2.1.P.5.3 Validation of Analytical Procedures

The following analytical procedures used for testing the drug product were validated:

- Identification, Assay and Uniformity of Dosage Unit ivermectin 3 mg tablet by HPLC
- Related substances
- Dissolution
- Microbial purity

1 Identification, Assay and Uniformity of Dosage Units of Ivermectin 3 mg by HPLC

1.1 The aim of the validation

Checking the performance characteristics of the method developed for the determination of the assay and identification of the ivermectin 3 mg tablet. Checking that the method meets the requirements of the routine quality control process and is suitable for its intended purpose.

1.2 Description of the analytical procedure

Diluent: methanol

Chromatographic conditions:

Mobile phase:	Acetonitril-methanol-purified water (53:35:12 V/V)
Flow:	1.2 ml/min
Column:	Zorbax SB-CN 5µm; 4,6 x 150 mm, or similar
Injection volume:	100 µl
Detection:	245 nm
Column temperature:	30°C
Run time:	min

Ivermectin standard solution:

Weight accurately approx. 30 mg of ivermectin standard material into a 100.00 ml volumetric flask, dissolve in 80.0 ml of diluent, and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.3 mg/ml*)

Sample solution:

Weight accurately 10 tablets material into a 100.00 ml volumetric flask, add 10 ml of purified water, sonicate for 10 minutes, shaking occasionally. Add 60.0 ml of diluent, sonicate for 5 min and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.3 mg/ml)*

System suitability:

Apply 5 consecutive injections of the standard solution. The RSD of the ivermectin peak area is not more than 2 %, the USP tailing for H_2B_{1a} peak is not more than 2.5 and the theoretical plate number is not less than 2500. The resolution between H_2B_{1a} H_2B_{1b} peaks is not less than 3.0.

Calculation:

$$\text{Ivermectin(mg)} = \frac{A_s}{A_{std}} \times \frac{W_{std}}{100} \times P_{std} \times \frac{100}{W_s} \times T$$

Where:

A_s: area of the ivermectin peak in the sample solution
A_{std}: area of the ivermectin peak in the standard solution
W_s: amount of sample taken (mg)
W_{std}: amount of ivermectin standard material taken (mg)
P_{std}: potency of the standard material, (%)
T: average mass of the tablets

Uniformity of Dosage Unit

Ivermectin standard solution:

Weight accurately approx. 15 mg of ivermectin standard material into a 50.00 ml volumetric flask, dissolve in 40.0 ml of diluent, and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.3 mg/ml*)

Sample solution:

Transfer 1 tablet material into a 10.00 ml volumetric flask, add 1 ml of purified water, sonicate for 10 minutes, shaking occasionally. Add 6.0 ml of diluent, sonicate for 5 min and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.3 mg/ml)*

Calculation:

$$\text{Ivermectin(mg)} = \frac{A_s}{A_{std}} \times \frac{W_{std}}{50} \times P_{std} \times \frac{10}{W_s}$$

Where:

A_s: area of the ivermectin peak in the sample solution
A_{std}: area of the ivermectin peak in the standard solution
W_s: amount of sample taken (mg)
W_{std}: amount of ivermectin standard material taken (mg)
P_{std}: potency of the standard material, (%)

1.3 Validation characteristics

The following validation characteristics were tested:

- Selectivity
- Linearity
- Range
- Accuracy
- Precision
- Ruggedness
- Stability of solutions

1.4 Tests

1.4.1 Selectivity

Procedure:

Ivermectin standard solution:

Weight accurately approx. 30 mg of ivermectin standard material into a 100.00 ml volumetric flask, dissolve in 80.0 ml of diluent, and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.3 mg/ml*)

Placebo solution:

Weight accurately 600 mg placebo into a 100.00 ml volumetric flask, add 10 ml of purified water, sonicate for 10 minutes, shaking occasionally. Add 60.0 ml of diluent, sonicate for 5 min and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml.

Measurement:

Analyse the placebo solution and the standard solution and record the peak responses detectable with the same retention time as the ivermectin peak.
To verify identity of the peaks, analyze standard and sample solutions prepared as directed in the method and perform PDA spectral analysis of the main peaks between 200 and 350 nm.

Requirement:

There are no peak(s) within the range of the peak corresponding to ivermectin on the chromatogram of the and placebo solution.
The retention time of the main peaks of the sample chromatogram is similar in retention time (± 0.5 min) to that of the main peaks of the standard chromatogram, and the PDA spectra are similar (absorption maximums of the main peaks of the sample solution do not differ more than ± 2 nm compared to the PDA spectrum of the main peaks of the standard solution).

Results:

No interference in detected. The retention times and PDA spectra are similar.

Conclusion:

Complies with requirement.

1.4.2 Linearity

Procedure:
Prepare standard samples as described in the method with the listed approximate final concentrations:

ivermectin:

