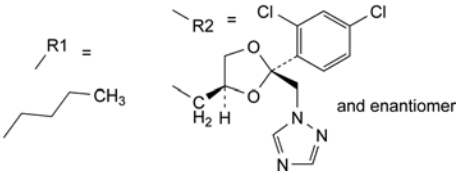
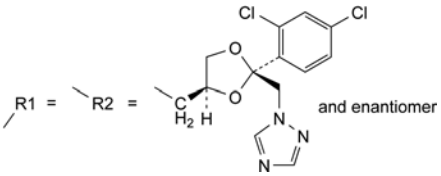


F. 2-butyl-4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one,

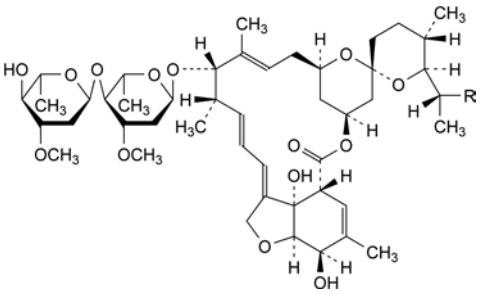


G. 4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

01/2008:1336
corrected 6.0

IVERMECTIN

Ivermectinum



Component	R	Molecular formula	M_r
H_2B_{1a}	CH_2-CH_3	$C_{48}H_{74}O_{14}$	875
H_2B_{1b}	CH_3	$C_{47}H_{72}O_{14}$	861

Ivermectin B1a: [70161-11-4]
Ivermectin B1b: [70288-86-7]

DEFINITION

Mixture of (2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-7-[[2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranosyl]oxy]-20,20b-dihydroxy-5′,6,8,19-tetramethyl-6′-[(1*S*)-1-methylpropyl]-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydrospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecene-13,2′-[2*H*]pyran]-17-one (or 5-*O*-demethyl-22,23-dihydroavermectin A_{1a}) (component H_2B_{1a}) and (2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,-20b*S*)-7-[[2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranosyl]oxy]-20,20b-dihydroxy-5′,6,8,19-tetramethyl-6′-(1-methylethyl)-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydrospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecene-13,2′-[2*H*]pyran]-17-one (or 5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-22,23-dihydroavermectin A_{1a}) (component H_2B_{1b}).

Semi-synthetic product derived from a fermentation product.

Content:

– ivermectin (H_2B_{1a} + H_2B_{1b}): 95.0 per cent to 102.0 per cent (anhydrous substance),

– ratio $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$ (areas by liquid chromatography): minimum 90.0 per cent.

CHARACTERS

Appearance: white or yellowish-white, crystalline powder, slightly hygroscopic.

Solubility: practically insoluble in water, freely soluble in methylene chloride, soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *ivermectin CRS*.

B. Examine the chromatograms obtained in the assay.
Results: the retention times and sizes of the 2 principal peaks in the chromatogram obtained with the test solution are similar to those of the 2 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 1.0 g in 50 mL of *toluene R*.

Specific optical rotation (*2.2.7*): – 17 to – 20 (anhydrous substance).

Dissolve 0.250 g in *methanol R* and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 40.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a). Dissolve 40.0 mg of *ivermectin CRS* in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 100.0 mL with *methanol R*.

Reference solution (c). Dilute 5.0 mL of reference solution (b) to 100.0 mL with *methanol R*.

Column:

– size: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 μm).

Mobile phase: *water R, methanol R, acetonitrile R* (15:34:51 *V/V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 μL.

System suitability:

– *resolution*: minimum of 3.0 between the first peak (component H_2B_{1b}) and the second peak (component H_2B_{1a}) in the chromatogram obtained with reference solution (a),
– *signal-to-noise ratio*: minimum of 10 for the principal peak in the chromatogram obtained with reference solution (c),
– *symmetry factor*: maximum of 2.5 for the principal peak in the chromatogram obtained with reference solution (a).

Limits:

– *impurity with a relative retention of 1.3 to 1.5* with reference to the principal peak: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent),
– *any other impurity* (apart from the 2 principal peaks): not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent),
– *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Ethanol and formamide. Gas chromatography (*2.2.28*).

Internal standard solution. Dilute 0.5 mL of *propanol R* to 100 mL with *water R*.

Test solution. In a centrifuge tube, dissolve 0.120 g of the substance to be examined in 2.0 mL of *m-xylene R* (if necessary heat in a water-bath at 40-50 °C). Add 2.0 mL of *water R*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 mL of *water R*. Discard the upper layer and combine the aqueous layers. Add 1.0 mL of the internal standard solution. Centrifuge and discard any remaining *m*-xylene.

Reference solution (a). Dilute 3.0 g of *ethanol R* to 100.0 mL with *water R*.

Reference solution (b). Dilute 1.0 g of *formamide R* to 100.0 mL with *water R*.

Reference solution (c). Dilute 5.0 mL of reference solution (a) and 5.0 mL of reference solution (b) to 50.0 mL with *water R*. Introduce 2.0 mL of this solution into a centrifuge tube, add 2.0 mL of *m-xylene R*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 mL of *water R*. Discard the upper layer and combine the aqueous layers. Add 1.0 mL of the internal standard solution. Centrifuge and discard any remaining *m*-xylene.

Reference solution (d). Dilute 10.0 mL of reference solution (a) and 10.0 mL of reference solution (b) to 50.0 mL with *water R*. Treat as prescribed for reference solution (c) (from "Introduce 2.0 mL of this solution...").

