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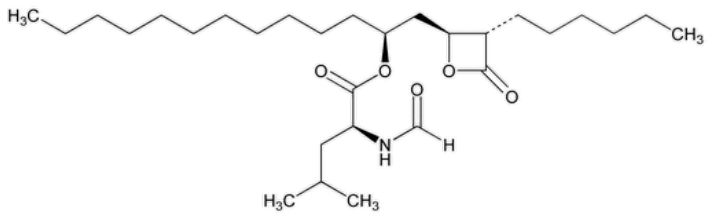
# Orlistat

***Change to read:***



$C_{29}H_{53}NO_5$ ▲495.75▲ (IRA 1-Jan-2022)

L-Leucine, *N*-formyl-, 1-[(3-hexyl-4-oxo-2-oxetanyl)methyl]dodecyl ester, [2*S*-[2α(*R**), 3β]]-;

*N*-Formyl-L-leucine, ester with (3*S*,4*S*)-3-hexyl-4-[(2*S*)-2-hydroxytridecyl]-2-oxetanone;

▲(*S*)-1-[(2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl]tridecan-2-yl formyl-L-leucinate▲ (IRA 1-Jan-2022) CAS RN®: 96829-58-2; UNII: 95M8R751W8.

## DEFINITION

Orlistat contains NLT 98.0% and NMT 101.5% of orlistat ($C_{29}H_{53}NO_5$), calculated on the anhydrous, solvent-free basis.

## IDENTIFICATION

***Change to read:***

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K or ▲197A▲ (IRA 1-Jan-2022)

• **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

***Change to read:***

• **Procedure**

[Note—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]

**Mobile phase:** Acetonitrile, phosphoric acid, and water (860: 0.05: 140)

**Standard solution:** 0.5 mg/mL of USP Orlistat RS in *Mobile phase*. Inject immediately after preparation or store at 5°.

**Sample solution:** 0.5 mg/mL of Orlistat in *Mobile phase*. Inject immediately after preparation or store at 5°.

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 195 ▲nm▲ (IRA 1-Jan-2022)

**Column:** 3.9-mm × 15-cm; 4-µm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 µL

▲**Run time:** NLT 5.5 times the retention time of orlistat▲ (IRA 1-Jan-2022)

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT ▲1.0%▲ (IRA 1-Jan-2022)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of orlistat ($C_{29}H_{53}NO_5$) in the portion of Orlistat taken:

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

$r_U$ = peak response of orlistat from the *Sample solution*

$r_S$ = peak response of orlistat from the *Standard solution*

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

$C_U$ = concentration of Orlistat in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–101.5% on the anhydrous, solvent-free basis

## IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Limit of Orlistat Related Compound A**

**Standard solution:** 0.1 mg/mL of USP Orlistat Related Compound A RS in acetone

**Sample solution:** 50 mg/mL of Orlistat in acetone

**Chromatographic system**

(See *Chromatography ⟨621⟩, General Procedures, Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Toluene and ethyl acetate (4:1)

**Detection solution:** Transfer 2.5 g of phosphomolybdic acid and 1 g of ceric sulfate into a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Remove the plate, and air-dry it thoroughly. Spray the dried plate with *Detection solution*, and place the plate in an oven at 120° for 30 min.

**Acceptance criteria:** Any secondary spot from the *Sample solution* corresponding to orlistat related compound A is not more intense than the corresponding spot from the *Standard solution* (0.2%).

- **Limit of Orlistat Related Compound B**

**Standard solution:** 0.025 mg/mL of USP Orlistat Related Compound B RS in methylene chloride

**Sample solution:** 50 mg/mL of Orlistat in methylene chloride

**Spiked sample solution:** 50 mg/mL of Orlistat in *Standard solution*

**Chromatographic system**

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

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 30-m fused silica, coated with a 0.25-µm G27 stationary phase

**Temperatures**

**Injector:** 270°

**Detector:** 280°

**Column:** See *Table 1*.

**Table 1**

| Initial Temperature (°) | Temperature Ramp (°/min) | Final Temperature (°) | Hold Time at Final Temperature (min) |
|---|---|---|---|
| 50 | 4 | 170 | — |
| 170 | 30 | 300 | 30 |

**Carrier gas:** Helium

**Flow rate:** 1 mL/min

**Injection type:** Split, split ratio 20:1

**Injection volume:** 2 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 10.0%

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Spiked sample solution*

Calculate the percentage of orlistat related compound B in the portion of Orlistat taken:

$$\text{Result} = \{r_U/[r_{SP} - r_U \times (C_T/C_U)]\} \times (C_S/C_U) \times 100$$

$r_U$ = peak response of orlistat related compound B from the *Sample solution*

$r_{SP}$ = peak response of orlistat related compound B from the *Spiked sample solution*

$C_T$ = concentration of Orlistat in the *Spiked sample solution* (mg/mL)

$C_U$ = concentration of Orlistat in the *Sample solution* (mg/mL)