0.20 mg/ml; 0.24 mg/ml; 0.30 mg/ml; 0.36 mg/ml and 0.40 mg/ml

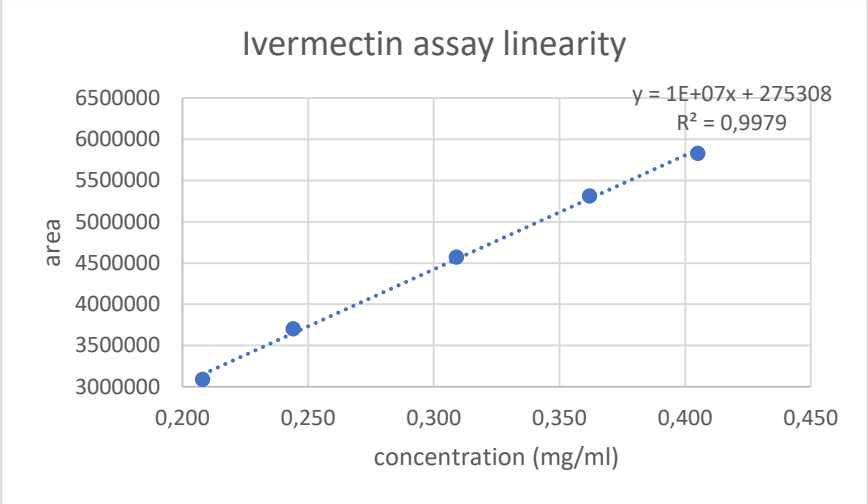
Measurement:
Separately inject 100 µL of the linearity solutions and record the peak responses of the ivermectin peaks.

Evaluation:
Plot the mean ivermectin peak areas against the concentrations and calculate the regression line by the method of the least squares.

Requirement:
The linear relationship is accepted if R^2 not less than 0.99.

Results:

IVERMECTIN concentration (mg/ml)	area
0.208	3088587
0.244	3703513
0.309	4572723
0.362	5311966
0.405	5829049



Clonclusion:
Complies with requirement.

1.4.3 Accuracy

Procedure:

Prepare sample mixtures with a known ivermectin content of 70 %, 100 %, and 130 % of the label claim.

Prepare three samples at each concentration levels, and analyze as described in the method of the assay test.

Evaluation:

Calculate the mean values and the relative standard deviations for each content.

Requirement:

The mean accuracy is between 98.0 – 102.0 %.

Results:

Theoretical ivermectin concentration (mg/ml)			**Area**			**Measured ivermectin concentration (mg/ml)**		
0.210	0.211	0.211	2979389	2945536	2930062	0.213	0.211	0.209
0.300	0.305	0.304	4121082	4205660	4071241	0.295	0.301	0.291
0.397	0.392	0.392	5400650	5382170	5400052	0.386	0.385	0.386

Accuracy (%)				
70%	101.44	99.81	99.29	**mean = 98.54%** **SD = 1.59** **95 % conf. = 1.04**
100 %	98.22	98.46	95.75	
130 %	97.26	98.17	98.49	

Conclusion:

Complies with requirement.

1.4.4 Precision (repeatability)

Procedure:

Analyze a standard solution prepared as described in the method five times consecutively and record the peak responses of the ivermectin peak.

Evaluation:

Calculate the mean and the relative standard deviation of the results.

Requirement:

The relative standard deviation is not more than 2.0 %.

Results:

Test #	IVERMECTIN concentration (µg/ml)	Peak area
1	0.31	4447066
2	0.31	4446976
3	0.31	4441329
4	0.31	4433222
5	0.31	4431243
6	0.31	4441208
Mean of peak areas:		**4440174**
Standard Deviation (SD):		**6697**
RSD %:		**0,15**
Confidence interval 95 %		**5359**

Conclusion:

Complies with requirement.

1.4.5 Intermediate precision

Procedure:

For testing the intermediate precision two different analyst measure the assay of a chosen batch of IVERMECTIN tablet as directed in the method, on two different days, using different instruments (see table below).

Analyst #1	DAY 1	Instrument #1
Analyst #2	DAY 2	Instrument #2

Prepare 5 samples for each measurement.

Evaluation:

Calculate the mean and the relative standard deviation of the results.

Requirement:

The relative standard deviation is not more than 2.0 %.

Results:

IVERMECTIN 3 mg tablet (% of LC)		
sample	**analyst #1 day #1 Instrument #1**	**analyst #2 day #2 Instrument #2**
1	100.32	99.52
2	99.37	98.21
3	100.38	100.20
4	99.39	99.08
5	99.97	99.29
Mean of results:	**99.57**	
SD:	**0.67**	
RSD %:	**0.67**	
Confidence interval 95 %	**0.38**	

Conclusion:

Complies with requirement.

1.4.6 Robustness (column temperature)

Procedure:
Determine the assay of a chosen batch of ivermectin tablet as described in the method applying the following column temperatures: 28°C; 30°C and 32°C. Prepare five samples for each test.

Evaluation:
Calculate the mean and the relative standard deviation of the results.

Requirement:
The relative standard deviation of the fifteen determinations is not more than 2.0 %

Results:

Ivermectin 3 mg tablets (% of LC)			
Sample #	**28°C**	**30°C**	**32°C**
1	100.00	100.32	100.65
2	100.31	99.37	100.26
3	99.31	100.38	99.82
4	100.50	99.39	101.91
5	95.12	99.97	96.51
Mean of results:	**99.59**		
SD:	**1.68**		
RSD %:	**1.69**		
Confidence interval 95 %	**0.85**		

Conclusion:

Complies with requirement.

1.4.7 Robustness (flow rate)

Procedure:
Determine the assay of a chosen batch of ivermectin 3 mg tablet as described in the method applying the following flow rates: 1.0 ml; 1.2 ml and 1.4 ml. Prepare five samples for each test.

Evaluation:
Calculate the mean and the relative standard deviation of the results.

Requirement:
The relative standard deviation of the fifteen determinations is not more than 2.0 %

Results:

Ivermectin 3 mg tablets (% of LC)			
Sample #	**1.0 ml**	**1.2 ml**	**1.4 ml**
1	98.80	100.32	99.03
2	98.70	99.37	99.66
3	97.86	100.38	98.38
4	99.46	99.39	99.16
5	94.53	99.97	94.60
Mean of results:	**98.64**		
SD:	**1.79**		
RSD %:	**1.81**		
Confidence interval 95 %	**0.90**		

Conclusion:

Complies with requirement.

