Table 1

Component	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
cis-1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-5,6,8-trifluoro-4(1*H*)-quinolinone	0.57	0.29	NMT 0.5
Orbifloxacin	1.0	1.00	—
cis-1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-5-hydroxy-4-oxo-3-quinolinecarboxylic acid	2.9	0.71	NMT 0.5
All other related compounds and impurities	—	0.11	NMT 0.5
Total known and unknown	—	—	NMT 1

Buffer to 200 mL. Pipet 10.0 mL of this solution and 10.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask. Dilute with *Buffer* to volume, and mix.

Assay preparation—Transfer 10 Tablets into a volumetric flask. Add *Buffer* to fill the flask about 70%, shake for 2 hours, and sonicate for 5 minutes. Dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a known concentration of about 0.02 mg per mL. Pass a portion of the solution through a 0.8-µm filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 290-nm detector and 4.6-mm × 3-cm column that contains 3-µm packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *System suitability preparation,* and record the peak response as directed for *Procedure:* the relative retention times are about 1.3 for methyl 4-aminobenzoate and 1.0 for orbifloxacin; the resolution, *R,* between methyl 4-aminobenzoate and orbifloxacin is not less than 2; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

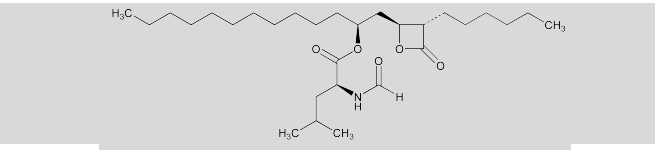
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of orbifloxacin ($C_{19}H_{20}F_3N_3O_3$) in the portion of Tablets taken by the formula:

$$C(D_U)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Orbifloxacin RS in the *Standard preparation;* D_U is the dilution factor of the *Assay preparation,* in mL; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Add the following:

▲Orlistat



$C_{29}H_{53}NO_5$ 495.73

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 [96829-58-2].

DEFINITION

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

IDENTIFICATION

- **A. Infrared Absorption** ⟨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**

[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

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

Flow rate: 1.0 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

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 from the *Sample solution*
r_S = peak response 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

Inorganic Impurities

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
- **Heavy Metals,** *Method II* ⟨231⟩**:** 20 ppm

Organic Impurities

- **Procedure 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⟩, *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 cerric sulfate into a 100-mL volumetric flask, 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%).

• **PROCEDURE 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 x 30-m fused silica, coated with a 0.25-µm G27 stationary phase
Column temperature: See the temperature program table below.

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

Temperature
Injector: 270°
Detector: 280°
Carrier gas: Helium
Flow rate: 30 mL/min
Split ratio: 10:1
Injection size: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Sample solution* and *Spiked sample solution*
Calculate the percentage of orlistat related compound B in the portion of Orlistat taken:

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

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_S = concentration of USP Orlistat Related Compound B RS in the *Standard solution* (mg/mL)
C_T = concentration of Orlistat in the *Spiked sample solution* (mg/mL)
Acceptance criteria: NMT 0.05% of orlistat related compound B is found.

• **PROCEDURE 3**
[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, 0.1 µg/mL of USP Orlistat Related Compound C RS, and 0.25 µg/mL of USP Orlistat Related Compound D RS in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Proceed as directed in the *Assay*, except to chromatograph the *System suitability solution*.
System suitability
Sample: *System suitability solution*
Suitability requirements
Signal-to-noise ratio: NLT 3 for the orlistat related compound C and orlistat related compound D peaks
Relative standard deviation: NMT 10.0% for the orlistat peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity 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 for each individual impurity from the *Sample solution*
r_S = peak response of USP Orlistat RS 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 as given in *Impurity Table 1*
Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

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*	0.90	—	Calculated in *Procedure 4*
Orlistat open ring amide[c]*	0.90	—	Calculated in *Procedure 4*
Orlistat	1.00	—	—
D-Leucine orlistat[d]	1.18	1.0	0.2
Individual unidentified impurity	—	1.0	0.1

* Coelutes in this LC system, determined using *Procedure 4*.
[a] *N*-Formyl-L-leucine.
[b] (2*S*,3*R*,5*S*)-5-[(*S*)-2-Formylamino-4-methyl-pentanoyloxy]-2-hexyl-3-hydroxy-hexadecanoic acid.
[c] *N*-Formyl-L-leucine (*S*)-1-[(2*S*,3*S*)-2-hydroxy-3-[1-phenyl-*R*-ethylcarbomoyl]nonyl]-dodecyl ester.
[d] *N*-Formyl-D-leucine (*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester or enantiomer.

• **PROCEDURE 4: LIMIT OF ORLISTAT RELATED COMPOUND D**
Mobile phase: Methanol and water (83:17)
System suitability solution: 4 mg/mL of USP Orlistat RS and 2.4 µg/mL of USP Orlistat Related Compound D RS in acetonitrile, respectively
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: 205 nm
Column: 4.0-mm × 25-cm; 5-µm packing L7
Flow rate: 0.6 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Signal-to-noise ratio: NLT 3 for the orlistat related compound D peak
Relative standard deviation: NMT 10.0% for the orlistat peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity 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 for each individual impurity from the *Sample solution*
r_S = peak response for USP Orlistat RS from the *Standard solution*
C_S = concentration of USP Orlistat RS in the *Standard solution* (µg/mL)
C_U = concentration of Orlistat in the *Sample solution* (µg/mL)
F = relative response factor as obtained in *Impurity Table 2*
Acceptance criteria: See *Impurity Table 2*.

