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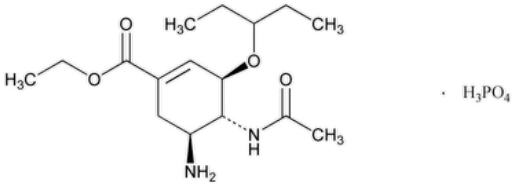
Oseltamivir Phosphate



$C_{16}H_{28}N_2O_4 \cdot H_3PO_4$ 410.40

[3*R*-(3α,4β,5α)]-Ethyl 4-(acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate (1:1);

Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate, phosphate (1:1) CAS RN®: 204255-11-8; UNII: 4A3O49NGEZ.

DEFINITION

Oseltamivir Phosphate contains NLT 98.0% and NMT 101.5% of $C_{16}H_{28}N_2O_4 \cdot H_3PO_4$, calculated on the anhydrous basis.

IDENTIFICATION

- **A.** **Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197M**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **Procedure**

Solution A: Dissolve 6.8 g of potassium dihydrogen phosphate in 980 mL of water. Adjust with 1 M potassium hydroxide solution to a pH of 6.0, and dilute with water to 1 L.

Mobile phase: Methanol, acetonitrile, and *Solution A* (245:135:620)

Diluent: Methanol, acetonitrile, and water (245:135:620)

Standard solution: 1 mg/mL of USP Oseltamivir Phosphate RS in *Diluent*

Sample solution: 1 mg/mL of Oseltamivir Phosphate in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 207 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 1.2 mL/min

Injection size: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{28}N_2O_4 \cdot H_3PO_4$ in the portion of Oseltamivir Phosphate taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

C_U = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous basis

IMPURITIES

Change to read:

Organic Impurities

- **Procedure 1**

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

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

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

r_S = peak response of oseltamivir phosphate from the *Standard solution*

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

C_U = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

F = relative response factor from *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.7%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oseltamivir acid[a]	0.17	1.4	0.3
Oseltamivir phenol[b]	0.51	2.7	0.1
Oseltamivir phosphate	1.00	1.0	—
Unspecified impurity	—	1.0	0.1
Total unspecified impurity	—	—	0.4

[a] (3*R*,4*R*,5*S*)-4-Acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid.

[b] 4-Acetylamino-3-hydroxybenzoic acid ethyl ester.

- **Procedure 2: Oseltamivir Related Compound A**

Buffer: 1.54 g/L of ammonium acetate in water

Mobile phase: Acetonitrile, water, and *Buffer* (3:6:1)

Stock solution A: 50 µg/mL of USP Oseltamivir Related Compound A RS, prepared as follows: Dissolve in alcohol, using 5% of final volume, and dilute with water to volume.

Solution A: 1 µg/mL of USP Oseltamivir Related Compound A RS in water from *Stock solution A*

Standard solution: 10 mg/mL of USP Oseltamivir Phosphate RS in *Solution A*

Sample solution: 10 mg/mL of Oseltamivir Phosphate in water

Chromatographic system

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

Mode: LC

Detectors: UV 210 nm and mass spectrometer

Column: 3.0-mm × 5-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 1 µL

Temperature: 40°

Use electrospray (+) ionization, a selected ion monitoring mode with m/z of 356.2 (protonated oseltamivir related compound A). Adjust the dwell time, fragmentation voltage, drying gas temperature, drying gas flow, nebulizer pressure, and capillary voltage as appropriate for an optimal response. [NOTE—A postcolumn flow splitter with a split ratio of about 3:1 is used.]

System suitability

Sample: *Standard solution*

[NOTE—The relative retention time for oseltamivir related compound A versus oseltamivir is about 2.6.]

Suitability requirements

Resolution: The oseltamivir related compound A peak (detected by MD-SIM mode) and the oseltamivir peak (detected by UV) are baseline resolved.

[NOTE—The resolution of the two components minimizes background noise and ion suppression effects for the trace of oseltamivir related compound A by the oseltamivir matrix.]

Relative standard deviation: NMT 15.0%, oseltamivir related compound A peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oseltamivir related compound A in the portion of Oseltamivir Phosphate taken:

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

r_U = peak response of oseltamivir related compound A from the *Sample solution*

r_S = peak response of oseltamivir related compound A from the *Standard solution*

C_S = concentration of USP Oseltamivir Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of oseltamivir phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.01%

- **PROCEDURE 3: LIMIT OF TRIBUTYL PHOSPHINE OXIDE**

Blank: Transfer 1.0 mL of suitable derivatizing reagent[1] to a vial. Close the vial, shake, and heat for 20 min at 60°. Centrifuge the pyridinium salt precipitate.

Standard stock solution 1: 21 mg/mL of USP Tributyl Phosphine Oxide RS in pyridine

Standard stock solution 2: 21 mg/mL of USP Oseltamivir Phosphate RS in suitable derivatizing reagent. Close the vial, mix, and heat for 20 min at 60°. Centrifuge the pyridinium salt precipitate.

Standard solution: 21 µg/mL each of USP Tributyl Phosphine Oxide RS and USP Oseltamivir Phosphate RS in pyridine from *Standard stock solution 1* and *Standard stock solution 2*, respectively

Sample solution: Transfer 15 mg of Oseltamivir Phosphate to a vial. Add 1.0 mL of suitable derivatizing reagent. Close the vial, mix, and heat for 20 min to 60°. Centrifuge the pyridinium salt precipitate.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m capillary column coated with a 0.25-µm phase G1

Split ratio: 1:50

Split flow: 64 mL/min

Injection size: 1 µL

Temperature

Detector: 260°

Injection port: 260°

Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
180	0	180	2
180	8	250	10

Linear velocity: 27 cm/s

Carrier gas: Helium

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for tributyl phosphine oxide and oseltamivir phosphate are about 0.54 and 1.00, respectively.]

Suitability requirements

Relative standard deviation: NMT 10.0% for the tributyl phosphine oxide and ▲oseltamivir▲ (ERR 1-Jun-2023) phosphate peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tributyl phosphine oxide in the portion of Oseltamivir Phosphate taken:

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

r_U = peak response of tributyl phosphine oxide from the *Sample solution*

r_S = peak response of tributyl phosphine oxide from the *Standard solution*

C_S = concentration of tributyl phosphine oxide in the *Standard solution* (mg/mL)

C_U = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **WATER DETERMINATION, *Method I*⟨921⟩:** NMT 0.5%
- **OPTICAL ROTATION, *Specific Rotation*⟨781S⟩**

Sample solution: 10 mg/mL in water

Acceptance criteria: Between −30.7 and −32.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Oseltamivir Phosphate RS

USP Oseltamivir Related Compound A RS

(3*S*,4*R*,5*S*)-Ethyl 4-acetamido-5-amino-2-azido-3-(pentan-3-yloxy)cyclohexanecarboxylate.

$C_{16}H_{29}N_5O_4$ 355.43

USP Tributyl Phosphine Oxide RS $C_{12}H_{27}OP$ 218.32

[1] Tri-Sil Reagent (product number: 48999 0049001) may be obtained from Pierce: www.piercenet.com.

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OSELTAMIVIR PHOSPHATE	Documentary Standards Support	SM12020 Small Molecules 1
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Chromatographic Database Information: Chromatographic Database

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