

The linearity was investigated with the drug substance BIWG 98 SE and the degradation product BIWG 98 D1 in the range of 1.6 – 30 ng $\hat{=}$ 0.1 – 2 %. A stock solution of drug substance and degradation product was diluted accordingly. Each mass was injected and measured three times. The mean out of three was used for linearity testing. (See figures 5, 6)

The data are summarised in the two following tables.

BIWG 98 SE drug substance

Injected mass [ng]	Integration units x_1	x_2	x_3	$\bar{x}_3$	RSD [%]	Theoretical value	deviation [%]
1.60	4.30	4.04	4.69	4.34	7.53	4.17	+ 4.1
3.20	8.27	8.10	7.99	8.12	1.74	8.16	- 0.5
6.40	15.81	16.03	15.75	15.86	0.93	15.96	- 0.6
9.60	23.99	24.25	24.12	24.12	0.54	24.09	+ 0.1
16.00	40.10	39.96	39.98	40.01	0.19	40.03	- 0.1
22.40	56.66	55.92	56.47	56.35	0.68	55.97	+ 0.7
32.00	79.54	79.98	79.52	79.68	0.33	79.87	- 0.2

Equation: $y = 2.49 x + 0.19$
Correlation coefficient: 0.99997

BIWG 98 D 1 degradation product

Injected mass [ng]	Integration units x_1	x_2	x_3	$\bar{x}_3$	RSD [%]	Theoretical value [IU]	deviation [%]
1.61	4.64	4.87	4.04	4.52	9.49	4.19	+ 7.8
3.21	7.68	8.99	8.84	8.50	8.43	8.62	- 1.4
6.43	17.96	16.95	16.74	17.22	3.79	17.54	- 1.8
9.64	26.40	26.85	26.74	26.67	0.90	26.44	+ 0.9
16.07	44.10	43.67	44.37	44.05	0.80	44.25	- 0.5
22.50	62.08	60.56	61.13	61.92	2.09	62.06	- 0.2
32.14	88.84	89.42	89.09	89.12	0.33	88.76	+ 0.4

Equation: $y = 2.77 x - 0.267$
Correlation coefficient: 0.99996

Both calibration curves are linear. The regression lines are not significantly different from a curve passing through the origin $y = ax$. The analysis can be performed using a test sample and a reference substance.

Reporting threshold

The reporting threshold is fixed according to the ICH guideline: Impurities in new drug products. The reporting limit is 0.1 % at maximum daily dose ≤ 1 g.

Therefore:
Reporing threshold BIWG 98 SE: 1.6 ng ≙ 0.1 % BIWG 98 SE
BIWG 98 D1: 1.6 ng

See regression lines figures 5, 6.
See figure 7 chromatogram at reporting limit

Accuracy

To verify the accuracy of the procedure the recovery was determined in the range of 0.1 %, 0.2 % 1 % of degradation by adding solution with the corresponding masses to a solution of a placebo tablet. For further procedure as under degradation of BIWG 98 SE. The determination was performed three times with 3 injections and measurements.

Table of recovery of BIWG 98 SE

Amount adjusted added (ng)	Degra-dation [%]	Amount determined ng X_1	X_2	X_3	$\bar{x}_3$	% of adjusted content $\bar{x}_3$	RSD [%]	Devia-tion [%]
1.61	0.1	1.52	1.49	1.69	1.57	97.52	6.88	- 2.48
1.64	0.1	1.72	1.51	1.69	1.64	100.0	6.93	0.0
1.62	0.1	1.48	1.69	1.51	1.56	96.30	7.28	- 3.70
3.20	0.2	3.24	3.31	3.12	3.22	100.73	2.98	+ 0.73
3.23	0.2	3.10	3.07	2.93	3.03	93.91	2.99	- 6.19
3.21	0.2	3.07	2.98	3.18	3.08	95.85	3.25	- 4.15
16.1	1.0	17.30	15.80	16.90	16.67	103.52	4.66	+ 3.52
16.4	1.0	17.10	15.90	15.30	16.10	98.17	5.69	- 1.83
16.2	1.0	15.90	16.50	17.00	16.47	101.65	3.34	+ 1.65
$\bar{x}$						98.63	3.13	- 1.37

Table of recovery of BIWG 98 D1

Amount adjusted added (ng)	Degradation [%]	Amount determined ng X_1	X_2	X_3	$\bar{x}_3$	% of adjusted content $\bar{x}_3$	RSD [%]	Deviation [%]
1.62	0.1	1.66	1.47	1.54	1.56	96.29	6.17	- 3.70
1.60	0.1	1.43	1.69	1.57	1.56	97.70	8.32	- 2.30
1.58	0.1	1.59	1.71	1.58	1.63	102.95	4.45	+ 2.95
3.24	0.2	3.18	3.42	3.31	3.30	101.95	3.64	+ 1.95
3.20	0.2	3.41	3.24	3.31	3.32	103.75	2.57	+ 3.75
3.16	0.2	3.01	3.32	3.14	3.16	100.00	4.93	0.00
16.2	1.0	16.40	15.90	16.13	16.14	99.65	1.55	- 0.35
16.0	1.0	16.32	16.61	16.12	16.35	102.19	1.51	+ 2.19
15.8	1.0	16.14	15.62	16.05	15.94	100.86	1.74	+ 0.86
$\bar{x}$						100.59	2.44	+ 5.35

The recovery of 9 determinations in the range of 0.1 % to 1.0 % degradation is 98.63 % for BIWG 98 SE and 100.59 % for BIWG 98 D1. Thereby the accuracy of both is verified.

