Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) dissolved by the same formula:

$$1170C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard solution;* and r_U and r_S are the responses of the corresponding analyte obtained from the *Test solution* and the *Standard solution,* respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$) is dissolved in 45 minutes, and not less than 70% *(Q)* of the labeled amount of chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Procedure for content uniformity—Proceed as directed in the *Assay,* except to prepare the *Assay preparation* as follows. Transfer 1 Tablet to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.25 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablet. Dilute with water to volume, and mix. Pass a portion of this solution through a filter having a 0.5- µm or finer porosity, and use the filtrate as the *Assay preparation.* Calculate the quantities, in mg, of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) in the Tablet taken by the formula:

$$CV(r_U / r_S)$$

in which V is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation;* and the other terms are as defined in the *Assay.*

Assay—

Mobile phase—Prepare a mixture of 740 mL of water, 250 mL of acetonitrile, 8 mL of 3.6 N sulfuric acid, and 930 mg of sodium octyl sulfate. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Atenolol RS and USP Chlorthalidone RS in a mixture of water and acetonitrile (3:1) to obtain a solution having known concentrations of about 0.25 mg of USP Chlorthalidone RS and 0.25J mg of USP Atenolol RS per mL, J being the ratio of the labeled amount, in mg, of atenolol to the labeled amount, in mg, of chlorthalidone per Tablet.

Assay preparation—Transfer 10 Tablets to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.5 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablets. Dilute with a mixture of water and acetonitrile (1:1) to volume, and mix. Pass a portion of this stock solution through a filter having a 0.5- µm or finer porosity. Transfer 25.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for atenolol and 1.0 for chlorthalidone; the resolution, R, between the atenolol and chlorthalidone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

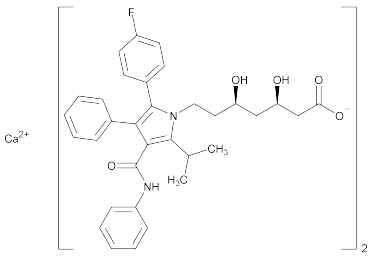
Procedure—Separately inject equal volumes (about 10 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) in each Tablet taken by the formula:

$$2C(V/10)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation; V* is the volume, in mL, of the volumetric flask used to prepare the stock solution for the *Assay preparation;* and r_U and r_S are the responses for the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation,* respectively.

NOTE—If a trailing peak or shoulder is observed on the chlorthalidone peak with a relative retention time of not more than 1.1 in the chromatograms of both the *Standard preparation* and the *Assay preparation,* sum the areas for the chlorthalidone peak with the trailing peak or shoulder to report the peak responses for chlorthalidone.

Atorvastatin Calcium



$C_{66}H_{68}CaF_2N_4O_{10} \cdot 3H_2O$ 1209.42

$C_{66}H_{68}CaF_2N_4O_{10}$ 1155.34

1*H*-Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-, calcium salt (2:1), trihydrate [*R*-(*R**,*R**)]-;
Calcium (β*R*,δ*R*)-2-(*p*-fluorophenyl)-β,δ-dihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrole-1-heptanoate (1:2), trihydrate [344423-98-9].
Anhydrous [134523-03-8].

DEFINITION

Atorvastatin Calcium contains NLT 98.0% and NMT 102.0% of $C_{66}H_{68}CaF_2N_4O_{10}$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩
- **B. Calcium**

Diluent: Methanol, water, and hydrochloric acid (75:25:2)
Blank: *Diluent*
Sample solution: 0.05 mg/mL of Atorvastatin Calcium in *Diluent*
Analysis
Samples: *Sample solution* and *Blank*
Spectrometric conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Calcium emission line at 422.7 nm
Flame: Air–acetylene
Acceptance criteria: The *Sample solution* exhibits a significant absorption at the calcium emission line at 422.7 nm.

ASSAY

- **Procedure**

Buffer: 3.9 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.0 ± 0.1.
Solution A: Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (21:12:67)

Solution B: Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (61:12:27)
Diluent: *N,N*-dimethylformamide
Mobile phase: See the gradient table below.
[NOTE—If necessary, adjust the *Mobile phase* by increasing or decreasing the percentage of acetonitrile or the pH of the ammonium acetate solution to achieve a retention time of 26–34 min for the atorvastatin peak.]