1.5 Summary and Conclusion

Performance characteristics	Requirements	Results
Selectivity	There are no peak(s) within the range of the peak corresponding to ivermectin in the chromatograms of the mobile phase, diluent and placebo solution.	No peak interference detected.
Linearity	$R^2 \geq 0.995$ (studied in the intended range of 70 % - 130 % of the declared content)	$R^2 = 0.998$
Accuracy	The recovery of the individual samples is between 95.0 – 105.0 %; the mean recovery is between 98.0 – 102.0 %. (studied in the intended range of 70 % - 130 % of the declared content)	individual recoveries: 95.75-101.44%; mean recovery: 98.54.%
Range	70 % - 130 % of the declared content	The range has been verified by the linearity and accuracy studies for the Assay determinations.
Precision (repeatability)	RSD≤2.0	RSD = 0.15 %
Intermediate precision	RSD≤2.0	RSD = 0.67 %
Ruggedness (column temperature)	RSD≤2.0	RSD = 1.69 %
Ruggedness (flow rate)	RSD≤2.0	RSD = 1.81.%
Stability of solutions	Solutions are stable (98,0 % - 102,0 %) at least for 8 hours stored in laboratory conditions.	Solutions are stable for: Standard solution 24 hours: % Sample solution 24 hours: %
Performance characteristics (system suitability)	RSD of five consecutive injections of a standard solution: NMT 2.0 Theoretical Plates/column length (USP) is NLT 5000 Tailing Factor is 0.8 – 1.5	The performance characteristics had been checked during the validation process and complied with the requirements.

According to the validation results the requirements for acceptance criteria were fulfilled. The applied method is reliable and suitable for routine test of product quality.

2 Ivermectin Related Substances

2.1 The aim of the validation

Checking the performance characteristics of the method developed for the determination of the IVERMECTIN related substances content of the IVERMECTIN tablet. Checking that the method meets the requirements of the routine quality control process and is suitable for its intended purpose.

2.2 Description of the analytical procedure

Diluent:

Mix methanol and purified water (2:8, V/V).

Chromatographic conditions:

Mobile phase:	Acetonitril-methanol-purified water (39:55:106 V/V)
Flow:	1.5 ml/min
Column:	Zorbax SB-CN 5µm; 4,6 x 150 mm, or similar
Injection volume:	20 µl
Detection:	245 nm and 280 nm for BHA (imp D) measurement
Column temperature:	30°C
Run time:	min

Ivermectin standard solution (1 %):

Weight accurately approx. 25 mg of ivermectin standard material into a 100.00 ml volumetric flask, dissolve in 80.0 ml of diluent, and dilute to volume with diluent. Dilute 1.00 ml of this solution to 100.00 ml with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.0025 mg/ml*)

3-tert-Butyl-hydroxyanisole (BHA) standard solution:

Weight accurately approx. 25 mg of BHA standard material into a 100.00 ml volumetric flask, dissolve in 80.0 ml of diluent, and dilute to volume with diluent. Dilute 1.00 ml of this solution to 50.00 ml with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(BHA concentration: 0.005 mg/ml*)

Impurity D peak identification solution:

Weight accurately approx. 11 mg of CuBr standard material into a 50.00 ml volumetric flask, add 25.0 ml of sample solution, and 0.5 ml of tert-butyl-hydroxy peroxide. Dilute to volume with diluent. Store the solution at room temperature for 20 minutes, and use not more than 2 hours after preparation. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml.

Sample solution:

Powder at least 10 tablets. Accurately weight approx. 250 mg of powdered tablet into a 50.00 ml volumetric flask, add 5 ml of purified water, sonicate for 10 minutes, shaking occasionally. Add 30.0 ml of diluent, sonicate for 5 min, allow to cool to room temperature and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.25 mg/ml)*

System suitability:

Inject from peak identification solution. The relative retention time of the impurity D peak calculated on the H_2B_{1a} peak is about 0.69. Inject 5 times consecutively from ivermectin standard solution (1 %). The RSD of the ivermectin peak area is not more than 2 %, the USP tailing for H_2B_{1a} peak is not more than 2.0 and the theoretical plate number is not less than 2500.

Injection order:

Peak identification solution – 1 injection
Ivermectin standard solution (1 %) – 5 injection
Sample solution – 1 injection
BHA standard solution – 1 injection **(detection: 280 nm!)**
Sample solution - 1 injection **(detection: 280 nm!)**

Calculation:

$$\text{Impurity (\%)} = \frac{A_s}{A_{std}} \times \frac{W_{std}}{100 \times 100} \times P_{std} \times \frac{50}{W_s} \times \frac{T}{AC}$$

Where:
A_s: area of an impurity peak in the sample solution
A_{std}: area of the ivermectin peak in the standard solution
W_s: amount of sample taken (mg)
W_{std}: amount of ivermectin standard material taken (mg)
P_{std}: potency of the standard material, (%)
AC: assay result (mg of ivermectin/ tablet)
T: average mass of tablets (mg)

Impurity D [BHA at 280 nm] (%) = $\frac{A_s}{A_{std}} \times \frac{W_{std}}{100 \times 50} \times P_{std} \times \frac{50}{W_s} \times \frac{T}{AC}$

Where:

A_s: area of an impurity peak in the sample solution
A_{std}: area of the BHA peak in the standard solution
W_s: amount of sample taken (mg)
W_{std}: amount of BHA standard material taken (mg)
P_{std}: potency of the standard material, (%)
AC: assay result (mg of ivermectin/ tablet)
T: average mass of tablets (mg)

Disregard peaks less than 0.1 %.