Range

Together with the accuracy the range was validated for 0.1 % - 1.0 % degradation.

Repeatability

The repeatability is derived from the 9 determinations of the accuracy.

BIWG 98 SE
Total mean: $\bar{x}_9$ 98.63 %
RSD repeatability: 3.13 %

BIWG 98 D1
Total mean: $\bar{x}_9$ 100.59 %
RSD repeatability: 2.44 %

Robustness

The robustness has been investigated by determination of degradation immediately after sample preparation and after storage for 48 hours at room temperature (23°C).

% degradation after sample preparation						% degradation after storage for 48 hours				
Prep. No.	Content [%]				RSD [%]	Content [%]				RSD [%]
	x_1	x_2	x_3	$\bar{x}_3$		x_1	x_2	x_3	$\bar{x}_3$	
1	0.21	0.20	0.22	0.21	4.76	0.21	0.22	0.21	0.21	2.71

4. Assay of BIWG 98 SE

The following validation parameters are included: specificity, linearity, accuracy, range, intermediate precision, robustness.

Specificity

The specificity was already treated under identification and decomposition of BIWG 98 SE. See figure 1.

Linearity

The linearity of BIWG 98 SE was investigated in the range of 0.13 - 1 µg $\triangleq$ 25 - 150 %.

To find out whether there is an influence of excipients, matrix effect of placebo, the drug substance and drug substance with placebo were investigated and compared.

A stock solution of the drug substance and a stock solution + placebo were prepared and diluted accordingly.

Each mass was injected and measured three times, the mean out of three was used for linearity testing as shown in figures 8 and 9.

The data are summarised in the following tables:

BIWG 98 SE drug substance

Injected mass [µg]	Integration units x_1	x_2	x_3	$\tilde{x}_3$	RSD [%]	Theoretical value [IU]	deviation [%]
0.133	335	334	333	334	0.30	345	- 3.3
0.267	664	667	663	665	0.31	673	- 1.2
0.400	1001	1001	1002	1001	0.06	998	+ 0.3
0.534	1330	1329	1331	1330	0.08	1325	+ 0.3
0.666	1667	1661	1663	1664	0.18	1648	+ 1.0
0.802	2012	2011	2011	2011	0.02	1980	+ 1.5
0.913	2250	2242	2241	2244	0.22	2252	- 0.4
1.065	2600	2592	2599	2597	0.17	2623	- 1.0

Equation: $y = 2443.74\ x + 20.43$
Correlation coefficient: 0.99976

BIWG 98 SE drug substance with placebo

Injected mass [µg]	Integration units x_1	x_2	x_3	$\tilde{x}_3$	RSD [%]	Theoretical value [IU]	deviation [%]
0.129	314	314	313	314	0.18	320	- 1.8
0.278	684	682	680	682	0.29	683	- 0.1
0.397	974	976	973	974	0.16	972	+ 0.2
0.528	1297	1293	1294	1295	0.16	1291	+ 0.3
0.662	1614	1618	1616	1616	0.12	1617	0.0
0.799	1957	1956	1955	1956	0.05	1950	+ 0.3
0.926	2275	2261	2267	2268	0.31	2259	+ 0.4
1.066	2580	2588	2592	2587	0.24	2600	- 0.5

Equation: $y = 2432.92\ x + 6.16$
Correlation coefficient: 0.9996

The calibration curves of BIWG 98 SE and BIWG 98 SE with placebo are linear.

The regression lines are not significantly different from a curve passing through the origin y = ax. They are superimposable and do not differ.

The excipients have no influence there is no matrix effect.

The analysis can be performed using a test sample and a reference substance.

Accuracy

To verify the accuracy of the procedure the recovery was determined in the range of 70 %, 100 %, 130 % of the stated content by adding 40 mg, 52 mg of BIWG 98 SE to corresponding placebo tablets. The further procedure as under assay.

The determination was performed three times with three injections and measurements.

Table of Recovery

Content adjusted		content determined				% of adjusted content	RSD	deviation
mg/tablet	% stated content	mg/tablet					[%]	[%]
		x_1	x_2	x_3	$\bar{x}_3$	x_3		
28.80	72.00	29.15	29.15	28.82	29.04	100.83	0.66	+ 0.83
28.63	71.58	28.25	28.53	28.43	28.40	99.21	0.50	- 0.79
28.71	71.28	28.20	28.14	28.21	28.18	98.17	0.13	- 1.83
39.36	98.40	38.64	38.65	38.60	38.63	98.15	0.06	- 1.85
40.00	100.00	39.24	39.12	39.48	39.28	98.20	0.47	- 1.80
39.34	98.35	39.01	38.67	38.81	38.83	98.70	0.43	- 1.30
50.62	126.55	49.99	50.13	50.12	50.08	98.93	0.16	- 1.07
51.74	129.30	51.30	51.32	50.96	51.19	98.94	0.40	- 1.06
52.71	131.78	52.53	52.29	52.10	52.31	99.23	0.41	- 0.77
$\bar{x}$						98.93	0.84	- 1.07

The recovery of 9 determinations is 98.93 % thereby the accuracy is verified.