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	100	0
70	20	80
85	0	100
100	0	100
105	100	0
115	100	0

System suitability solution: 0.05 mg/mL of USP Atorvastatin Calcium RS and 0.06 mg/mL of USP Atorvastatin Related Compound B RS in *Diluent*
Standard solution: 0.4 mg/mL of USP Atorvastatin Calcium RS in *Diluent*. [NOTE—Use sonication if necessary.]
Sample solution: 0.4 mg/mL of Atorvastatin Calcium in *Diluent*. [NOTE—Use sonication if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—If significant fronting of the peaks for atorvastatin related compound B and atorvastatin is observed, use the following *Diluent* to prepare the *Sample solution*, *Standard solution*, and *System suitability solution*: acetonitrile, stabilizer-free tetrahydrofuran, and water (1:1:2).]
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the peaks for atorvastatin related compound B and atorvastatin, *System suitability solution*
Tailing factor: NMT 1.6, *Standard solution*
Relative standard deviation: NMT 0.6%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{66}H_{68}CaF_2N_4O_{10}$ in the portion of Atorvastatin Calcium 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 Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Atorvastatin Calcium in the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

• **HEAVY METALS**
Diluent: Methanol and water (9:1)
Sample solution: Dissolve 250 mg of the sample in 30 mL of *Diluent*.
Standard lead solution: Prepared as directed under *Heavy Metals* ⟨231⟩.
Reference solution: Dilute 0.5 mL of the *Standard lead solution* with *Diluent* to 30 mL.
Blank solution: 20 mL of *Diluent*
Monitor solution: Dissolve 250 mg of Atorvastatin Calcium in 0.5 mL of the *Standard lead solution*, and dilute with *Diluent* to 30 mL.
Analysis
Samples: *Sample solution, Reference solution, Blank solution,* and *Monitor solution*
To each solution, add 2 mL of *pH 3.5 Acetate Buffer*, prepared as directed under *Heavy Metals* ⟨231⟩. Mix, add to 1.2 mL of thioacetamide–glycerin base TS, and mix immediately. Pass the solutions through a membrane filter of 0.45-µm pore size. Compare the spots on the filters obtained with the different solutions: the brown color of the spot from the *Sample solution* is not more intense than that of the spot from the *Reference solution*. The test is invalid if the *Reference solution* does not show a slight brown color compared to the *Blank solution*, or if the color of the *Monitor solution* is not at least as intense as the color of the *Reference solution*.
Acceptance criteria: NMT 20 ppm

Organic Impurities

• **PROCEDURE**
Buffer, Solution A, Solution B, Diluent, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed for the *Assay*.
Standard solution: 1.5 µg/mL each of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound C RS, and USP Atorvastatin Related Compound D RS in *Diluent*
Sample solution: 1 mg/mL of Atorvastatin Calcium in *Diluent*. [NOTE—Use sonication if necessary.]
Analysis
Samples: *Standard solution* and *Sample solution*
Chromatograph the *Standard solution,* and identify the components on the basis of their relative retention times, given in *Impurity Table 1*.
Calculate the percentage of each of the atorvastatin related compounds A, B, C, and D in the portion of Atorvastatin Calcium taken:

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

r_U = peak response of the relevant atorvastatin related compound from the *Sample solution*
r_S = peak response of the relevant atorvastatin related compound from the *Standard solution*
C_S = concentration of the relevant atorvastatin related compound in the *Standard solution* (mg/mL)
C_U = concentration of Atorvastatin Calcium in the *Sample solution* (mg/mL)
Calculate the percentage of any other individual impurity in the portion of Atorvastatin Calcium taken:

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

r_U = peak response of any other individual impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*
[NOTE—Disregard any peak observed in the blank; the reporting level for impurities is 0.05%.]
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%. [NOTE—This total does not include atorvastatin related compound E, as determined in the test for *Enantiomeric Purity*.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atorvastatin related compound A [a]	0.8	0.3
Atorvastatin related compound B [b]	0.9	0.3
Atorvastatin	1.0	n/a
Atorvastatin related compound C [c]	1.2	0.3
Atorvastatin related compound D [d, e]	2.1	0.1
Any other individual impurity	—	0.1

[a] Desfluoro impurity.
[b] 3*S*,5*R* isomer.
[c] Difluoro impurity.
[d] Epoxide impurity.
[e] Atorvastatin related compound D may undergo a transformation equilibrium with its cyclic hemiketal form. The cyclic hemiketal of atorvastatin related compound D elutes about 1–2 min before ator vastatin related compound D. Use the sum of the areas of the two peaks as a peak response for ator vastatin related compound D in the *Standard solution* and the *Sample solution*.