$C_S$ = concentration of USP Orlistat Related Compound B RS in the *Standard solution* (mg/mL)

**Acceptance criteria:** NMT 0.05%

***Change to read:***

• **Organic Impurities**

[Note—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]

**Mobile phase, Standard solution,** and **Sample solution:** Prepare as directed in the *Assay*.

**System suitability solution:** 10 µg/mL of USP Orlistat RS and 0.1 µg/mL of USP Orlistat Related Compound C RS in *Mobile phase*

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 195 nm

**Column:** 3.9-mm × 15-cm; 4-µm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 µL

▲**Run time:** NLT 5.5 times the retention time of orlistat▲ (IRA 1-Jan-2022)

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Relative standard deviation:** NMT ▲5.0%▲ (IRA 1-Jan-2022) for the orlistat peak

**Signal-to-noise ratio:** NLT 3 for orlistat related compound C

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity other than orlistat related compound A, orlistat related compound B, orlistat related compound D, orlistat open ring amide, and orlistat related compound E in the portion of Orlistat 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 orlistat from the *Standard solution*

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

$C_U$ = concentration of Orlistat in the *Sample solution* (mg/mL)

$F$ = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| Formylleucine[a] | 0.10 | 4.0 | 0.2 |
| Orlistat related compound C | 0.13 | 33 | 0.05 |
| Orlistat open ring epimer[b] | 0.44 | 1.0 | 0.2 |
| Orlistat related compound D[c] | 0.90 | — | see *Table 3* |
| Orlistat open ring amide[c,d] | 0.90 | — | see *Table 3* |
| Orlistat | 1.00 | — | — |
| D-Leucine orlistat[e] | 1.18 | 1.0 | 0.2 |
| ▲Any individual unspecified impurity▲ (IRA 1-Jan-2022) | — | 1.0 | 0.1 |
| Total impurities[f] | — | — | 1.0 |

[a] *N*-Formyl-L-leucine.

[b] ▲(2*S*,3*R*,5*S*)-5-[(Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.▲ (IRA 1-Jan-2022)

[c] Coelutes in this LC system, determined using *Limits of Orlistat Related Compound D and Orlistat Open Ring Amide*.

[d] ▲(7*S*,8*S*,10*S*)-8-Hydroxy-7-{[(*R*)-1-phenylethyl]carbamoyl}henicosan-10-yl formyl-L-leucinate.▲ (IRA 1-Jan-2022)

[e] ▲(*S*)-1-[(2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl]tridecan-2-yl formyl-D-leucinate.▲ (IRA 1-Jan-2022)

[f] Sum of results obtained in the tests for *Limit of Orlistat Related Compound A*, *Limit of Orlistat Related Compound B*, *Organic Impurities*, *Limits of Orlistat Related Compound D and Orlistat Open Ring Amide*, and *Limit of Orlistat Related Compound E*.

***Change to read:***

• **Limits of Orlistat Related Compound D and Orlistat Open Ring Amide**

**Mobile phase:** Methanol and water (830:170)

**System suitability solution:** ▲4 mg/mL of USP Orlistat RS and 2.5 µg/mL of orlistat related compound D prepared by dissolving a suitable amount of USP Orlistat RS and diluting USP Orlistat Related Compound D Solution RS in acetonitrile▲ (IRA 1-Jan-2022)

**Standard solution:** 5.0 mg/mL of USP Orlistat RS in acetonitrile

**Sample solution:** 5.0 mg/mL of Orlistat in acetonitrile

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.0-mm × 25-cm; 5-µm packing L7

**Flow rate:** 0.6 mL/min

**Injection volume:** 20 µL

▲**Run time:** NLT 1.4 times the retention time of orlistat▲ (IRA 1-Jan-2022)

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Relative standard deviation:** NMT ▲5.0%▲ (IRA 1-Jan-2022) for the orlistat peak

**Signal-to-noise ratio:** NLT 3 for the orlistat related compound D peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of orlistat related compound D and orlistat open ring amide in the portion of Orlistat taken:

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

$r_U$ = peak response of orlistat related compound D or orlistat open ring amide from the *Sample solution*

$r_S$ = peak response of orlistat from the *Standard solution*

$C_S$ = concentration of USP Orlistat RS in the *Standard solution* (▲mg/mL▲ (IRA 1-Jan-2022))

$C_U$ = concentration of Orlistat in the *Sample solution* (▲mg/mL▲ (IRA 1-Jan-2022))

$F$ = relative response factor (see *Table 3*)

**Acceptance criteria:** See *Table 3*.

**Table 3**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| Orlistat related compound D | 0.94 | 1.0 | 0.2 |
| Orlistat | 1.00 | — | — |
| Orlistat open ring amide[a] | 1.25 | 4.3 | 0.1 |

a ▲(7*S*,8*S*,10*S*)-8-Hydroxy-7-{[(*R*)-1-phenylethyl]carbamoyl}henicosan-10-yl formyl-L-leucinate.▲ (IRA 1-Jan-2022)

***Change to read:***

• **Limit of Orlistat Related Compound E**

**Buffer:** 0.4 N borate solution, adjusted to a pH of 10.2

**Derivatizing agent:** o-Phthalaldehyde (OPA) solution. [Note—If unable to obtain commercially, the *Derivatizing agent* can be prepared as 1% each of 3-mercaptopropionic acid and o-phthalaldehyde in 0.4 M borate buffer solution.]