Acceptance criteria:

Impurity D	NMT 2.0 %
Impurity at RRT 1.4	NMT 2.7 %
Any individual unspecified degradation product	NMT 1.0 %
Total impurities	NMT 6.0 %

2.3 Validation characteristics

The following validation characteristics were tested:

- Selectivity
- Linearity
- Range
- Accuracy
- Precision
- Ruggedness
- Stability of solutions
- LOQ/LOD

2.4 Tests

2.4.1 Selectivity

Procedure:

Ivermectin standard solution (1 %):

Weight accurately approx. 25 mg of ivermectin standard material into a 100.00 ml volumetric flask, dissolve in 80.0 ml of diluent, and dilute to volume with diluent. Dilute 1.00 ml of this solution to 100.00 ml with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.0025 mg/ml*)

Placebo solution:

Accurately weight approx. 250 mg of powdered tablet into a 50.00 ml volumetric flask, add 5 ml of purified water, sonicate for 10 minutes, shaking occasionally. Add 30.0 ml of diluent, sonicate for 5 min, allow to cool to room temperature and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm PTFE syringe filter, discharging the first 1-2 ml.

Measurement:

Analyse the placebo solution and the standard solution, and record the peak responses of the peak or the peaks detectable with the same retention time.

Requirement:

There are no peak(s) within the range of the peak corresponding known impurities the chromatograms of the placebo solution.

Results:

No peak interference is observed.

Conclusion:

Complies with requirement.

2.4.2 Linearity

Procedure:
Prepare samples as described in the method with the listed final ivermectin concentrations:
0.125 µg/ml; 1.25 µg/ml; 2.5 µg/ml; 5.0 µg/ml and 12.5 µg/ml

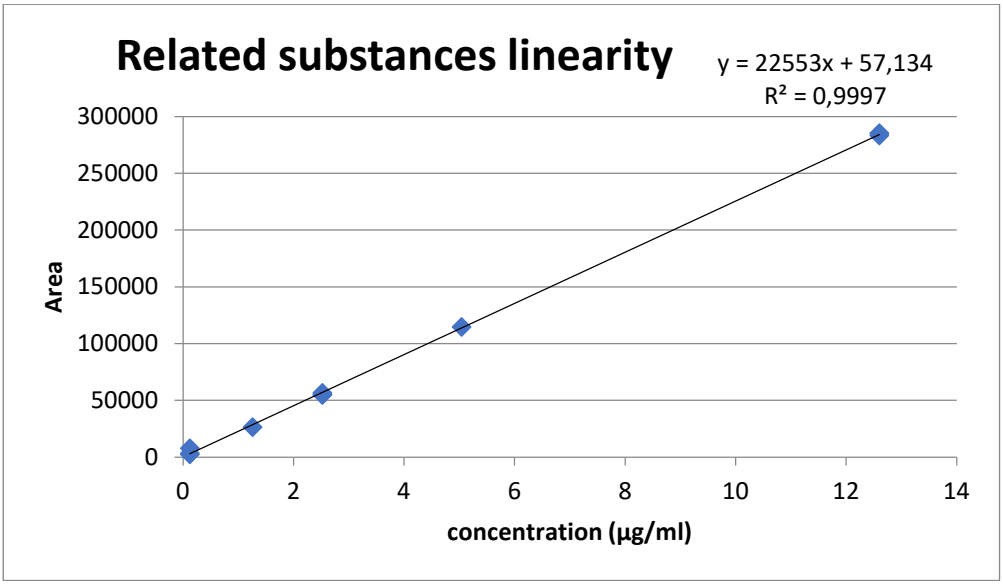
Measurement:
Separately inject 20 µL of the linearity solutions and record the peak responses of the ivermectin peak.

Evaluation:
Plot the peak areas against the concentrations and calculate the regression line by the method of the least squares.

Requirement:
The linear relationship is accepted if R^2 not less than 0.99.

Results:

Ivermectin concentration (µg/ml)	area
0.126	3318
1.26	26434
2.52	54821
5.04	114510
12.60	284990



Conclusion: Complies with requirement.

2.4.3 Accuracy

Procedure:

Prepare sample mixtures with a known ivermectin content, equivalent of 0.125 µg/ml; 2.5 µg/ml and 12.5 µg/ml final ivermectin concentration.

Prepare three samples at each concentration levels, and analyze as described in the method of the assay test.

Evaluation:

Calculate the mean values and the relative standard deviations for each content.

Requirement:

The accuracy of the individual samples is between 90.0 – 110.0 %; the mean recovery is between 95.0 – 105.0 %.

Results:

IVERMECTIN 3 mg tablets								
Theoretical ivermectin concentration (µg/ml)			**Area**			**Measured ivermectin concentration (µg/ml)**		
0.126	0.126	0.126	3382	3318	2618	0.137	0.135	0.117
2.52	2.52	2.52	54955	54821	56540	2.463	2.457	2.534
12.6	12.6	12.6	283249	284990	285168	12.694	12.772	12.780

Accuracy (%)				
0.05 %	109.11	107.04	93.12	**mean = 100.95%** **RSD = 4.53** **95 % conf. = 2.99**
1.0 %	97.73	97.49	100.55	
5.0 %	100.75	101.37	101.43	

Conclusion:

Complies with requirement.