Column:

– *material*: fused silica,

– *size*: *l* = 30 m, Ø = 0.53 mm,

– *stationary phase*: *macrogol 20 000 R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 7.5 mL/min.

Split ratio: 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	50 → 80
	2 - 8	80 → 240
Injection port		220
Detector		280

Detection: flame ionisation.

Injection: 1 µL; inject the test solution and reference solutions (c) and (d).

Limits:

– *ethanol*: maximum 5.0 per cent,

– *formamide*: maximum 3.0 per cent.

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

1.0 g complies with limit test C. Prepare the standard using 2 mL of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 1.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances.

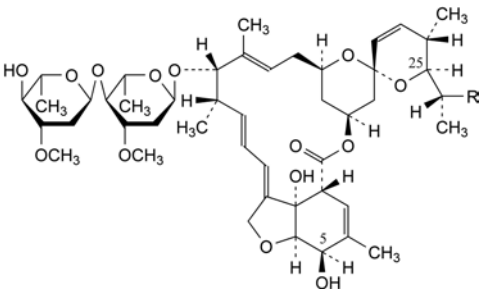
Injection: 20 µL; inject the test solution and reference solution (a).

Calculate the percentage contents of ivermectin (H_2B_{1a} + H_2B_{1b}) and the ratio H_2B_{1a}/(H_2B_{1a} + H_2B_{1b}) using the declared contents of *ivermectin CRS*.

STORAGE

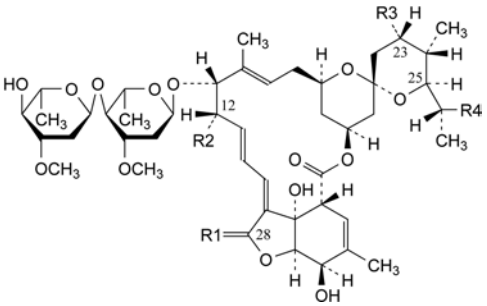
In an airtight container.

IMPURITIES



A. R = C_2H_5: 5-*O*-demethylavermectin A_{1a} (avermectin B_{1a}),

B. R = CH_3: 5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)avermectin A_{1a} (avermectin B_{1b}),

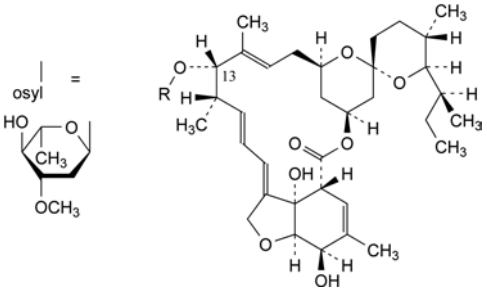


C. R1 = H_2, R2 = CH_3, R3 = OH, R4 = C_2H_5: (23*S*)-5-*O*-demethyl-23-hydroxy-22,23-dihydroavermectin A_{1a} (avermectin B_{2a}),

D. R1 = O, R2 = CH_3, R3 = H, R4 = C_2H_5: 5-*O*-demethyl-28-oxo-22,23-dihydroavermectin A_{1a} (28-oxoH_2B_{1a}),

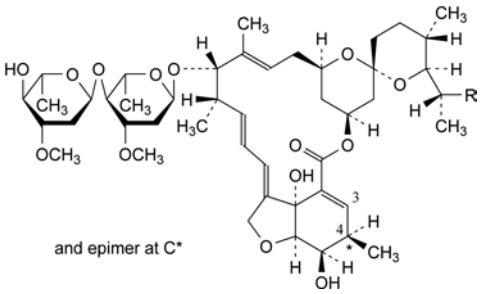
E. R1 = H_2, R2 = C_2H_5, R3 = H, R4 = C_2H_5: 5-*O*,12-didemethyl-12-ethyl-22,23-dihydroavermectin A_{1a} (12-demethyl-12-ethyl-H_2B_{1a}),

F. R1 = H_2, R2 = C_2H_5, R3 = H, R4 = CH_3: 5-*O*,12-didemethyl-25-de(1-methylpropyl)-12-ethyl-25-(1-methylethyl)-22,23-dihydroavermectin A_{1a} (12-demethyl-12-ethyl-H_2B_{1b}),



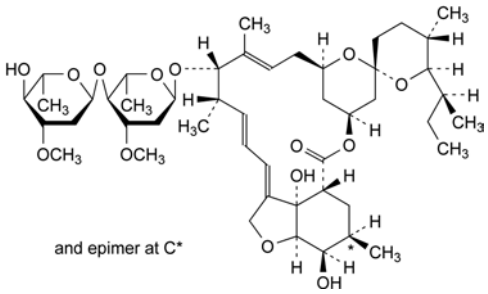
G. R = H: (6*R*,13*S*,25*R*)-5-*O*-demethyl-28-deoxy-6,28-epoxy-13-hydroxy-25-[(1*S*)-1-methylpropyl]milbemycin B (H_2B_{1a} aglycone),

H. R = osyl: 4′-*O*-de(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-5-*O*-demethyl-22,23-dihydroavermectin A_{1a},



I. R = C_2H_5: 2,3-didehydro-5-*O*-demethyl-3,4,22,23-tetrahydroavermectin A_{1a} ($\Delta^{2,3}$ H_2B_{1a}),

J. R = CH_3: 2,3-didehydro-5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-3,4,22,23-tetrahydroavermectin A_{1a} ($\Delta^{2,3}$ H_2B_{1b}),



K. (4*R*) and (4*S*)-5-*O*-demethyl-3,4,22,23-tetrahydroavermectin A_{1a} (H_4B_{1a} isomers).