SPECIFIC TESTS

- **ENANTIOMERIC PURITY**

Mobile phase: Hexane, dehydrated alcohol, and trifluoroacetic acid (940:60:1)

System suitability stock solution: 5 mg/mL of USP Atorvastatin Calcium RS and 37.5 µg/mL of USP Ator vastatin Related Compound E RS in methanol. [NOTE—Atorvastatin related compound E is the 3*S*,5*S* enantiomer of ator vastatin.]

System suitability solution: Transfer 2.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.

Sample solution: Transfer 10 mg of Ator vastatin Calcium to a 10-mL volumetric flask, dissolve in 2.0 mL of methanol, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm × 25-cm; packing L51
Flow rate: 1.0 mL/min
Injection size: 20 µL

System suitability
Samples: *System suitability solution*
[NOTE—The elution order of the peaks is ator vastatin related compound E followed by ator vastatin.]
Resolution: NLT 2.0 between the peaks for ator vastatin related compound E and ator vastatin

Analysis
Samples: *Sample solution*
Calculate the per centage of ator vastatin related compound E in the portion of Ator vastatin Calcium taken:

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

r_U = peak response for ator vastatin related compound E
r_T = sum of the responses of the peaks for atorvastatin related compound E and atorvastatin

Acceptance criteria: NMT 0.3% of ator vastatin related compound E

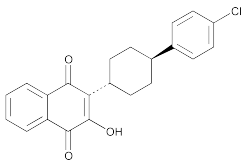
- **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** 3.5%–5.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Atorvastatin Calcium RS
USP Atorvastatin Related Compound A RS
Desfluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-2-isopropyl-4,5-diphenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.
$C_{66}H_{70}CaN_4O_{10}$ 1119.38
USP Atorvastatin Related Compound B RS
3*S*,5*R* Isomer, or (3*S*,5*R*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.
$C_{66}H_{68}CaF_2N_4O_{10}$ 1155.34
USP Atorvastatin Related Compound C RS
Difluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-4,5-bis(4-fluorophenyl)-2-isopropyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.
$C_{66}H_{66}F_4N_4O_{10}$ 1191.34
USP Atorvastatin Related Compound D RS
Epoxide impurity, or 3-(4-fluorobenzoyl)-2-isobutyr yl-3-phenyl-oxirane-2-carboxylic acid phenylamide.
$C_{26}H_{22}FNO_4$ 431.46
USP Atorvastatin Related Compound E RS
3*S*,5*S* Enantiomer, or (3*S*,5*S*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.
$C_{66}H_{68}CaF_2N_4O_{10}$ 1155.34

Atovaquone



$C_{22}H_{19}ClO_3$ 366.84
1,4-Naphthalenedione, 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-, *trans*-.
2-[*trans*-4-(*p*-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone [95233-18-4].

» Atovaquone contains not less than 97.5 percent and not more than 101.5 per cent of $C_{22}H_{19}ClO_3$, calculated on the anhydrous and organic solvent-free basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Atovaquone RS
USP Atovaquone Related Compound A RS
cis-2[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone.

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Water, *Method I* ⟨921⟩: not more than 1.0%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals—

Test preparation—Thoroughly mix 1.0 g of Atovaquone with 0.5 g of magnesium oxide in a silica crucible. Ignite to dull redness until a homogeneous white or grayish-white mass is obtained. If the mixture remains colored after 30 minutes, allow to cool, mix using a fine glass rod, and repeat the ignition. If necessary, repeat the operation. Heat the residue at 800 ° for about 1 hour. Cool, take up the residue in two 5-mL portions