2.4.4 Precision (repeatability)

Procedure:

Analyze a standard solution prepared as described in the method six times consecutively and record the peak responses of the IVERMECTIN peak.

Evaluation:

Calculate the mean and the relative standard deviation of the results.

Requirement:

The relative standard deviation is not more than 5.0 %.

Test #	IVERMECTIN concentration (µg /ml)	Peak area
1	5.0	112881
2	5.0	110409
3	5.0	109522
4	5.0	106849
5	5.0	105610
6	5.0	112881
Mean of peak areas:		**109054.20**
Standard Deviation (SD):		**2890.68**
RSD %:		**2.65**
Confidence interval 95 %		**2533.75**

Conclusion:

Complies with requirement.

2.4.5 Intermediate precision

Procedure:

For testing the intermediate precision two different analyst measure the IVERMECTIN related substances content (%) of a chosen batch of IVERMECTIN tablet spiked with a known impurity as directed in the method, on two different days, using different instruments (see table below).

Analyst #1	DAY 1	Instrument #1
Analyst #2	DAY 2	Instrument #2

Prepare 5 samples for each measurement and for each strength.

Evaluation:

Calculate the mean and the relative standard deviation of the results.

Requirement:

The relative standard deviation is not more than 10.0 %.

Results:

IVERMECTIN 3 mg tablets % RRT1,4 related substance		
sample	**analyst #1 day #1 Instrument #1**	**analyst #2 day #2 Instrument #2**
1	1.73	1.65
2	1.69	1.72
3	1.67	1.61
4	1.63	1.67
5	1.61	1.64
Mean of results:	**1.66**	
SD:	**0.04**	
RSD %:	**2.53**	
Confidence interval 95 %	**0.03**	

Conclusion:

Complies with requirement.

2.4.6 Robustness (injection volume)

Procedure:
Determine the related substances content of a chosen batch of IVERMECTIN tablets spiked with a known impurity as described in the method, with applying the following injection volumes: 15 µl; 20 µl and 25 µl. Prepare five samples for each test.

Evaluation:
Calculate the mean and the relative standard deviation of the results.

Requirement:
The relative standard deviation of the fifteen determinations is not more than 10.0 %

Results:

IVERMECTIN 3 mg tablets % of RRT1,4 related substances			
Sample #	**15 µl**	**20 µl**	**25 µl**
1	1.68	1.73	1.55
2	1.67	1.69	1.56
3	1.69	1.67	1.55
4	1.68	1.63	1.52
5	1.68	1.61	1.54
Mean of results:	**1.63**		
SD:	**0.07**		
RSD %:	**4.22**		
Confidence interval 95 %	**0.03**		

Conclusion:

Complies with requirement.

2.4.7 Robustness (detection wavelength)

Procedure:
Determine the related substances content of a chosen batch of IVERMECTIN tablet as described in the method applying the following column temperature: 243 nm; 245 nm and 248 nm. Prepare five samples for each test for each strength.

Evaluation:
Calculate the mean and the relative standard deviation of the results.

Requirement:
The relative standard deviation of the fifteen determinations is not more than 10.0 %

Results:

IVERMECTIN 3 mg tablets % of spiked related substances			
Sample #	**243 nm**	**245 nm**	**247 nm**
1	1.71	1.73	1.73
2	1.71	1.69	1.69
3	1.69	1.67	1.67
4	1.72	1.63	1.63
5	1.73	1.61	1.61
Mean of results:	**1.68**		
SD:	**0.04**		
RSD %:	**2.57**		
Confidence interval 95 %	**0.02**		

Conclusion:

Complies with requirement.

2.4.8 Limit of Detection/Limit of Quantitation

Procedure:

Determine the noise level on a blank chromatogram. Dilute standard solutions to until the average pear area is at least 3 times (LOQ level) and at least ten times (LOD level) of the noise.

Evaluation:

Based on signal to noise ratio and linearity/accuracy tests.

Requirement:

Signal to noise ratio is at least 3 for LOD and 10 for LOQ. The recovery at LOQ concentration level is between 95.0 – 105.0 %.

Results:

IVERMECTIN recovery at LOQ concentration level:

IVERMECTIN tablets								
Theoretical IVERMECTIN concentration (µg/ml)			**Area**			**Measured IVERMECTIN concentration (µg/ml)**		
0.126	0.126	0.126	3382	3318	2618	0.137	0.135	0.117

Recovery (%)			
109.11	107.04	93.12	**mean = 103.09 %**

0.125 µg/ml had been established and verified as a limit of quantitation and 0.06µg/ml as a limit of detection. Booth limits fulfills the requirements for signal-to-noise ratio, and at LOQ concentration level the determination of the analyte is accurate and reproducible.

Conclusion:

Complies with requirement.