Impurity Table 2

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] *N*-Formyl-L-leucine (*S*)-1-[(2*S*,3*S*)-2-hydroxy-3-[1-phenyl-*R*-ethylcarbomoyl]nonyl]-dodecyl ester.

• **PROCEDURE 5: LIMIT OF ORLISTAT RELATED COMPOUND E**
Buffer: 0.4 N borate solution, adjusted to a pH of 10.2
Derivitizing agent: *o*-Phthaldehyde (OPA) solution.
[NOTE—If unable to obtain commercially, the *Derivitizing agent* can be prepared as 1% each of 3-mercaptopropionic acid and *o*-phthaldialdehyde 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 the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	96.7	3.3
20	60	40
24	0	100
38	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 head-space 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*, but instead 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; 50-µm packing L1
Analytical: 2.1-mm × 20-cm; packing L1
Flow rate: 0.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 6.0% for the orlistat related compound E peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of this impurity in the portion of Orlistat taken:

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

r_U = peak response for orlistat related compound E in the *Sample solution*
r_S = peak response for USP Orlistat Related Compound E RS in 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
Individual impurity: NMT 0.2% of orlistat related compound E is found.
Total impurities: NMT 1.0% of total impurities is found, the results for *Procedures 1, 2, 3, 4,* and *5* being added.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781**⟩
Sample solution: 30 mg/mL in dehydrated alcohol
Acceptance criteria: Between –48.0° and –51.0°, at 20°
• **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 0.2%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers between 2° and 8°.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Orlistat RS
USP Orlistat Related Compound A RS
USP Orlistat Related Compound B RS
USP Orlistat Related Compound C RS
USP Orlistat Related Compound D RS
USP Orlistat Related Compound E RS▲*USP35*

Add the following:

▲Orlistat Capsules

DEFINITION

Orlistat Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of orlistat ($C_{29}H_{53}NO_5$).

IDENTIFICATION

- 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**

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

Standard solution: 0.6 mg/mL of USP Orlistat RS in *Mobile phase*

Sample solution: Transfer the contents of NLT 10 Capsules into a suitable container, weigh, and mix. Transfer an accurately weighed portion of the powder, equivalent to 120 mg of orlistat, into a 200-mL volumetric flask. Add 140 mL of *Mobile phase*, and sonicate for 1 min. Shake the resulting solution mechanically for 15 min, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter of 0.45-µm or finer pore size, discarding the first few mL of filtrate.

Chromatographic system

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

Mode: LC

Detector: 195 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.0 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

System suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of orlistat ($C_{29}H_{53}NO_5$) in the portion of Capsules 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 Orlistat RS in the *Standard solution* (mg/mL)
C_U = concentration of orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩

Medium: 3% Sodium lauryl sulfate and 0.5% sodium chloride in water. To each 10 L of media add 1–2 drops of *n*-octanol, and adjust with phosphoric acid to a pH of 6.0; 900 mL.

Apparatus 2: 75 rpm, with coil wire sinker

Time: 45 min

Mobile phase: Acetonitrile and water (860:140)

Standard solution: Transfer about 13 mg of USP Orlistat RS to a 100-mL volumetric flask. Dissolve in 2 mL of acetonitrile, and dilute with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.2-µm pore size.

Chromatographic system

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

Mode, Detector, and **Column:** Proceed as directed in the *Assay.*

Flow rate: 2.0 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

System suitability requirements

Relative standard deviation: NMT 2.0%

Calculate the percentage of the labeled amount of orlistat ($C_{29}H_{53}NO_5$) dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of orlistat is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

- **PROCEDURE**

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

System suitability solution: 0.025 mg/mL of USP Orlistat Related Compound D RS in *Mobile phase*. Transfer 1 mL of this solution to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

Chromatographic system

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

System suitability

Sample: *System suitability solution*

System suitability requirements

Resolution: NLT 1.4 between USP Orlistat RS and USP Orlistat Related Compound D RS

Relative standard deviation: NMT 2.0% for the orlistat peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules 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 in the *Sample solution*
r_S = peak response of orlistat in 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 *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Orlistat open-ring epimer[a]	0.45	1.0	1.5
Orlistat open ring[b]	0.5	1.0	0.3
Orlistat related compound D	0.9	1.0	1.0
Orlistat	1.0	—	—
Hexyl undecyl pyranone[c]	2.0	1.0	0.2

[a] (2*S*,3*R*,5*S*)-5-[(*N*-Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.
[b] (2*S*,3*S*,5*S*)-5-[(*N*-Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.
[c] (*S*)-3-Hexyl-5,6-dihydro-6-undecyl-2*H*-pyran-2-one.
[d] (*S*)-[(*S*,*E*)-Henicos-7-en-10-yl] *N*-formyl-L-leucinate.