Range

Together with the accuracy the range was validated for 70 % to 130 % together with accuracy.

Intermediate precision

The intermediate precision of the assay was determined for each strength by the following procedure:

Number of technicians: n = 2
Number of replicates: m = 6
Number of multiple measurements: l = 3

The sample preparation was performed as described in the assay, 4 tablets for each preparation.

40 mg

Technician No. 1						Technician No. 2				
Prep. No.	mg/tablet x_1	x_2	x_3	$\bar{x}_3$	RSD [%]	mg/tablet x_1	x_2	x_3	$\bar{x}_3$	RSD [%]
1	40.88	40.63	40.57	40.70	0.40	40.95	40.99	40.87	40.94	0.15
2	40.80	40.72	40.69	40.74	0.13	40.85	40.89	40.95	40.90	0.12
3	40.47	40.28	40.37	40.37	0.22	40.62	40.60	40.68	40.63	0.10
4	40.12	40.20	40.13	40.15	0.11	39.71	39.79	39.87	39.79	0.20
5	40.52	40.35	40.26	40.38	0.32	40.76	40.75	40.67	40.73	0.12
6	39.84	39.95	39.86	39.88	0.13	40.35	40.48	40.32	40.38	0.21
$\bar{x}$				40.37	0.80				40.56	1.06

Total mean mg/tablet : 40.47
SD of intermediate precision : 0.375 %
RSD of intermediate precision : 0.93 %

The procedure for the assay determination corresponds exactly to the information in the Testing Specifications. Therefore always 4 tablets are applied with the consequence that partly the intermediate precision is influenced by the content uniformity.

The alternative would have been to apply at least 10 tablets in a 100 or 250 ml flask and ultrasonicate with a final RSD of about 1 %.

The testing specifications cover both Release Samples and Stability Testing.

For the release samples the content uniformity has to be determined and the mean is taken as assay, if it is within release specifications.

In stability testing according to the ICH Guideline "Stability Testing of New Drug Substances and Products" the need for the extend of replication will depend on the results of validation studies. The necessity can only be elucidated if the procedure for the intermediate precision follows exactly the information in the Testing Specifications.

The extend of replication will depend on the RSD of the assay procedure.

RSD $\leq$ 1.5 % single analysis
RSD > 1.5 % 3 fold analysis

With an RSD: 0.93 % a single analysis is performed.

Robustness

The robustness has been investigated by determination of the content immediately after sample preparation and after storage of 48 hours.

No change took place. Furthermore no decomposition at the beginning and after 48 hours.

Content after sample preparation						Content after storage for 48 hours				
Prep. No.	Content [%]			~	RSD [%]	Content [%]			~	RSD [%]
	x_1	x_2	x_3	$\bar{x}_3$		x_1	x_2	x_3	$\bar{x}_3$	
1	100.47	100.01	100.08	100.19	0.3	100.03	99.31	100.23	99.86	0.5

Figure 10 demonstrates a chromatogram for assay.

The robustness has been extended to pH and composition of the eluent solution with the following data:

Method	**Substance**	**Chromatographic data**			
		t_R	k’	As	Rs
Methanol : buffer pH 3.0 650 : 350	BIWG 98 D1	0.73 min	0.45	2.0	4.17
	BIWG 98 SE	1.44 min	1.86	1.3	
Methanol : buffer pH 2.5 650 : 350	BIWG 98 D1	0.63 min	0.25	2.37	5.41
	BIWG 98 SE	1.37 min	1.37	1.47	
Methanol : buffer pH 3.5 650 : 350	BIWG 98 D1	0.91 min	0.81	1.53	2.85
	BIWG 98 SE	1.66 min	2.29	1.47	
Methanol : buffer pH 3.0 710 : 300	BIWG 98 D1	0.66 min	0.31	1.97	2.89
	BIWG 98 SE	0.96 min	0.91	1.47	
Methanol : buffer pH 3.0 580 : 300	BIWG 98 D1	0.81 min	0.61	2.04	5.77
	BIWG 98 SE	2.88 min	3.53	1.19	

The method is not robust against changes in the pH of the buffer and the change in the organic phase of the eluent solution. Both should be kept constant

But in all cases Rs > 1.5 and the elution order is unchanged.

5. Content uniformity

The validation parameters specificity, linearity, accuracy range and robustness are covered under assay of BIWG 98 SE, since the procedure is the same besides 1 tablet is dissolved in 50 ml instead of 4 tablets. The dilution is correspondingly different. Therefore the repeatability had been determined.