2.5 Summary and Conclusion

Performance characteristics	Requirements	Results
Selectivity	There are no peak(s) within the range of the peak corresponding to impurities in the chromatograms of the mobile phase, diluent and placebo solution.	No peak interference detected.
Linearity	IVERMECTIN: $R^2 \geq 0.99$ (studied in the intended range)	$R^2 = 0.999$
Accuracy	The recovery of the individual samples is between 90.0 – 110.0 %; the mean recovery is between 95.0 – 105.0 %. (studied in the intended range)	Individual recoveries: 93.12-109.11 %; Mean recovery: 100.95 %
Range	20 % - 150 % of the declared content	The range has been verified by the linearity and accuracy studies.
Precision (repeatability)	RSD≤10.0	RSD = 2.65 %
Intermediate precision	RSD≤10.0	RSD = 2.53 %
Ruggedness (injection volume)	RSD≤10.0	RSD = 2.65 %
Ruggedness (detection vawelength)	RSD≤10.0	RSD = 2.53%
LOQ/LOD	Signal-to-noise ratio: LOD ≥ 3 LOQ≥10 (recovery: 95-105%)	LOD: 0.06 µg/ml LOQ:0.126 µg/ml (103.09 %)
Stability of solutions	Solutions are stable (98,0 % - 102,0 %) at least for 8 hours stored in the prescribed conditions.	Use freshly prepared solutions.
Performance characteristics (system suitability)	RSD of six consecutive injections of a standard solution: NMT 2.0 Theoretical Plates/column length (USP) is NLT 5000 Tailing Factor is between 0.8 – 2.0	The performance characteristics had been checked during the validation process and complied with the requirements.

According to the validation results the requirements for acceptance criteria were fulfilled. The applied method is reliable and suitable for routine test of product quality.

3 Dissolution

3.1 The aim of the validation

Checking the performance characteristics of the method developed for the determination of the dissolution of the Ivermectin 3 mg tablet. Checking that the method meets the requirements of the routine quality control process and is suitable for its intended purpose.

3.2 Description of the analytical procedure

Method: Ph.Eur.2.9.3

Test:

Apparatus:	App. 2 (paddle), Ph. Eur. / USP
Temperature:	37 ± 0.5 °C
Rotation speed:	50 rpm
Sample amount:	1 tablet per vessel
Dissolution medium:	900 ml of 0.5% sodium lauryl sulphate in pH 7 phosphate buffer
Time:	15 min
Sample amount:	10 ml

Preparation of pH 7 phosphate buffer:

Dissolve 1.36 g of KH_2PO_4 in 1000 ml of purified water. Dissolve 26.81 g of NaH_2PO_4 in 1000 ml of purified water. Mix 389 ml of KH_2PO_4 solution and 611 ml of NaH_2PO_4 solution. Check pH and degas.

Dissolution medium:

Dissolve 5.0 g of sodium dodecyl sulphate in 900 ml of pH7 phosphate buffer and dilute to 1000 ml with the same solvent, and mix well by stirring for 10 min. Check pH and degas.

Sample solution:

Place one tablet into each of the six vessels before starting rotation of the blade.

After 15 minutes of agitation withdraw 10 ml sample. A portion of the solution is filtered through a 0.45µm CA syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.0033 mg/ml*)

Standard solution:

Weight accurately approx. 33 mg of ivermectin standard into a 250.00 ml volumetric flask, dissolve in 200.0 ml of dissolution medium, and dilute to volume with dissolution media. Dilute 5.00 ml of this solution to 200.00 ml with diluent. Prior to injection filter a portion of the solution through a 0.45µm CA syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.0033 mg/ml*)

Chromatographic conditions:

Mobile phase:	Acetonitril-methanol-purified water (53:35:12 V/V)
Flow:	1.2 ml/min
Column:	Zorbax SB-CN 5µm; 4,6 x 150 mm, or similar
Injection volume:	100 µl
Detection:	245 nm
Column temperature:	30°C
Run time:	min

System suitability:

Apply 5 consecutive injections of the standard solution. The RSD of the ivermectin peak area is not more than 2 %, the USP tailing for H_2B_{1a} peak is not more than 2.5 and the theoretical plate number is not less than 2500. The resolution between H_2B_{1a} H_2B_{1b} peaks is not less than 3.0.

Calculation (ivermectin):

$$\text{Ivermectin (\%)} = \frac{A_s}{A_{std}} \times \frac{W_{std}}{250 \times 40} \times P_{std} \times \frac{900}{3}$$

Where:

A_s: area of the ivermectin peak in the sample solution
A_{std}: area of the ivermectin peak in the standard solution
W_s: amount of sample taken (mg)
W_{std}: amount of ivermectin standard material taken (mg)
P_{std}: potency of the standard material, (%)

3.3 Validation characteristics

The following validation characteristics were tested:

- Selectivity
- Linearity
- Range
- Accuracy
- Precision
- Ruggedness
- Stability of solutions

3.4 Tests

3.4.1 Selectivity

Procedure:

Placebo solution:

Place 60 mg of placebo into 900 ml of dissolution medium, and stir for 15 minutes.

After 15 minutes of agitation withdraw 10 ml sample. A portion of the solution is filtered through a 0.45µm CA syringe filter, discharging the first 1-2 ml. Dilute 5.0 ml of the filtrate to 100.0 ml with the diluent.

Standard solution:

Weight accurately approx. 33 mg of ivermectin standard into a 250.00 ml volumetric flask, dissolve in 200.0 ml of dissolution medium, and dilute to volume with dissolution media. Dilute 5.00 ml of this solution to 200.00 ml with diluent. Prior to injection filter a portion of the solution through a 0.45µm CA syringe filter, discharging the first 1-2 ml. *(ivermectin concentration: 0.0033 mg/ml*)

Measurement:

Analyse the placebo solution and the standard solutions, and record the peak responses of the ivermectin peak or the peaks detectable with the same retention time.

Requirement:

There are no peak(s) within the range of the peak corresponding to ivermectin in the chromatograms of the dissolution medium and placebo solution.

Results:

No interference detected.

Conclusion:

Complies with requirement.

