the centre and a loosely arranged spongy parenchyma; fragments of leaf epidermis; upper epidermal cells with straight anticlinal walls and a striated cuticle; lower epidermal cells with sinuous anticlinal walls; stomata of the diacytic type (*2.8.3*) more numerous on the lower surface; glandular trichomes with a short unicellular stalk and a globular head composed of 8, sometimes up to 16 cells or with a unicellular head; covering trichomes conical, uniseriate, up to about 300 µm long, occasionally up to 1500 µm, composed of from 2 to 8 cells with slight swellings at the junctions and a warty or striated cuticle; fragments of the calyx containing small, cluster crystals of calcium oxalate; spherical pollen grains, about 25 µm to 30 µm in diameter, with 3 pores and 3 furrows and a smooth exine; thick-walled, lignified fibres and spirally and annularly thickened vessels from the stem; occasional brown fragments of pericarp with single crystals of calcium oxalate.

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 0.5 g of the powdered drug (355) (*2.9.12*) add 5 ml of *methanol R*. Heat in a water-bath at 65 °C for 5 min with shaking. Cool and filter.

Reference solution. Dissolve 5 mg of *naphthol yellow S R* and 2.0 mg of *catalpol R* in 5.0 ml of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *glacial acetic acid R, water R, ethyl acetate R* (20:20:60 *V/V/V*).

Application: 20 µl as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with *dimethylaminobenzaldehyde solution R2*, using about 5 ml for a plate 200 mm square; heat at 100-105 °C for 10 min until the spots appear; examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, further weak greyish-blue zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A wide white zone
	A greyish-blue zone (iridoid)
———	———
Naphthol yellow S: an intense yellow zone Catalpol: a greyish-blue zone	1 or 2 greyish-blue zones (iridoid)
———	———
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 2 per cent of brown or yellow leaves and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 12.0 per cent.

ASSAY

Stock solution. In a 100 ml round-bottomed flask, place 1.00 g of the powdered drug (355) (*2.9.12*), add 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool, filter each extract through the plug of absorbent cotton into the flask. After cooling, filter the combined acetone extracts through a paper filter into a volumetric flask, dilute to 100.0 ml with *acetone R* by rinsing the flask and the paper filter. Introduce 20.0 ml of the solution into a separating funnel, add 20 ml of *water R* and shake the mixture with 1 quantity of 15 ml and then with 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, wash with 2 quantities, each of 50 ml, of *water R*, and filter the extracts over 10 g of *anhydrous sodium sulphate R* into a volumetric flask and dilute to 50.0 ml with *ethyl acetate R*.

Test solution. To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation liquid. Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Measure the absorbance (*2.2.25*) of the test solution after 30 min, by comparison with the compensation liquid at 425 nm. Calculate the percentage content of flavonoids, calculated as hyperoside, from the expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

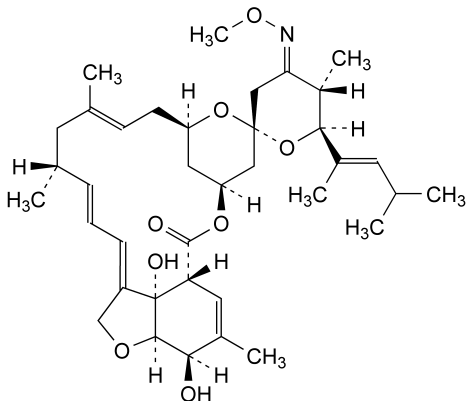
A = absorbance at 425 nm,

m = mass of the substance to be examined, in grams.

01/2008:1656

MOXIDECTIN FOR VETERINARY USE

Moxidectinum ad usum veterinarium



$C_{37}H_{53}NO_8$ M_r 640

[113507-06-5]

DEFINITION

(2a*E*,2′*R*,4*E*,4′*E*,5′*S*,6*R*,6′*S*,8*E*,11*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-[(1*E*)-1,3-dimethylbut-1-enyl]-20,20b-dihydroxy-4′-(methoxyimino)-5′,6,8,19-tetramethyl-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydrospiro[2*H*,17*H*-11,15-methanofuro[4,3,2-*pq*][2,6]benzodioxacyclooctadecine-13,2′-pyran]-17-one ((6*R*,23*E*,25*S*)-5*O*-demethyl-28-deoxy-25-[(1*E*)-1,3-dimethylbut-1-enyl]-6,28-epoxy-23-(methoxyimino)milbemycin B).