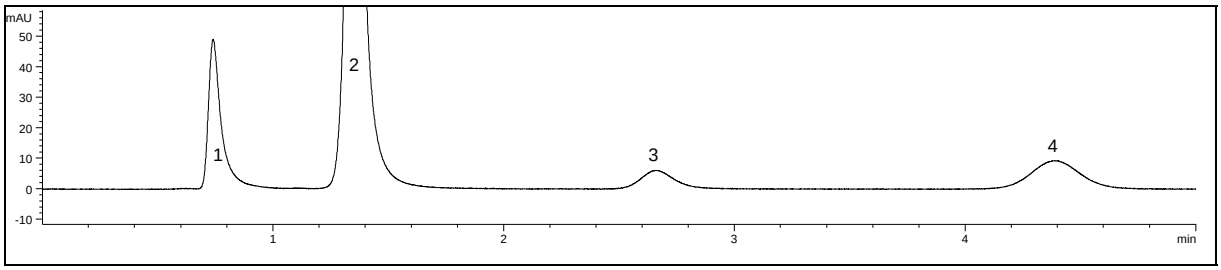
10 tablets where placed in a 500 ml flask, 400 ml solvent were added and ultrasonicated for 10 minutes. Then the flask is made up to the mark with solvent at room temperature. Then proceeded as under content uniformity.

Repeatability					
Prep. No.	mg/tablet				RSD [%]
	x_1	x_2	x_3	$\tilde{x}_3$	
1	39.71	40.19	39.89	39.93	0.56
2	40.19	39.93	99.94	40.02	0.80
3	40.65	40.33	40.29	40.41	0.48
4	40.03	40.22	39.81	40.02	0.54
5	40.05	40.94	40.35	40.45	1.15
6	39.85	40.06	39.86	39.92	0.30
$\bar{x}$				40.13	

Total mean mg/tablet:	40.13
SD of repeatability %:	0.24
RSD of repeatability %:	0.60

6. Figures and structural formulae

Figure 1: Degradation and assay of BIWG 98 SE: Validation of Procedure



Peak No.	Substance
1	BIWG 98 SE D1
2	BIWG 98 SE
3	BIWG 98 O
4	BIWG 98 L

Chromatographic Conditions

Eluent solution

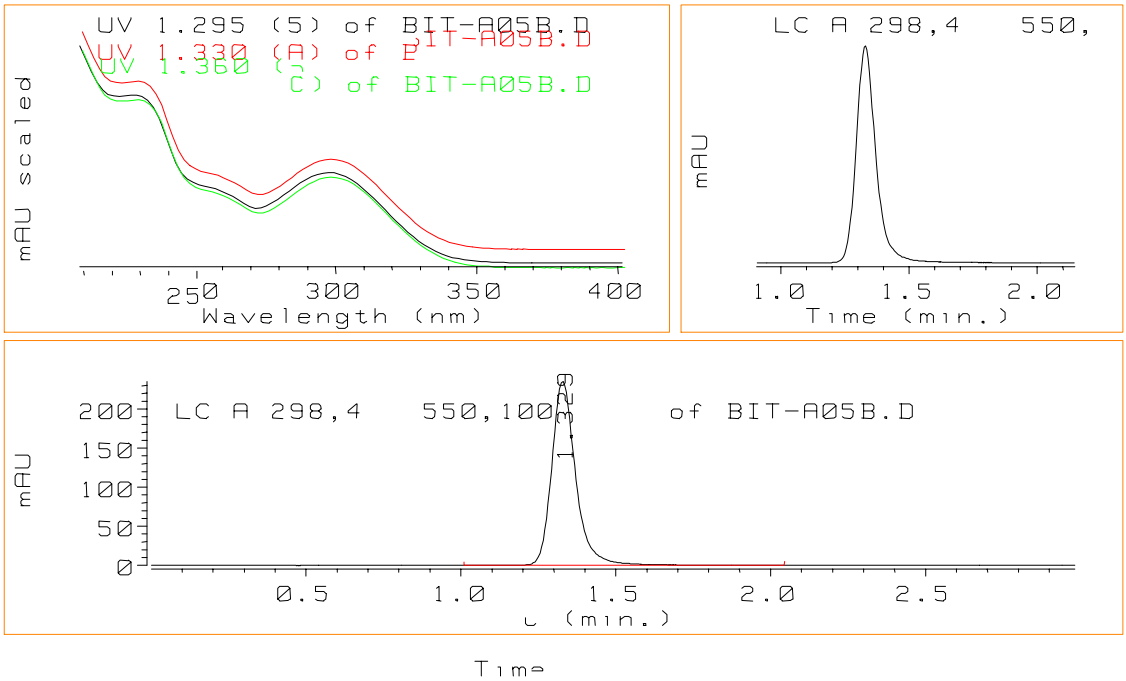
Methanol	(65 Vol%)
Buffer solution	(35 Vol%)

Flow rate : 0.7 ml/min
Column temperature : 40°C
Wavelength : 298 nm

Column

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

Figure 2: Investigation of peak purity BIWG 98 SE peak at half heights and maximum (HP 1090 program).