3.4.2 Linearity

Procedure:
Prepare standard samples as described in the method with the listed final ivermectin and ivermectin concentrations.
Ivermectin: 0.0015 mg/ml; 0.0025 mg/ml; 0.0030 mg/ml; 0.0033 mg/ml; and 0.0040 mg/ml

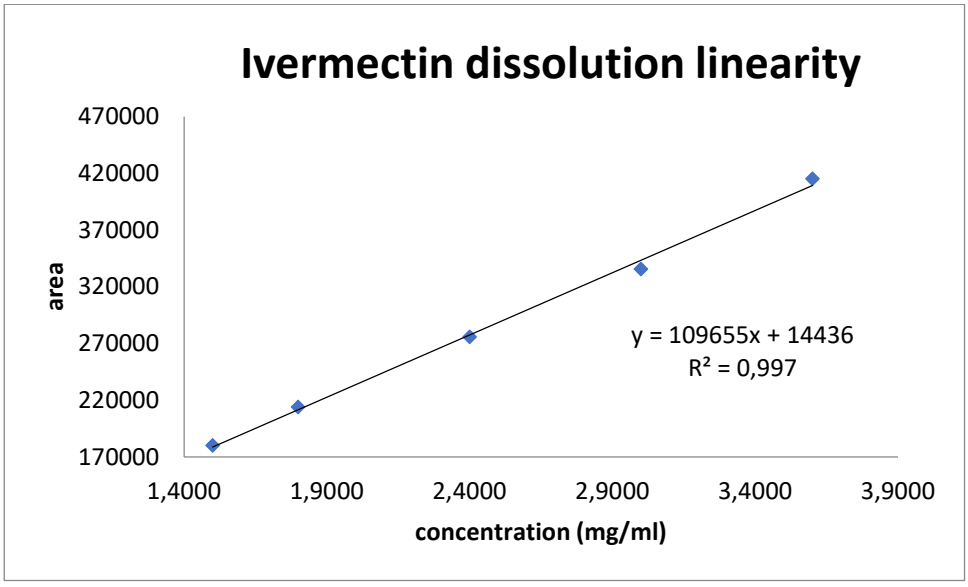
Measurement:
Separately inject 100 µL of the linearity solutions and record the peak responses of the Ivermectin peak.

Evaluation:
Plot the mean ivermectin and ivermectin peak areas against the concentrations and calculate the regression line by the method of the least squares.

Requirement:
The linear relationship is accepted if R^2 not less than 0.99.

Results:

Ivermectin concentration (µg/ml)	area
1.5	180288
1.8	214004
2.4	275858
3.0	335542
3.6	415246



Conclusion: Complies with requirement.

3.4.3 Accuracy

Procedure:

Prepare samples with a known ivermectin content of 50 %, 100 % and 120 % of the label claim.

Prepare three samples at each concentration levels, and analyze as described in the method of the assay test.

Evaluation:

Calculate the mean values and the relative standard deviations for each content.

Requirement:

The accuracy of the individual samples is between 95.0 – 105.0 %; the mean recovery is between 97.0 – 103.0 %.

Results:

Ivermectin tablets								
Theoretical ivermectin concentration (µg/ml)			**Area**			**Measured ivermectin concentration (µg/ml)**		
1.66	1.66	1.66	315931	316397	315971	1.65	1.66	1.66
3.35	3.35	3.35	392868	395889	400737	3.40	3.43	3.47
4.02	4.02	4.02	465756	480354	468819	4.03	4.16	4.06

Accuracy (%)				
50%	99.60	99.81	99.67	**mean = 101.27%** **RSD= 1.57%** **95 % conf. = 1.04**
100%	101.58	102.36	103.61	
120%	100.30	103.50	101.01	

Conclusion:

Complies with requirement.

3.4.4 Precision (repeatability)

Procedure:

Analyze a standard solution prepared as described in the method six times consecutively and record the peak responses of the Ivermectin peak.

Evaluation:

Calculate the mean and the relative standard deviation of the results.

Requirement:

The relative standard deviation is not more than 2.0 %.

Test #	Ivermectin concentration (mg /ml)	Peak area
1	0.033	13161739
2	0.033	13161631
3	0.033	13167629
4	0.033	13169093
5	0.033	13166831
6	0.033	13167225
Mean of peak areas:		**13165691**
Standard Deviation (SD):		**3196**
RSD %:		**0.02**
Confidence interval 95 %		**2558**

Conclusion:

Complies with requirement.

3.4.5 Intermediate precision

Procedure:

For testing the intermediate precision two different analyst measure the dissolution of a chosen batch of Ivermectin tablet as directed in the method, on two different days, using different instruments (see table below).

Analyst #1	DAY 1	Instrument #1
Analyst #2	DAY 2	Instrument #2

Prepare 6 samples for each measurement.

Evaluation:

Calculate the mean and the relative standard deviation of the results.

Requirement:

The relative standard deviation is not more than 5.0 %.

Results:

Ivermectin tablets ivermectin dissolution (% of LC)		
sample	**analyst #1 day #1 Instrument #1**	**analyst #2 day #2 Instrument #2**
1	101.06	100.29
2	107.37	106.34
3	97.17	100.03
4	99.63	105.02
5	99.01	106.22
6	97.83	105.43
Mean of results:	**102.12**	
SD:	**3.68**	
RSD %:	**3.61**	
Confidence interval 95 %	**2.08**	

Conclusion:

Complies with requirement.

2.4.6 Robustness (detection wavelength)

Procedure:

Determine the ivermectin dissolution of a chosen batch of Ivermectin tablet as described in the method, with applying the following detection wavelengths: 318 nm; 320 nm and 322 nm. Prepare five samples for each test.