Semi-synthetic product derived from a fermentation product.

It may contain suitable stabilisers such as antioxidants.

Content: 92.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or pale yellow, amorphous powder.

Solubility: practically insoluble in water, very soluble in ethanol (96 per cent), slightly soluble in hexane.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *moxidectin CRS*.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_5 (*2.2.2, Method II*).

Dissolve 0.40 g in *benzyl alcohol R* and dilute to 20 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

A. *Test solution*. Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml *acetonitrile R*.

Reference solution (b). Dissolve 5 mg of *moxidectin for system suitability CRS* (containing impurities A, B, C, D, E, F, G, H, I, J and K) in 5 ml of *acetonitrile R*.

Reference solution (c). Dissolve 25.0 mg of *moxidectin CRS* in *acetonitrile R* and dilute to 25.0 ml with *acetonitrile R*.

Column:

- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (4 µm);
- *temperature*: 50 °C.

Mobile phase: dissolve 7.7 g of *ammonium acetate R* in 400 ml of *water R*, adjust to pH 4.8 with *glacial acetic acid R* and add 600 ml of *acetonitrile R*.

Flow rate: 2.5 ml/min.

Detection: spectrophotometer at 242 nm.

Injection: 10 µl of the test solution and reference solutions (a) and (b).

Run time: 2 times the retention time of moxidectin.

Identification of impurities: use the chromatogram supplied with *moxidectin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D, E + F and G.

Relative retention with reference to moxidectin (retention time = about 12 min): impurity A = about 0.5; impurity B = about 0.7; impurity C = about 0.75; impurity D = about 0.94; impurities E + F = about 1.3-1.5; impurity G = about 1.6.

System suitability: reference solution (b):

- *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to moxidectin.

Limits:

- *impurity D*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent);
- *sum of impurities E and F*: not more than 1.7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.7 per cent);
- *impurities A, C, G*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- *impurity B*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *any other impurity eluting before impurity G*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard the peak due to the stabiliser (identify this peak, where applicable, by injecting a suitable reference solution).

B. *Test solution*. Dissolve 75.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*.

Reference solution (b). Dissolve 5 mg of *moxidectin for system suitability CRS* (containing impurities A, B, C, D, E, F, G, H, I, J and K) in 5 ml of *acetonitrile R*.

Column:

- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);
- *temperature*: 35 °C.

Mobile phase: dissolve 3.8 g of *ammonium acetate R* in 250 ml of *water R*, adjust to pH 4.2 with *acetic acid R* and add 750 ml of *acetonitrile R*.

Flow rate: 2.0 ml/min.

Detection: spectrophotometer at 242 nm.

Injection: 10 µl.

Run time: 10 times the retention time of moxidectin.

Identification of impurities: use the chromatogram supplied with *moxidectin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities H + I, J and K.

Relative retention with reference to moxidectin (retention time = about 4 min): impurity G = about 1.4; impurities H + I = about 2.0; impurity J = about 2.2; impurity K = about 3.4.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to impurities H + I and J.

Limits:

– *sum of impurities H and I*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

– *impurities J, K*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *any other impurity eluting after impurity G*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard the peak due to the stabiliser (identify this peak, where applicable, by injecting a suitable reference solution).

Total of all impurities. Calculate the sum of the impurities eluting from the start of the run to impurity G in test A, and from impurities H + I to the end of the run in test B. The total of all impurities is not more than 7.0 per cent.

Heavy metals (*2.4.8*): maximum 20 ppm.

Prescribed solution. Dissolve 0.50 g in 20 ml of *ethanol (96 per cent) R.*

Reference solution. Mix 6 ml of a 1 ppm Pb standard solution with 2 ml of the prescribed solution and 4 ml of *water R.*

To each solution add 2 ml of *buffer solution pH 3.5 R.* Mix. Add 1.2 ml of *thioacetamide reagent R.* Mix immediately. Filter the solutions through a membrane filter (pore size 0.45 µm). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston. Compare the spots on the filters obtained with the different solutions. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution. The substance to be examined complies if the test solution is not more intense than that in the reference solution.