Chromatographic Conditions

Eluent solution

Methanol (65 Vol%) Buffer solu

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

Figure 3: UV spectrum of BIWG 98 SE to derive the wavelength 298 nm.

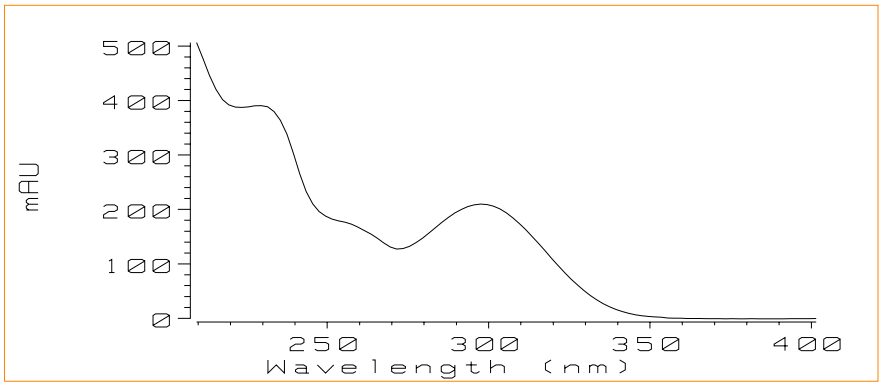
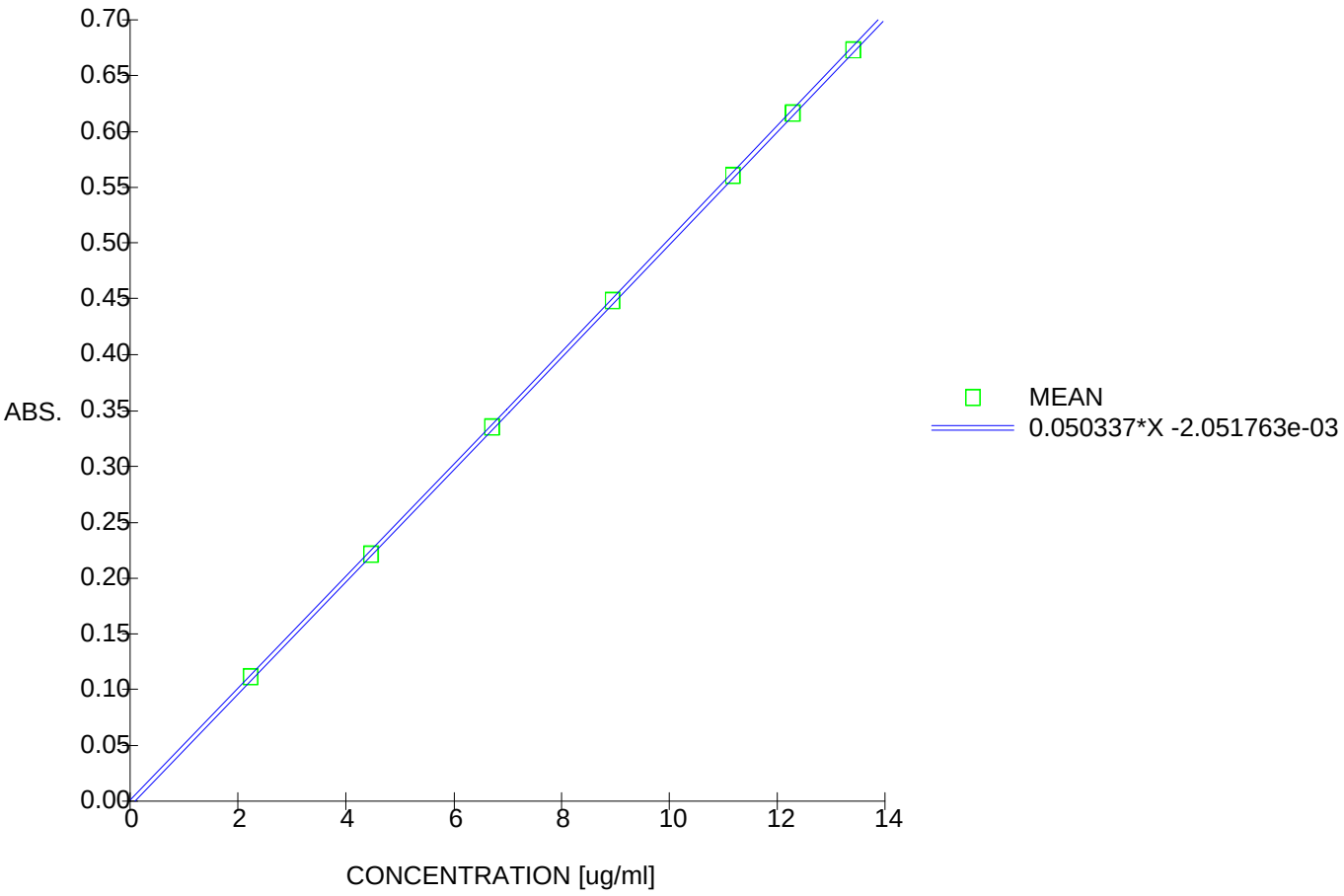
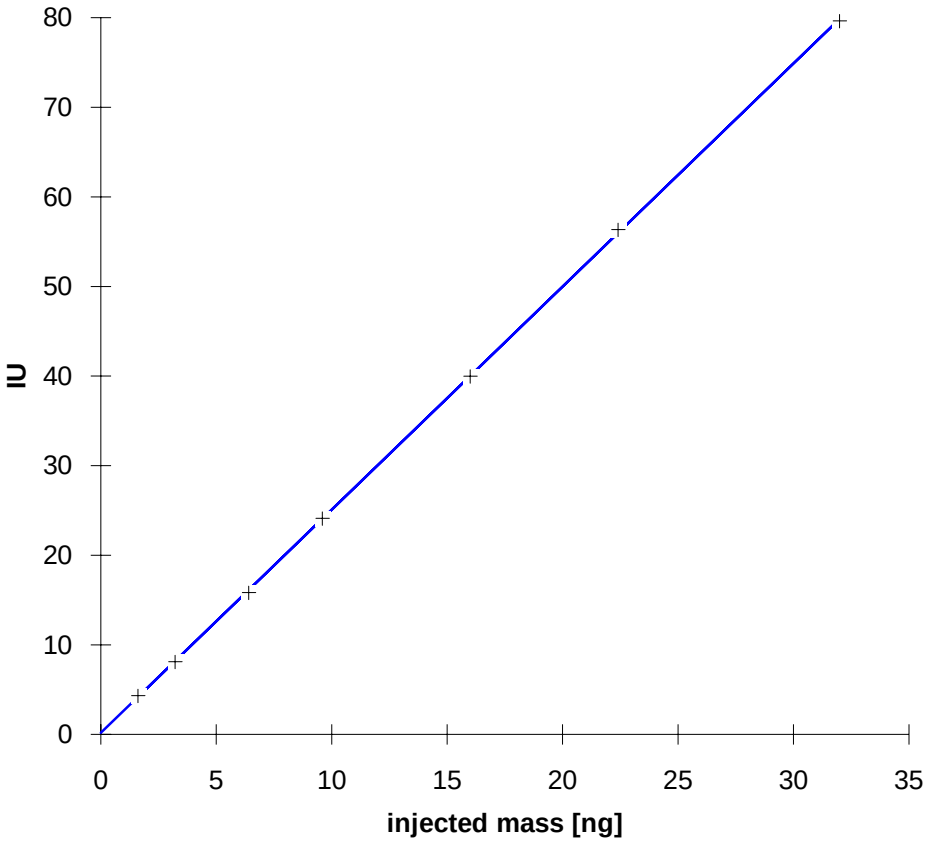


