2.1.P.5.2 Analytical Procedures

1 Appearance

Procedure: Checked by visual examination.

Requirement: White or almost-white, round, biconvex, tablets, "I" on one side

2 Identification-Ivermectin

HPLC

Procedure:

As described in the assay test. The retention time of the main peak of the sample chromatogram is similar in retention time (± 0.5 min) to that of the main peak of the standard chromatogram.

Requirement: Identical

3 Average mass and uniformity of mass

Method: Ph.Eur.2.9.5

Test:

Weigh individually 20 tablets taken at random.

Evaluation:

Not more than 2 of the individual masses deviate from the average mass by more than ± 10.0 % and none deviates more than ± 20.0 %.

Requirement:

Average mass:	60.0 mg ± 5 %
Individual mass:	Average mass ± 10 % (18/20)
	Average mass ± 20 % (20/20)

4 Dimensions

Procedure:

Test 20 tablets by tablet tester instrument.

Requirement:

Diameter: 6.0 ± 0.2 mm
Thickness: 1.5 – 2.2 mm

5 Hardness

Method: Ph.Eur.2.9.8

Test:

Determine the hardness individually 20 tablets by a suitable hardness tester instrument.

Evaluation:

Express the results as the mean, minimum and maximum values of the forces measured.

Requirement: not less than 25 N

6 Friability

Method: Ph.Eur.2.9.7

Test:

Take approximately 6.5 g tablets on a sieve no. 1000 and remove any loose dust with the aid of a soft brush. Accurately weigh the tablet sample and place the tablets in the drum. Rotate the drum 100 times and remove the tablets. Remove any loose dust from the tablets as before. If no tablets is cracked, broken or split, weigh the tablets again.

Requirement: not more than 1.0 %

7 Disintegration

Method: Ph.Eur.2.9.1

Test:

Determine the disintegration individually 6 tablets by a suitable disintegration tester with no disks, using 37 ± 0.5 °C water as medium.

Requirement: not more than 15 min

8 Dissolution

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, (%)

Acceptance criteria: Not less than 80 % (Q) of the label claim is dissolved in 15 minutes.

9 Assay

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

Acceptance criteria: 3.0 mg ± 5 %/tablet

10 Uniformity of dosage units

Test:

Select not less than 30 tablets taken at random. Accurately weigh 10 tablets individually (w_1, w_2, …w_{10}).

Determine the individual ivermectin content of the tablets.

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. 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)*

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:

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

Variable	Definition	Conditions	Value
T	Target content per dosage unit at time of manufacture, expressed as a percentage of label claim. T=100 % in our case.		
n	Sample size. (Number of the tablets in the sample)		
k	Acceptability constant	If n = 10	2.4
		If n = 30	2.0
s	Sample standard deviation		$\left[\frac{\sum_{i=1}^{n}\left(x_i-\overline{X}\right)^2}{n-1}\right]^{1/2}$
RSD	Relative standard deviation		$\frac{100\times s}{\overline{X}}$
M If T ≤ 101.5 %	Standard value	If 98.5 % ≤ $\overline{X}$ ≤ 101.5%	M = $\overline{X}$ (AV = ks)
		If $\overline{X}$ ≤ 98.5 %	M = 98.5 % (AV = 98.5- $\overline{X}$ + ks)
		If $\overline{X}$ > 101.5 %	M =101.5 % (AV = $\overline{X}$ - 101.5+ ks)
M If T > 101.5 %	Standard value	If 98.5 % ≤ $\overline{X}$ ≤ T	M = $\overline{X}$ (AV = ks)
		If $\overline{X}$ ≤ 98.5 %	M = 98.5 % (AV = 98.5- $\overline{X}$ + ks)
		If $\overline{X}$ > T	M =T % (AV = $\overline{X}$ - T+ ks)
AV	Acceptance value		AV = $\left\lvert M-\overline{X}\right\rvert + k\times s$
L1	Maximum allowed acceptance value		L1 = 15.0
L2	Maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than 0.75M while on the high side, no dosage unit result can be greater than 1.25 M.	L2 = 25.0

Evaluation:

The acceptance value of the first 10 dosage units is less than L1. If the acceptance value is greater than L1, test 20 additional dosage units and calculate the acceptance value. The final acceptance value is less than L1, and no individual content of the dosage unit is less than (1-L2x0.01) M, or more than (1+L2x0.01) M. L1 is 15, L2 is 25.

Acceptance criteria: AV ≤ 15.0

11 Related substances

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)

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

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 %

12 Loss on Drying

Test:

Finely powder the content of at least 10 tablets. Dry 2.0 g of the powder to constant mass in a automated dryer.

Acceptance criteria: NMT 6.0 %

13 Microbiological purity

Procedure:

According to Ph. Eur. [2.6.12.; 2.6.13.]. Detailed test conditions are available separately.

Requirement:
TAMC ≤ 10^3 CFU/g
TYMC ≤ 10^2 CFU/g
Absence of *Escherichia coli*/g