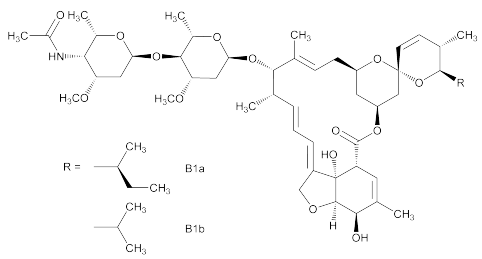
Eprinomectin



$C_{50}H_{75}NO_{14}$ (Component B_{1a}) 914.13

$C_{49}H_{73}NO_{14}$ (Component B_{1b}) 900.10

Component B_{1a}

Avermectin A_{1a}, 4″-(acetylamino)-5-*O*-demethyl-4″-deoxy-, (4″*R*)-;

(2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-(*S*)-*sec*-butyl-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5′,6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl-4-*O*-(4-acetamido-2,4,6-trideoxy-3-*O*-methyl-α-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranoside [or (4″*R*)-4″-(acetylamino)-5-*O*-demethyl-4″-deoxyavermectin A_{1a}] [133305-88-1].

Component B_{1b}

Avermectin A_{1a}, 4″-(acetylamino)-5-*O*-demethyl-25-de(1-methylpropyl)-4″-deoxy-25-(1-methylethyl)-, (4″*R*)-;

(2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6′-isopropyl-5′,6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl-4-*O*-(4-acetamido-2,4,6-trideoxy-3-*O*-methyl-α-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranoside [or (4″*R*)-4″-(acetylamino)-5-*O*-demethyl-25-de(1-methyl-propyl)-4″-deoxy-25-(1-methylethyl)avermectin A_{1a}] [133305-89-2].

DEFINITION

Eprinomectin is a mixture of component B_{1a} ($C_{50}H_{75}NO_{14}$) and component B_{1b} ($C_{49}H_{73}NO_{14}$). It contains NLT 90.0% of component B_{1a} ($C_{50}H_{75}NO_{14}$) and NLT 95.0% of components B_{1a} ($C_{50}H_{75}NO_{14}$) and B_{1b} ($C_{49}H_{73}NO_{14}$), calculated on the anhydrous, solvent-free, and antioxidant-free basis. It may contain small amounts of a suitable antioxidant.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197M⟩
- **B.** The retention times of the component B_{1a} peak and the component B_{1b} peak of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% (v/v) solution of perchloric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	45	55
15	45	55
25	5	95
30	45	55
35	45	55

Diluent: Methanol and water (4:1)

Standard solution: 0.500 mg/mL of USP Eprinomectin RS in *Diluent*

System suitability solution: Transfer 4 mL of *Standard solution* to an LC vial. Add 2 drops of 1 M sodium hydroxide and let stand for 20 min prior to injection.

Sample solution: 0.500 mg/mL of Eprinomectin in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 15 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—For relative retention times, see *Table 2*.]

Table 2

Components of the System Suitability Solution	Relative Retention Time
Impurity A	0.55
Component B_{1b}	0.77
Component B_{1a}	1.00
Impurities C + D	1.05
Impurity E	1.28

Suitability requirements

Resolution: NLT 3 between component B_{1b} and component B_{1a}; NLT 1 between component B_{1a} and impurities C + D, *System suitability solution*

Column efficiency: NLT 4,500 theoretical plates for component B_{1a}, *System suitability solution*

Symmetry factor: NMT 1.5 for component B_{1a}, *System suitability solution*

Relative standard deviation: NMT 1.0% from five injections for component B_{1a}, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of component B_{1a} ($C_{50}H_{75}NO_{14}$) in the portion of Eprinomectin taken:

$$\text{Result} = [r_{1a}/(r_{1a} + r_{1b})] \times 100$$

r_{1a} = peak area of component B_{1a} from the *Sample solution*

r_{1b} = peak area of component B_{1b} from the *Sample solution*

Calculate the percentage of component B_{1a} ($C_{50}H_{75}NO_{14}$) and component B_{1b} ($C_{49}H_{73}NO_{14}$) in the portion of Eprinomectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of component B_{1a} or component B_{1b} from the *Sample solution*

r_S = peak area of component B_{1a} or component B_{1b} from the *Standard solution*
C_S = concentration of component B_{1a} or component B_{1b} in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NLT 90.0% of component B_{1a} and NLT 95.0% of components B_{1a} and B_{1b}, on the anhydrous, solvent-free, and antioxidant-free basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%

Delete the following:

•• **Heavy Metals,** *Method II* ⟨**231**⟩**:** not more than 10 ppm.● (Official 1-Jan-2018)

Delete the following:

•• **Limit of Residual Solvents**

Standard solution A: Transfer 3.0 mL each of acetonitrile, methanol, isopropyl acetate, and heptane to a 50-mL volumetric flask. Dilute with dimethylacetamide to volume.

Standard solution B: Transfer 1.0 mL of *Standard solution A* to a 100-mL volumetric flask. Dilute with dimethylacetamide to volume. Further dilute 10.0 mL of this solution with dimethylacetamide to 50.0 mL.

Sample solution: 100 mg/mL of Eprinomectin in dimethylacetamide

Sensitivity solution A: Transfer 3.0 mL each of methanol, isopropyl acetate, and heptane to a 50-mL volumetric flask. Dilute with dimethylacetamide to volume. Further dilute 50 µL of this solution with dimethylacetamide to 25 mL.