Figure 4: Calibration curve of BIWG 98 SE for dissolution.



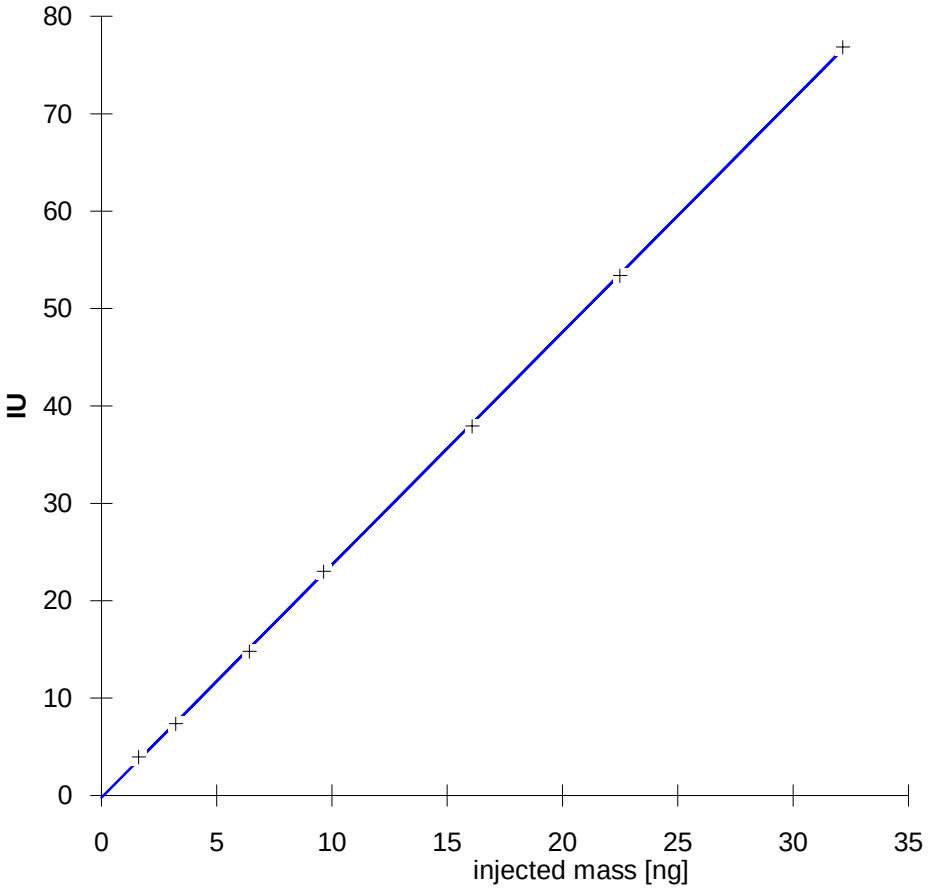
CORRELATION COEFFICIENT R = 0.999994

Figure 5: Calibration curve of BIWG 98 SE (range 1.6 – 30 ng).



