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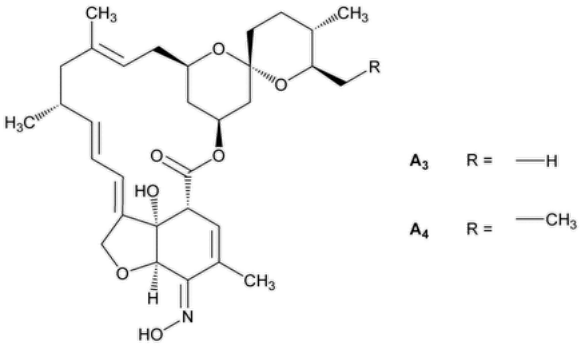
Milbemycin Oxime

Change to read:



Mixture of milbemycin A_3 oxime and milbemycin A_4 oxime CAS RN®: 129496-10-2.

Milbemycin A_3 Oxime $C_{31}H_{43}NO_7$ ▲541.69

Milbemycin B, 5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyamino)-25-methyl-, (6*R*,25*R*)-; (2*R*,2a'*E*,2a1'*S*,4'*E*,5*S*,6*R*,6'*R*,8'*E*,11'*R*,15'*S*,17a'*R*,20'*Z*,20a'*R*)-2a1'-Hydroxy-20'-(hydroxyimino)-5,6,6',8',19'-pentamethyl-2a1',3,4,5,6,6',7',10',11',14',15',17a',20',20a'-tetradecahydro-2'*H*,17'*H*-spiro[pyran-2,13'-[11,15]methano[1,5]dioxacyclooctadecino[9,8,7-*cd*]benzofuran]-17'-one.▲ (ERR 1-Jun-2023)

Milbemycin A_4 Oxime

$C_{32}H_{45}NO_7$ ▲555.71

Milbemycin B, 5-demethoxy-28-deoxy-6,28-epoxy-25-ethyl-5-(hydroxyamino)-, [6*R*,25*R*]-; (2*R*,2a'*E*,2a1'*S*,4'*E*,5*S*,6*R*,6'*R*,8'*E*,11'*R*,15'*S*,17a'*R*,20'*Z*,20a'*R*)-6-Ethyl-2a1'-hydroxy-20'-(hydroxyimino)-5,6',8',19'-tetramethyl-2a1',3,4,5,6,6',7',10',11',14',15',17a',20',20a'-tetradecahydro-2'*H*,17'*H*-spiro[pyran-2,13'-[11,15]methano[1,5]dioxacyclooctadecino[9,8,7-*cd*]benzofuran]-17'-one.▲ (ERR 1-Jun-2023)

DEFINITION

Milbemycin Oxime is a mixture of milbemycin A_4 oxime and milbemycin A_3 oxime. It contains NLT 95.0% and NMT 102.0% of the sum of milbemycin A_4 oxime and milbemycin A_3 oxime, calculated on the anhydrous basis. The ratio of milbemycin A_4 oxime is NLT 0.75.

IDENTIFICATION

- **A.** **Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Solution A: Water

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	26	74
2	26	74
27	24.5	75.5

Time (min)	Solution A (%)	Solution B (%)
47	24.5	75.5
47.1	26	74
60	26	74

Diluted phosphoric acid solution: 0.3–0.5 mL/L of phosphoric acid in water

Diluent: Acetonitrile and *Diluted phosphoric acid solution* (75:25)

Standard solution: 0.2 mg/mL of USP Milbemycin Oxime RS in *Diluent*. Sonicate if necessary to facilitate dissolution.

Sample solution: 0.2 mg/mL of Milbemycin Oxime in *Diluent*. Sonicate if necessary to facilitate dissolution. This solution must be stored at 5° protected from light.

Chromatographic system

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

Mode: LC

Detector: UV 240 nm

Column: 3.0-mm × 10-cm; 3-µm packing L1

Column temperature: 35°

Flow rate: 0.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The elution order is milbemycin A_3 oxime followed by milbemycin A_4 oxime. (See *Table 2* for the relative retention times.)]

Suitability requirements

Resolution: NLT 9 between milbemycin A_3 oxime and milbemycin A_4 oxime

Tailing factor: NMT 2.0 each for milbemycin A_3 oxime and milbemycin A_4 oxime

Relative standard deviation: NMT 0.73% each for milbemycin A_3 oxime and milbemycin A_4 oxime

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of milbemycin A_3 oxime ($C_{31}H_{43}NO_7$) in the portion of Milbemycin Oxime taken:

$$\text{Result} = (r_U/r_S) \times [(C_S \times F_{A3})/C_U] \times 100$$

r_U = peak response of milbemycin A_3 from the *Sample solution*

r_S = peak response of milbemycin A_3 from the *Standard solution*

C_S = concentration of USP Milbemycin Oxime RS in the *Standard solution* (mg/mL)

F_{A3} = potency of milbemycin A_3 oxime in USP Milbemycin Oxime RS (mg/mg)

