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*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩**—**

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ dissolved by employing the following method.

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*.

Procedure—Separately inject equal volumes (about 50 µL) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ dissolved in comparison with a Standard solution having a known concentration of USP Loperamide Hydrochloride RS in the same *Medium* and similarly chromatographed.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ is dissolved in 30 minutes.

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

Assay—

Buffer solution—Transfer 3.0 g of triethylamine hydrochloride and 1.0 mL of phosphoric acid to a 1-L flask, add 550 mL of water, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (45:55). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Loperamide Hydrochloride RS in methanol to obtain a solution having a known concentration of about 2 mg per mL. Quantitatively dilute this solution with water to obtain a solution having a known concentration of about 0.2 mg per mL. Transfer 10.0 mL of this solution to a 250-mL volumetric flask, add 5.0 mL of 5% phosphoric acid solution and 25 mL of methanol, dilute with water to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 16 mg of loperamide hydrochloride, to a 2000-mL volumetric flask. Add 40 mL of 5% phosphoric acid solution and 200 mL of methanol, dilute with water to volume, and mix.

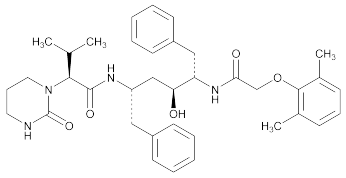
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4-mm × 8-cm column that contains 5-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) in the portion of Tablets taken by the formula:

$$2000C(r_U / r_S)$$

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

Lopinavir



$C_{37}H_{48}N_4O_5$ 628.80

[1*S*-[1*R**(*R**),3*R**,4*R**]]-*N*-[4[[(2,6-Dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]-tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide; (α*S*)-Tetrahydro-*N*-[(α*S*)-α-[(2*S*,3*S*)-2-hydroxy-4-phenyl-3-[2-(2,6-xylyloxy)acetamido]butyl]phenethyl]-α-isopropyl-2-oxo-1(2*H*)-pyrimidineacetamide [192725-17-0].

DEFINITION

Lopinavir contains NLT 98.0% and NMT 102.0% of $C_{37}H_{48}N_4O_5$ calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197S⟩
  Sample solution: Dissolve 50 mg in 1.0 mL of deuterated chloroform.
- **B.** The retention time of the lopinavir peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
  Buffer: 2.7 g/L of monobasic potassium phosphate and 0.9 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0. Pass the solution through a suitable filter of 0.45-µm pore size.
  Diluent: Acetonitrile and water (1:1)
  Solution A: Acetonitrile and *Buffer* (9:11)
  Mobile phase: *Solution A*
  Standard solution: 0.025 mg/mL of USP Lopinavir RS in *Diluent*
  Sample solution: 0.025 mg/mL in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.6-mm × 25-cm; 4-µm packing L1
  Column temperature: 50°
  Flow rate: 1 mL/min
  Injection size: 20 µL
  Run time: 60 min
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 8000 theoretical plates
  Capacity factor: NLT 15
  Tailing factor: 0.8–1.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{37}H_{48}N_4O_5$ in the portion of Lopinavir taken:

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

r_U = peak response of lopinavir from the *Sample solution*

r_S = peak response of lopinavir from the *Standard solution*

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

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

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

- **PROCEDURE 1**

[NOTE—For early-eluting impurities.]

Buffer, Diluent, and **Solution A:** Proceed as directed in the *Assay*.

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
60	100	0
61	0	100
81	0	100
82	100	0
100	100	0

System suitability solution: 0.5 mg/mL of USP Lopinavir System Suitability Mixture RS in *Diluent*

Standard solution: 0.005 mg/mL of USP Lopinavir RS in *Diluent*

Sample solution: 0.5 mg/mL in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection size: 20 µL

Run time: 100 min

[NOTE—Data collection is only for the first 60 min. The remaining gradient steps wash out the late eluting impurities and re-equilibrate the column.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Impurity Table 1*.]

Suitability requirements

Resolution: NLT 1.2 between lopinavir *N*-formylphenoxyacetamide and lopinavir *N*-acetylphenoxyacetamide, *System suitability solution*

Capacity factor: NLT 15, *Standard solution*

Column efficiency: NLT 8000, *Standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Diluent*, *System suitability solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of each lopinavir related impurity and unidentified impurity in the portion of Lopinavir taken:

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

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

r_S = peak response of lopinavir from the *Standard solution*

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

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

F = relative response factor (see *Impurity Table 1*)

Impurity Table 1

Name	Relative Retention Time[o]	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir free amine[a]	0.03	0.61	0.1
Lopinavir *N*-formylaminoalcohol[b]	0.07	0.80	0.2
Lopinavir divalinate[c]	0.10	0.65	0.1
Sulfolopinavir[d]	0.13	0.76	0.1
Lopinavir phenoxyacetamide[e]	0.25	0.96	0.1
Lopinavir *N*-formylphenoxyacetamide[f]	0.59	1.3	0.1
Lopinavir *N*-acetylphenoxyacetamide[g]	0.62	1.2	0.1
Lopinavir oxazine[h]	0.90	1.1	0.1
Lopinavir	1.00	—	—
Isolopinavir[i]	1.10	0.99	0.2
Lopinavir 2,4-phenoxy isomer[j]	1.13	0.97	0.1
Lopinavir D-leucine diastereomer[k]	1.25	1.1	0.1
Z-Diacylethenediamine[l]	1.28	1.4	0.1
Lopinavir (2*R*,4*R*) diastereomer[m]	1.32	1.0	0.1
Lopinavir (4*R*) epimer[n]	1.38	0.97	0.1
Any other individual impurity	—	1.0	0.1

[a] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[b] (*S*)-*N*-[(2*S*,4*S*,5*S*)-5-Formamido-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[c] (2*S*,2′*S*)-*N*,*N*′-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis{3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide}.