Evaluation:

Calculate the mean and the relative standard deviation of the results.

Requirement:

The relative standard deviation of the eighteen determinations is not more than 5.0 %

Results:

Ivermectin tablets ivermectin dissolution (% of LC)			
Sample #	**243 nm**	**245 nm**	**247 nm**
1	97.62	101.06	100.76
2	98.64	107.37	101.55
3	98.12	97.17	103.76
4	101.75	99.63	100.04
5	104.45	99.01	106.58
6	103.50	97.83	105.53
Mean of results:	**100.35**		
SD:	**3.19**		
RSD %:	**3.18**		
Confidence interval 95 %	**1.48**		

Conclusion:

Complies with requirement.

3.4.7 Robustness (column temperature)

Procedure:

Determine the ivermectin dissolution of a chosen batch of Ivermectin tablet as described in the method applying the injection volumes 28°C; 30°C and 32°C. Prepare five samples for each test.

Evaluation:
Calculate the mean and the relative standard deviation of the results.

Requirement:
The relative standard deviation of the eighteen determinations is not more than 5.0 %

Results:

Ivermectin tablets ivermectin dissolution (% of LC)			
Sample #	**28°C**	**30°C**	**32°C**
1	99.76	101.06	103.36
2	100.55	107.37	105.61
3	99.08	97.17	101.83
4	101.87	99.63	100.91
5	104.57	99.01	103.46
6	96.06	97.83	101.59
Mean of results:	**100.35**		
SD:	**2.95**		
RSD %:	**2.94**		
Confidence interval 95 %	**1.36**		

Conclusion:

Complies with requirement.

3.4.8 Robustness (agitation rate)

Procedure:
Determine the ivermectin dissolution of a chosen batch of Ivermectin tablet as described in the method applying agitation rates of 45 rpm, 50 rpm and 55 rpm. Prepare six samples for each test.

Evaluation:
Calculate the mean and the relative standard deviation of the results.

Requirement:
The relative standard deviation of the eighteen determinations is not more than 5.0 %

Results:

Ivermectin tablets ivermectin dissolution (% of LC)			
Sample #	**45 rpm**	**50 rpm**	**55 rpm**
1	101.99	101.06	100.29
2	97.36	107.37	106.34
3	91.59	97.17	100.03
4	91.72	99.63	105.02
5	103.95	99.01	106.22
6	96.44	97.83	105.43
Mean of results:	**100.35**		
SD:	**4.71**		
RSD %:	**4.70**		
Confidence interval 95 %	**2.18**		

Conclusion:

Complies with requirement.

3.4.9 Robustness (dissolution medium temperature)

Procedure:
Determine the ivermectin dissolution of a chosen batch of Ivermectin tablet as described in the method applying dissolution medium temperatures of 35°C, 37°C and 39°C. Prepare six samples for each test.

Evaluation:
Calculate the mean and the relative standard deviation of the results.

Requirement:
The relative standard deviation of the eighteen determinations is not more than 5.0 %

Results:

Ivermectin			
Sample #	**35°C**	**37°C**	**39°C**
1	98.63	101.06	91.25
2	100.75	107.37	101.06
3	96.96	97.17	96.07
4	87.50	99.63	106.10
5	97.25	99.01	98.30
6	98.61	97.83	102.97
Mean of results:	**100.35**		
SD:	**4.61**		
RSD %:	**4.59**		
Confidence interval 95 %	**2.13**		

Conclusion:

Complies with requirement.

3.5 Summary and Conclusion

Performance characteristics	Requirements	Results
Selectivity	There are no peak(s) within the range of the peak corresponding to ivermectin and ivermectin in the chromatograms of the mobile phase, diluent and placebo solution.	No peak interference detected.
Linearity	$R^2 \geq 0.99$ (studied in the intended range)	$R^2 = 0.997$
Accuracy	The recovery of the individual samples is between 95.0 – 105.0 %; the mean recovery is between 97.0 – 103.0 %. (studied in the intended range of 50 % - 120 % of the declared content)	Individual recoveries: 99.60-103.61% Mean recovery: 101.27%
Range	50 % - 120 % of the declared content	The range had been verified by the linearity and accuracy studies.
Precision (repeatability)	RSD≤2.0	RSD = 0.2
Intermediate precision	RSD≤5.0	RSD = 3.68
Ruggedness **Effect of changes in detection wavelength**	RSD≤5.0	RSD = 3.18
Ruggedness **Effect of changes in column temperature**	RSD≤5.0	RSD = 2.94
Ruggedness **Effect of changes in agitation rate**	RSD≤5.0	RSD = 4.70
Ruggedness **Effect of changes in dissolution medium temperature**	RSD≤5.0	RSD = 4.59
Stability of solutions	Solutions are stable (98,0 % -102,0 %) at least for 12 hours stored in laboratory conditions.	Solutions are stable for hours. Standard solution: Sample solution:
Performance characteristics (system suitability)	RSD of six consecutive injections of a standard solution: NMT 2.0 Theoretical Plates/column length (USP) is NLT 2000 for ivermectin and 2500 for ivermectin. Tailing Factor is NMT 2.0.	The performance characteristics had been checked during the validation process and complied with the requirements.

According to the validation results the requirements for acceptance criteria were fulfilled. The applied method is reliable and suitable for routine test of product quality.

4 Microbial purity

Validation of microbial purity has been carried out by our contract laboratory Pharmavalid Pharmaceutical Measurement and Services Ltd. Microbiological Laboratory. (*Procedure and results are attached.)*