Water (*2.5.12*): maximum 1.3 per cent, determined on 0.50 g.

Sulphated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

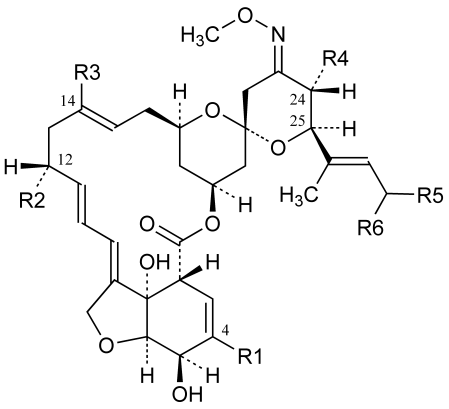
Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of $C_{37}H_{53}NO_8$ using the declared content of *moxidectin CRS.*

IMPURITIES

Specified impurities: A, B, C, D, E, F, G, H, I, J, K.

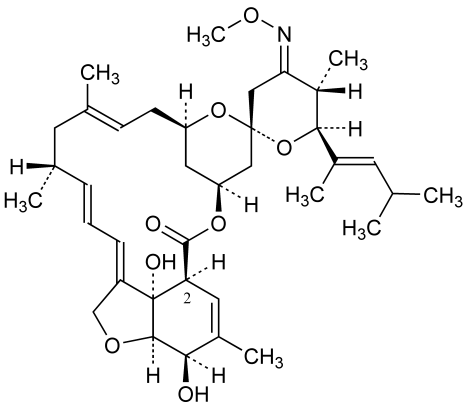


A. R1 = R2 = R3 = R4 = CH_3, R5 = R6 = H:
25-des[(1*E*)-1,3-dimethylbut-1-enyl]-25-[(1*E*)-1-methylprop-1-enyl]moxidectin,

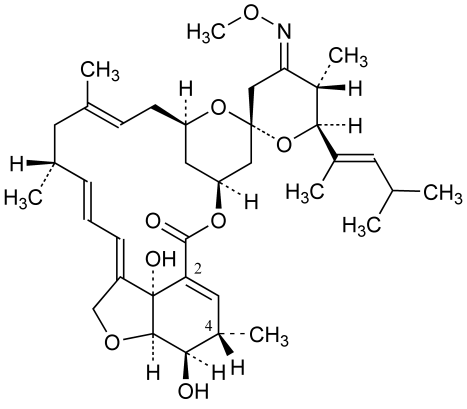
B. R1 = R2 = R3 = R5 = R6 = CH_3, R4 = H:
24-desmethylmoxidectin,

C. R1 = R2 = R3 = R4 = R5 = CH_3, R6 = H:
25-des[(1*E*)-1,3-dimethylbut-1-enyl]-25-[(1*E*)-1-methylbut-1-enyl]moxidectin,

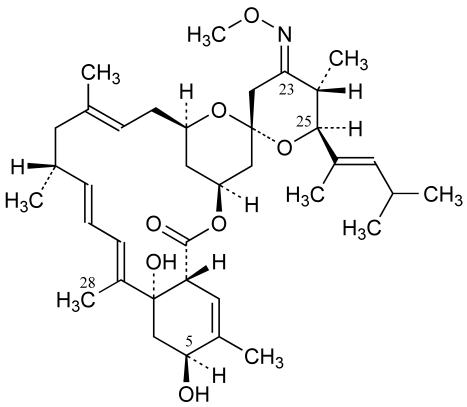
F. one of groups R1 to R6 is C_2H_5, the others are CH_3:
x-desmethyl-x-ethylmoxidectin,