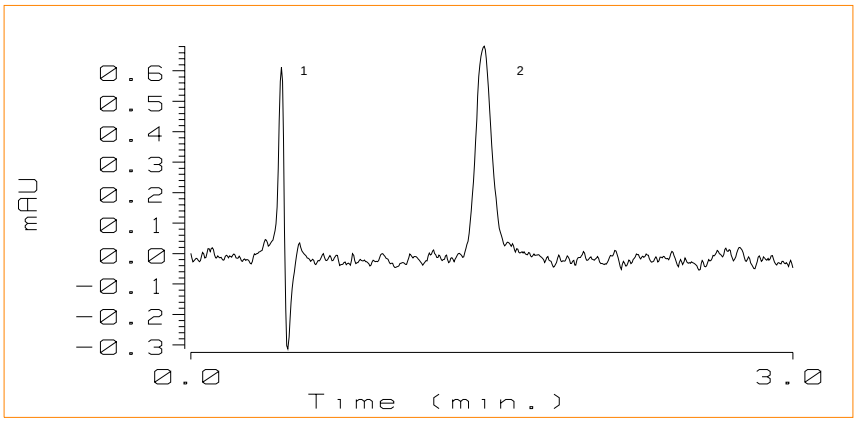
\+ = $\bar{x}_3$
y = 2.49 x + 0.19
Correlation coefficient: 0.99997

Figure 6: Calibration curve of BIWG 98 D1 (range 1.6 – 30ng).



\+ = $\overline{x}_3$
y = 2.39 x - 0.23
Correlation coefficient: 0.99996

Figure 7: HPLC chromatogram of reporating threshold 1.6 ng ≙ 0.1 % BIWG 98 SE



Peak No.	Substance
1	dead volume
2	BIWG 98 SE 1.6 ng

Chromatographic Conditions

Eluent solution

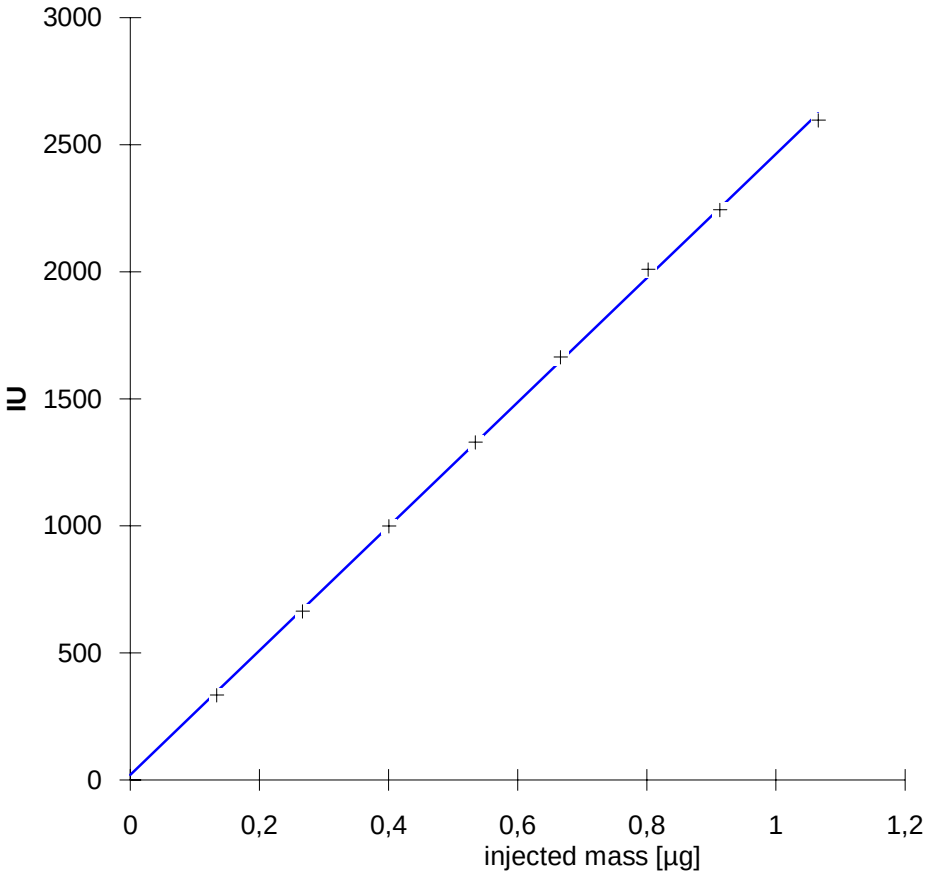
Methanol	(65 Vol%)
Buffer solution	(35 Vol%)