Sensitivity solution B: Transfer 3.0 mL of acetonitrile to a 50-mL volumetric flask. Dilute with dimethylacetamide to volume. Further dilute 50 µL of this solution with dimethylacetamide to 25 mL.

Sensitivity solution C: Transfer 5.0 mL of *Sensitivity solution A* and 1.0 mL of *Sensitivity solution B* to a 50-mL volumetric flask. Dilute with dimethylacetamide to volume. [NOTE—This solution contains 100 ppm (m/m) of methanol, isopropyl acetate, and heptane and 20 ppm (m/m) of acetonitrile.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 25-m fused-silica analytical column coated with a 20-µm S3 stationary phase
Temperatures
Injection port: 200°
Detector: 220°
Column: See *Table 3*

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
110	5	160	5
160	30	220	25

Carrier gas: Helium
Flow rate: 20 mL/min
Injection volume: 1 µL

System suitability
Samples: *Standard solution B* and *Sensitivity solution C*
[NOTE—The relative retention times for methanol, acetonitrile, isopropyl acetate, and heptane are 1, 2.1, 7.6, and 8.6, respectively, *Sensitivity solution C*.]
Suitability requirements
Sensitivity: The peaks for methanol, acetonitrile, isopropyl acetate, and heptane are detectable, *Sensitivity solution C*.
Relative standard deviation: NMT 5.0% for six injections, *Standard solution B*

Analysis
Samples: *Standard solution B* and *Sample solution*. Reinject *Standard solution B* in duplicate after every six sample injections. The individual values for the area response of the two injections agree within ±5% of their corresponding average response.
Calculate the percentage of each solvent present in the portion of Eprinomectin taken:

$$\text{Result} = 0.12D \times (r_U/r_S)$$

r_U = solvent peak area from the *Sample solution*
r_S = solvent peak area from *Standard solution B*
D = density, mg/mL, of acetonitrile (0.787), isopropyl acetate (0.870), methanol (0.796), and heptane (0.684)

Acceptance criteria
Acetonitrile: NMT 0.005%
Total impurities: NMT 0.5% for the sum of all solvents● (IRA 1-Jan-2017)

• **Limit of 8a-oxo-B_{1a}**

Solution A, Solution B, Diluent, Standard solution, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and *Solution A* (13:7)
Butylated hydroxytoluene stock solution: 0.5 mg/mL of butylated hydroxytoluene in methanol. Sonicate to dissolve, if necessary.
Butylated hydroxytoluene solution: 0.01 mg/mL of butylated hydroxytoluene from *Butylated hydroxytoluene stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 15 µL

System suitability 1
Samples: *Standard solution* and *System suitability solution*
System suitability determination: Use the conditions as directed for *Chromatographic system* and the suitability requirements for *System suitability* as directed in the *Assay*.

System suitability 2
Samples: *Sample solution* and *Butylated hydroxytoluene solution*
Suitability requirements
Relative standard deviation: NMT 3.0% from six injections, *Butylated hydroxytoluene solution*

Analysis
Samples: *Sample solution* and *Butylated hydroxytoluene solution*
[NOTE—The retention time for 8a-oxo-B_{1a} is 4–9 min from the *Sample solution*, and the retention time for

butylated hydroxytoluene is 12–17 min from *Butylated hydroxytoluene solution*.]

Calculate the percentage of 8a-oxo-B_{1a}, on the anhydrous, solvent-free, and antioxidant-free basis in the portion of Eprinomectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times P \times 100$$

r_U = peak area of 8a-oxo-B_{1a} from the *Sample solution*
r_S = peak area of butylated hydroxytoluene from the *Butylated hydroxytoluene solution*
C_S = concentration of butylated hydroxytoluene in the *Butylated hydroxytoluene solution* (mg/mL)
C_U = concentration of Eprinomectin in the *Sample solution* (mg/mL)
F = relative response factor for butylated hydroxytoluene with respect to 8a-oxo-B_{1a}, 0.4
P = purity of butylated hydroxytoluene used to prepare the *Butylated hydroxytoluene solution*

Acceptance criteria: NMT 0.5%

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Eprinomectin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual related substance from the *Sample solution*
r_T = sum of the responses of all the peaks

Acceptance criteria: [NOTE—See *Table 2* for the relative retention times of impurity A and impurity E.]
Impurities with relative retention times of 0.23, 0.93, and 1.16 with respect to the B_{1a} peak: NMT 1.0%
Impurity A: NMT 1.0%
Impurity E: NMT 1.0%
All other known impurities: NMT 0.5%
Total unknown impurities: NMT 1.0
Total impurities: NMT 5.0%

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨**781S**⟩**,** *Procedures, Specific Rotation*

Sample solution: 5 mg/mL of Eprinomectin in chloroform
Acceptance criteria: +132° to +140°, determined at 405 nm on the anhydrous, solvent-free, and antioxidant-free basis

- **WATER DETERMINATION** ⟨**921**⟩**,** *Method 1, Method Ia*

Sample: 0.250 g
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 2° and 8° at ambient humidity.
- **LABELING:** Label it to state the name(s) and amount(s) of any added substance(s). Label to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Eprinomectin RS