[d] (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-{(*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido}-1,6-diphenylhexan-3-yl hydrogen sulfate.

[e] *N*-[(2*S*,3*S*,5*S*)-5-Amino-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

[f] 2-(2,6-Dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.

[g] *N*-[(2*S*,3*S*,5*S*)-5-Acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

[h] *N*-{(*S*)-1-[(4*S*,6*S*)-4-Benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl}-2-(2,6-dimethylphenoxy)acetamide.

[i] (*S*)-*N*-{(2*S*,3*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-3-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[j] (*S*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,4-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[k] (*R*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[l] (*Z*)-*N*,*N*′-(Ethene-1,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].

[m] (*S*)-*N*-{(2*R*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[n] (*S*)-*N*-{(2*S*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

[o] (See *Chromatography* ⟨621⟩*, Interpretation of Chromatograms*.)

- **PROCEDURE 2**

[NOTE—For late-eluting impurities.]

Buffer, Diluent, and **Solution A:** Proceed as directed in the *Assay*.

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: *Solution A* and *Solution B* (3:7)

System suitability solution: 0.5 mg/mL of USP Lopinavir System Suitability Mixture RS in *Diluent*

Standard solution: 0.005 mg/mL of USP Lopinavir RS in *Diluent*
Sample solution: 0.5 mg/mL in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 4-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: 50 min
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times are listed in *Impurity Table 2*.]
Suitability requirements
Capacity factor: NLT 1.5
Column efficiency: NLT 3000
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Diluent*, *System suitability solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of each lopinavir related impurity and unidentified impurity in the portion of Lopinavir taken:

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

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of lopinavir from the *Standard solution*
C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)
C_U = concentration of Lopinavir in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 2*)

Impurity Table 2

Name	Relative Retention Time[g]	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir	1.00	—	—
Lopinavir *O*-acyl[a]	1.49	0.77	0.1
Lopinavir (2*R*) epimer[b]	1.91	1.1	0.1
Lopinavir diamide[c]	4.39	1.4	0.1
Lopinavir *N*-acyl[d]	6.01	1.3	0.1
Lopinavir *O*-phenoxyactyl[e]	7.14	1.1	0.1
Lopinavir amino-alcohol urea[f]	8.46	1.3	0.1
Any other individual impurity	—	1.0	0.1

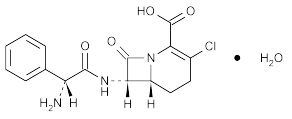
[a] (*S*)-{(2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(*S*)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)butanamido]-1,6-diphenylhexan-3-yl} 3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoate.
[b] (*S*)-*N*-{(2*R*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl] butanamide.
[c] *N*,*N*′-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].
[d] (*S*)-*N*-{(2*S*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl}-2-{3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2*H*)-yl}-3-methylbutanamide.
[e] (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-{(S)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido}-1,6-diphenylhexan-3-yl 2-(2,6-dimethylphenoxy)acetate.
[f] *N*,*N*′-(2*S*,2′*S*,3*S*,3′*S*,5*S*,5′*S*)-5,5′-Carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].
[g] (See *Chromatography* ⟨621⟩, *Interpretation of Chromatograms*.)

Acceptance criteria
Total impurities: NMT 0.7%
[NOTE—Total impurities from *Procedure 1* and *Procedure 2*.]

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 4.4%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Lopinavir RS
  USP Lopinavir System Suitability Mixture RS
  Lopinavir System Suitability Mixture contains lopinavir *N*-formylphenoxyacetamide, lopinavir *N*-acetylphenoxyacetamide, and several other minor components.
  Lopinavir *N*-formylphenoxyacetamide is (2-(2,6-dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.
  $C_{29}H_{34}N_2O_4$ 474.59
  Lopinavir *N*-acetylphenoxyacetamide is (*N*-[(2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.
  $C_{30}H_{36}N_2O_4$ 488.62

Loracarbef



$C_{16}H_{16}ClN_3O_4 \cdot H_2O$ 367.79
1-Azabicyclo[4.2.0][oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-, monohydrate, [6*R*-[6α,7β(*R**)]]-.
(6*R*,7*S*)-7-[(*R*)-2-Amino-2-phenylacetamido]-3-chloro-8-oxo-l-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monohydrate [121961-22-6].
Anhydrous 349.78

» Loracarbef contains not less than 960 µg and not more than 1020 µg of anhydrous loracarbef