Flow rate : 0.7 ml/min
Column temperature : 40°C
Wavelength : 298 nm

Column

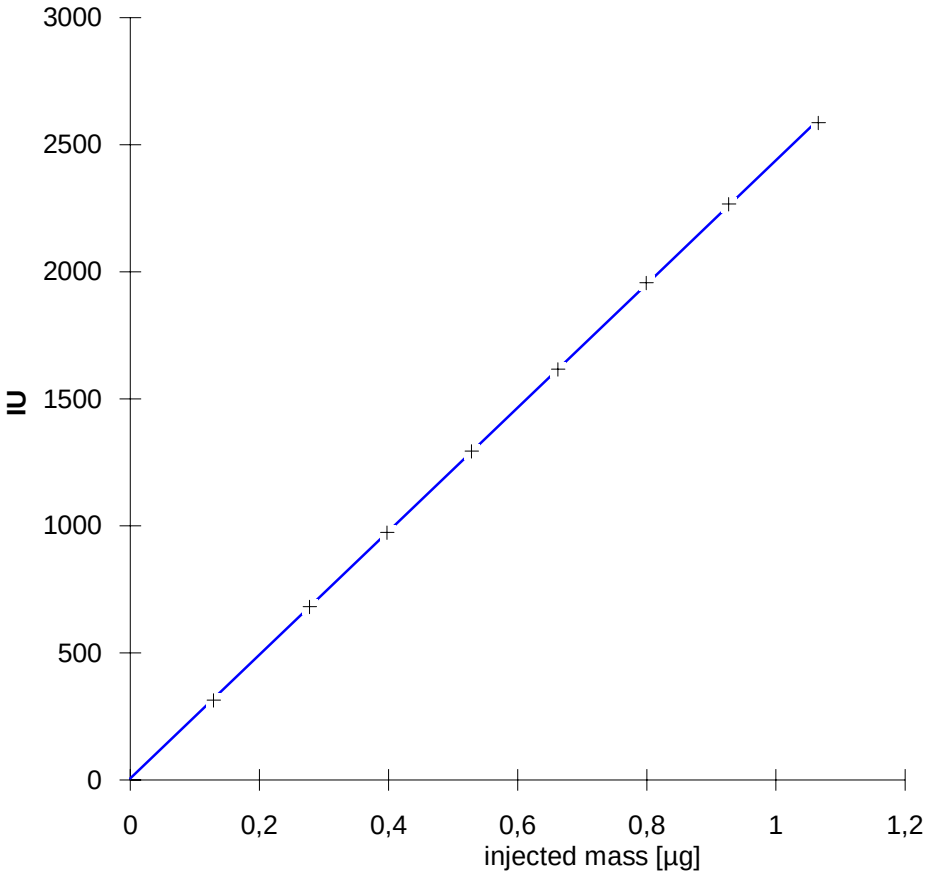
Material : Nucleosil 100, C 18, 5 µm
Length : 4.0 cm
Diameter : 4.0 mm

Figure 8: Calibration curve of BIWG 98 SE (range 0.13 – 1 µg)



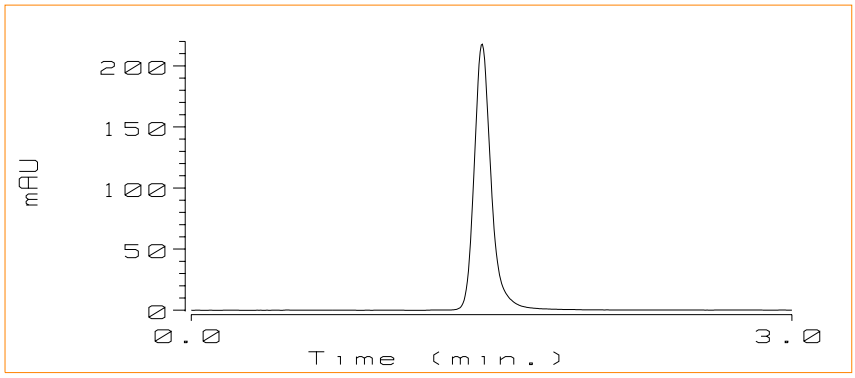
\+ = $\bar{x}_3$
y = 2443.74 x + 20.43
Correlation coefficient: 0.99976

Figure 9: 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

Figure 10: Chromatogram of BIWG 98 SE assay, 0.53 µg



Chromatographic Conditions

Eluent solution

Methanol	(65 Vol%)
Buffer solution	(35 Vol%)
Flow rate	**:** 0.7 ml/min
Column temperature	**:** 40°C
Wavelength	**:** 298 nm

Column

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

Structural formulae

BIWG 98 SE

BIWG 98 SE DEC

7. Literature

1. Grimm W., Schepky G., Stabilitätsprüfung in der Pharmazie Editor Cantor Verlag Aulendorf pp 37