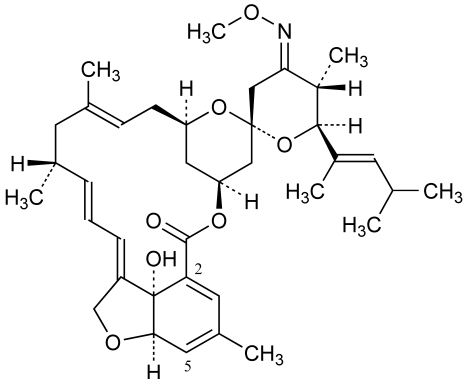
D. 2-*epi*-moxidectin,



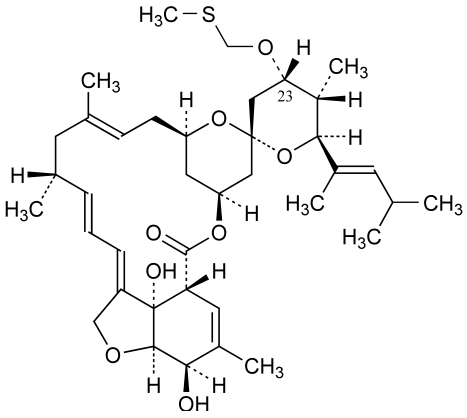
E. (4*S*)-2-dehydro-4-hydromoxidectin,



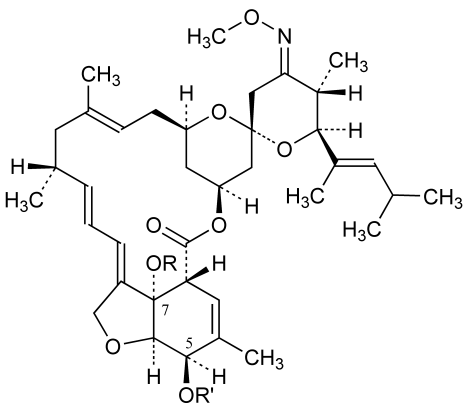
G. (23*E*,25*S*)-5*O*-desmethyl-28-deoxy-25-[(1*E*)-1,3-dimethylbut-1-enyl]-23-(methoxyimino)milbemycin B,



H. 2,5-didehydro-5-deoxymoxidectin,

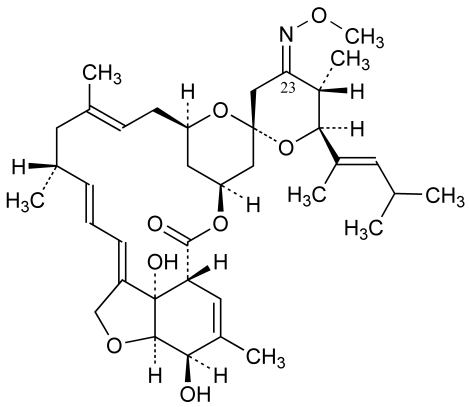


I. (23*S*)-23-des(methoxyimino)-23-[(methylsulphanyl)methoxy]moxidectin,



J. R = CH_2-S-CH_3, R′ = H: 7-*O*-[(methylsulphanyl)methyl]-moxidectin,

K. R = H, R′ = CO-C_6H_4-*p*NO_2: 5-*O*-(4-nitrobenzoyl)moxidectin,

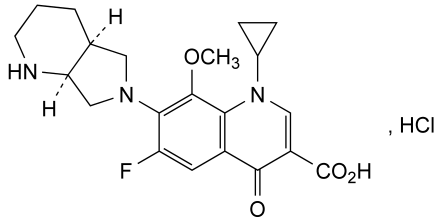


L. (23*Z*)-moxidectin.

01/2008:2254

MOXIFLOXACIN HYDROCHLORIDE

Moxifloxacini hydrochloridum



$C_{21}H_{25}ClFN_3O_4$ M_r 437.9

DEFINITION

1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The production method is validated to demonstrate the satisfactory enantiomeric purity of the final product.

CHARACTERS

Appearance: light yellow or yellow powder or crystals, slightly hygroscopic.

Solubility: sparingly soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *moxifloxacin hydrochloride CRS*.

C. Dissolve 50 mg in 5 ml of *water R*, add 1 ml of *dilute nitric acid R*, mix, allow to stand for 5 min and filter. The filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution. The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_2 (*2.2.2, Method II*). If intended for use in the manufacture of parenteral dosage forms, the solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_2 (*2.2.2, Method II*).

Dissolve 1.0 g in 20 ml of *dilute sodium hydroxide solution R*.