C_U = concentration of Milbemycin Oxime in the *Sample solution* (mg/mL)

Calculate the percentage of milbemycin A_4 oxime ($C_{32}H_{45}NO_7$) in the portion of Milbemycin Oxime taken:

$$\text{Result} = (r_U/r_S) \times [(C_S \times F_{A4})/C_U] \times 100$$

r_U = peak response of milbemycin A_4 from the *Sample solution*

r_S = peak response of milbemycin A_4 from the *Standard solution*

C_S = concentration of USP Milbemycin Oxime RS in the *Standard solution* (mg/mL)

F_{A4} = potency of milbemycin A_4 oxime in USP Milbemycin Oxime RS (mg/mg)

C_U = concentration of Milbemycin Oxime in the *Sample solution* (mg/mL)

Calculate the percentage of the sum of milbemycin A_3 oxime and milbemycin A_4 oxime in the portion of Milbemycin Oxime taken:

$$\text{Result} = P_{A3} + P_{A4}$$

P_{A3} = percentage of milbemycin A_3 oxime

P_{A4} = percentage of milbemycin A_4 oxime

Calculate the ratio of milbemycin A_3 oxime in the portion of Milbemycin Oxime taken:

$$\text{Result} = P_{A3}/(P_{A3} + P_{A4})$$

P_{A3} = percentage of milbemycin A_3 oxime

P_{A4} = percentage of milbemycin A_4 oxime

Calculate the ratio of milbemycin A_4 oxime in the portion of Milbemycin Oxime taken:

$$\text{Result} = P_{A4}/(P_{A3} + P_{A4})$$

P_{A4} = percentage of milbemycin A_4 oxime

P_{A3} = percentage of milbemycin A_3 oxime

Acceptance criteria

Sum of milbemycin A_4 oxime and milbemycin A_3 oxime: 95.0%–102.0% on the anhydrous basis

Ratio of milbemycin A_4 oxime: NLT 0.75

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.5%
- **Organic Impurities**

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

Diluted standard solution: 0.002 mg/mL of USP Milbemycin Oxime RS in *Diluent* from *Standard solution*

Analysis

Samples: *Sample solution* and *Diluted standard solution*

Calculate the percentage of each impurity in the portion of Milbemycin Oxime taken:

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

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the peak responses of milbemycin A_3 oxime and milbemycin A_4 oxime from the *Diluted standard solution*

C_S = concentration of USP Milbemycin Oxime RS in the *Diluted standard solution* (mg/mL)

C_U = concentration of Milbemycin Oxime in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.10%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Milbemycin A_3 oxime	1.00	—
11'-Desmethylmilbemycin A_4 oxime[a]	1.17	0.7
(20'*R*)-Hydroxymilbemycin A_4 keto form[b]	1.33	0.5
Milbemycin A_4 oxime	1.43	—
Milbemycin A_4 keto form[c]	1.51	0.7
Milbemycin D oxime[d]	2.18	3.0
Any other individual impurity	—	0.5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities (excluding milbemycin D oxime)	—	3.5

[a] (1'*R*,2*R*,4'*S*,5*S*,6*R*,8'*R*,10'*E*,13'*R*,14'*E*,16'*E*,20'*R*,21'*Z*,24'*S*)-6-Ethyl-24'-hydroxy-21'-(hydroxyimino)-5,13',22'-trimethyl-3,4,5,6-tetrahydrospiro[pyran-2,6'-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2'-one.

[b] (1'*R*,2*R*,4'*S*,5*S*,6*R*,8'*R*,10'*E*,13'*R*,14'*E*,16'*E*,20'*R*,21'*Z*,24'*S*)-6-Ethyl-20',24'-dihydroxy-21'-(hydroxyimino)-5,11',13',22'-tetramethyl-3,4,5,6-tetrahydrospiro[pyran-2,6'-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2'-one.

[c] (1'*R*,2*R*,4'*S*,5*S*,6*R*,8'*R*,10'*E*,13'*R*,14'*E*,16'*E*,20'*S*,24'*S*)-6-Ethyl-24'-hydroxy-5,11',13',22'-tetramethyl-3,4,5,6-tetrahydrospiro[pyran-2,6'-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2',21'-dione.

[d] (1'*R*,2*R*,4'*S*,5*S*,6*R*,8'*R*,10'*E*,13'*R*,14'*E*,16'*E*,20'*R*,21'*Z*,24'*S*)-24'-Hydroxy-21'-(hydroxyimino)-6-(propan-2-yl)-5,11',13',22'-tetramethyl-3,4,5,6-tetrahydrospiro[pyran-2,6'-[3,7,19]trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa[10,14,16,22]tetraene]-2'-one.

SPECIFIC TESTS

- **Water Determination ⟨921⟩, *Method I*:** NMT 3.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at room temperature, protected from light.
- **Labeling:** Label it to indicate that it is for veterinary use only.
- **USP Reference Standards ⟨11⟩**
  USP Milbemycin Oxime RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
MILBEMYCIN OXIME	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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