**Solution A:** Transfer 4.1 g of sodium acetate trihydrate and 40 mg of ethylenediaminetetraacetic acid (EDTA) into a 1-L volumetric flask. Dissolve in 950 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 7.2. Dilute with water to volume, add 2.5 mL of tetrahydrofuran, and mix. Filter, and degas.

**Solution B:** Transfer 2.7 g of sodium acetate trihydrate and 40 mg of EDTA into a 1-L volumetric flask. Dissolve in 200 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 7.2. Add 800 mL of acetonitrile, filter, and degas.

**Mobile phase:** See *Table 4*.

**Table 4**

| Time (min) | Solution A (%) | Solution B (%) |
|---|---|---|
| 0 | 96.7 | 3.3 |
| 20 | 60 | 40 |
| 24 | 0 | 100 |
| ▲34▲ (IRA 1-Jan-2022) | 0 | 100 |
| 38 | 96.7 | 3.3 |
| 45 | 96.7 | 3.3 |

**Standard solution:** Transfer a weighed quantity of about 0.2 mg of USP Orlistat Related Compound E RS into a 20-mL headspace vial. Add 10 mL of 4 N sodium hydroxide, and close the vial. Heat the vial to 100° for 1 h, then allow to cool to room temperature. Transfer 2 mL of the resulting solution into a 50-mL volumetric flask, and dilute with water to volume. To 0.5 mL of this solution add 2.0 mL of *Buffer* and 0.5 mL of *Derivatizing agent*.

**Sample solution:** Proceed as directed for the *Standard solution*, except use 25 mg of Orlistat to replace the 0.2 mg of USP Orlistat Related Compound E RS.

**Chromatographic system**

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

**Mode:** LC

**Detector:** Fluorescence 340 nm (excitation); 450 nm (emission)

**Columns**

**Guard:** 2.1-mm × 2-cm; 5-µm packing L1

**Analytical:** 2.1-mm × 20-cm; 5-µm packing L1

**Flow rate:** 0.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 6.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of orlistat related compound E in the portion of Orlistat taken:

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

$r_U$ = peak response of orlistat related compound E from the *Sample solution*

$r_S$ = peak response of orlistat related compound E from the *Standard solution*

$C_S$ = concentration of USP Orlistat Related Compound E RS in the *Standard solution* (mg/mL)

$C_U$ = concentration of Orlistat in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.2%

## SPECIFIC TESTS

- **Optical Rotation ⟨781S⟩, *Procedures, Specific Rotation***

**Sample solution:** 30 mg/mL in dehydrated alcohol

**Acceptance criteria:** Between −48.0° and −51.0°, at 20°

- **Water Determination ⟨921⟩, *Method I, Method Ic*:** NMT 0.2%

## ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers between 2° and 8°.

***Change to read:***

- **USP Reference Standards ⟨11⟩**

USP Orlistat RS

USP Orlistat Related Compound A RS

▲(3*S*,4*S*)-3-Hexyl-4-[(*R*)-2-hydroxytridecyl]oxetan-2-one.▲ (IRA 1-Jan-2022)

$C_{22}H_{42}O_3$ ▲354.58▲ (IRA 1-Jan-2022)

USP Orlistat Related Compound B RS

Diisopropyl hydrazine-1,2-dicarboxylate.

$C_8H_{16}N_2O_4$ ▲204.23▲ (IRA 1-Jan-2022)

USP Orlistat Related Compound C RS

Triphenylphosphine oxide.

$C_{18}H_{15}OP$ ▲278.29

USP Orlistat Related Compound D Solution RS

Orlistat related compound D;
(3*S*,4*R*,6*S*)-3-Hexyl-2-oxo-6-undecyltetrahydro-2*H*-pyran-4-yl formyl-L-leucinate.

$C_{29}H_{53}NO_5$ 495.75▲ (IRA 1-Jan-2022)

USP Orlistat Related Compound E RS

▲(*S*)-1-[(2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl]tridecan-2-yl formyl-L-isoleucinate.▲ (IRA 1-Jan-2022)

$C_{29}H_{53}NO_5$ ▲495.75▲ (IRA 1-Jan-2022)

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

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| ORLISTAT | Documentary Standards Support | SM22020 Small Molecules 2 |
| REFERENCE STANDARD SUPPORT | RS Technical Services<br>RSTECH@usp.org | SM22020 Small Molecules 2 |

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