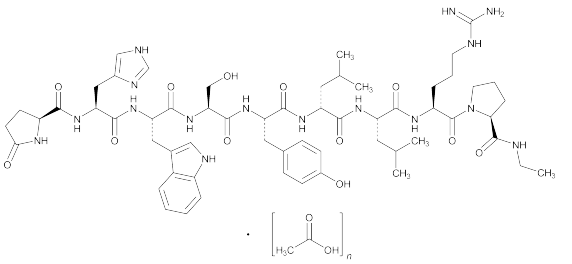
Leuprolide Acetate



$C_{59}H_{84}N_{16}O_{12} \cdot (C_2H_4O_2)n$, n = 1 or 2 1209.41 (as free base)
Luteinizing hormone-releasing factor, 6-D-leucine-9-(*N*-ethyl-L-prolinamide)-10-deglycinamide acetate (salt);
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide acetate (salt) [74381-53-6].

DEFINITION

Leuprolide Acetate is a synthetic nonapeptide agonist analog of luteinizing hormone-releasing factor. It contains NLT 97.0% and NMT 103.0% of leuprolide ($C_{59}H_{84}N_{16}O_{12}$), calculated on the anhydrous, acetic acid-free basis.
[NOTE—Due to the hygroscopic nature of this material, analyses are performed immediately after opening the container in a glove box under dry nitrogen purge.]
[**CAUTION—**Leuprolide Acetate is a potent hormonal manipulator. Avoid skin contact and inhalation of dusts and mists.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197K⟩
- **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**

Solution A: 15.2 mg/mL of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile and *n*-propyl alcohol (3:2)
Mobile phase: *Solution A* and *Solution B* (17:3)
Standard stock solution: 1 mg/mL of USP Leuprolide Acetate RS in *Mobile phase*
Standard solution: 50 µg/mL. Dilute 5.0 mL of the *Standard stock solution* with *Mobile phase* to 100.0 mL.
Degradation standard solution: Dilute 5.0 mL of the *Standard stock solution* with water to 50.0 mL. Transfer 5 mL of the solution to a scintillation vial. Add 100 µL of 1 N sodium hydroxide solution, cap tightly, and shake vigorously. Place in an oven at 100° for 60 min, remove, allow to cool, add 50 µL of 1 M phosphoric acid, recap, and shake vigorously to mix.
Sample solution: 50 µg/mL of Leuprolide Acetate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1–1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Mobile phase*, *Standard solution*, and *Degradation standard solution*
[NOTE—Chromatograph the *Mobile phase*, and verify that no extraneous peaks are present.]
[NOTE—The relative retention times for the degradation product and leuprolide are about 0.90 and 1.0, respectively.]
Suitability requirements
Retention time: 41–49 min for leuprolide, *Degradation standard solution*
Resolution: NLT 1.5 between leuprolide and the degradation product, *Degradation standard solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of leuprolide ($C_{59}H_{84}N_{16}O_{12}$) in the portion of Leuprolide Acetate taken:

$$\bullet\text{Result} = [(r_U/r_S) \times (C_S/C_U) \times P \times M \times 100]/(100 - AC - WC)\bullet_{\text{(IRA 1-Feb-2011)}}$$

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
$\bullet C_S\bullet_{\text{(IRA 1-Feb-2011)}}$ = concentration of USP Leuprolide Acetate RS in the *Standard solution* (µg/mL)
$\bullet C_U\bullet_{\text{(IRA 1-Feb-2011)}}$ = concentration of Leuprolide Acetate in the *Sample solution* (µg/mL)
P = designated purity of USP Leuprolide Acetate RS (%)
$\bullet M$ = (100 − H)/100 where H is equal to the water content of USP Leuprolide Acetate RS$\bullet_{\text{(IRA 1-Feb-2011)}}$
AC = acetic acid content (%)
WC = water content (%)
Acceptance criteria: 97.0%–103.0% on the anhydrous and acetic acid-free basis

OTHER COMPONENTS

Change to read:

- **CONTENT OF ACETIC ACID**

Diluent: Methanol, adjusted with phosphoric acid to a pH of 2.5
Standard solution: Pipet 2.0 mL of glacial acetic acid into a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 4.0 mL of the solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix to obtain a solution having a known concentration of about 0.08 mg/mL.
Sample solution: Transfer about 100 mg of Leuprolide Acetate, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica capillary column that contains a 1.2-µm film of phase G35
Temperature
Column: 100°
Injection port: 200°
Detector: 250°
Carrier gas: Helium
Flow rate: 10 mL/min
Injection size: 1.0 µL
Injection type: Splitless mode
System suitability
Samples: *Diluent* and *Standard solution*
Suitability requirements
$\bullet\bullet_{\text{(IRA 1-Feb-2011)}}$
Blank: Chromatograph the *Diluent*, and verify that there are no interfering peaks.
Column efficiency: NLT 15,000 theoretical plates, *Standard solution*

Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 2.0% for glacial acetic acid, for replicate injections of the *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetic acid ($C_2H_4O_2$) in the portion of Leuprolide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (839.2/W_U)$$

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
W_U = amount of Leuprolide Acetate taken to prepare the *Sample solution* (mg)

Acceptance criteria: 4.7%–9.0%

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.3%
- **Chromatographic Purity**

Solution A: 15.2 mg/mL of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0 prior to final dilution.
Solution B: Acetonitrile and *n*-propyl alcohol (3:2)
Mobile phase: *Solution A* and *Solution B* (17:3)
Standard stock solution: 1 mg/mL of USP Leuprolide Acetate RS in *Mobile phase*
Standard solution: Dilute 1.0 mL of the *Standard stock solution* with *Mobile phase* to 100.0 mL.
Degradation standard solution: Dilute 5 mL of *Standard stock solution* with water to 50.0 mL. Transfer 5 mL of the solution to a scintillation vial. Add 100 µL of 1 N sodium hydroxide solution, tightly cap, and shake vigorously. Place in an oven at 100° for 60 min, remove, allow to cool, add 50 µL of 1 M phosphoric acid, recap, and shake vigorously to mix.
Sample solution: Transfer about 100 mg of Leuprolide Acetate to a 100-mL volumetric flask, and dissolve in and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1–1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Mobile phase*, *Standard solution*, *Degradation standard solution*, and *Sample solution*
[NOTE—Chromatograph the *Mobile phase*, and verify that no extraneous peaks are present.]
Suitability requirements
Retention time: 41–49 min for leuprolide, *Degradation standard solution*
Resolution: NLT 1.5 between leuprolide and the degradation product, *Degradation standard solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Record the chromatograms for 90 min.]
Calculate the percentage of each impurity in the portion of leuprolide acetate [$C_{59}H_{84}N_{16}O_{12} \cdot (C_2H_4O_2)n$] taken:

$$\text{Result} = 0.01 \times (r_U/r_S) \times (W_S/W_U) \times P \times {}^{\bullet}M_{\bullet\,(\text{IRA 1-Feb-2011})}$$

r_U = peak response for each impurity from the *Sample solution*
r_S = leuprolide peak response from the *Standard stock solution*
W_S = weight of USP Leuprolide Acetate RS in the *Standard stock solution* (mg)
W_U = weight of Leuprolide Acetate in the *Sample solution* (mg)
P = designated purity of USP Leuprolide Acetate RS (%)
${}^{\bullet}M$ = (100 − *H*)/100 where *H* is equal to the water content of USP Leuprolide Acetate RS● (IRA 1-Feb-2011)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetyl-leuprolide	1.5	1.0
D-His-leuprolide	0.9	0.5
L-Leu⁶-leuprolide	1.2	0.5
D-Ser-leuprolide	0.8	0.5
Leuprolide	1.0	—
Any other impurity	—	0.5
Total impurities	—	2.5

SPECIFIC TESTS

- **Amino Acid Content**

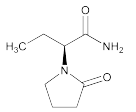
[NOTE—Use a suitable, validated procedure (see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩).]
Standard solutions: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. For the validation of the method, an appropriate internal standard, such as norleucine, is used. Prepare a separate, equimolar solution of L-tryptophan.
Sample solution: Transfer 64 mg of Leuprolide Acetate to a suitable vessel. Dissolve in 1.0 mL of water. Transfer 0.10 mL of this solution to a vacuum hydrolysis tube, add 2.0 mL of 6 N hydrochloric acid, evacuate the tube, and heat for 16 h at 120°. Transfer 0.10 mL of the hydrolysate so obtained to a suitable vessel, add 1 mL of water, and lyophilize. Dissolve in and dilute to a suitable volume in a buffer solution suitable for amino acid analysis.
Analysis: Inject equal volumes of the *Standard solution* and the *Sample solution* into the amino acid analyzer, and record and measure the responses for each amino acid peak. Express the content of each amino acid in moles.
Calculate the relative proportions of the amino acids in the *Sample solution*, taking one-seventh of the sum of the number of moles of histidine, glutamic acid, leucine, proline, tyrosine, and arginine as equal to one.
Acceptance criteria: 0.85–1.1 moles each of glutamic acid, proline, tyrosine, histidine, and arginine per mole of Leuprolide Acetate; 1.8–2.2 moles of leucine per mole of Leuprolide Acetate; serine and tryptophan are also present.

- **Optical Rotation, *Specific Rotation* ⟨781S⟩:** −38.0° to −42.0°
expressed on an anhydrous, acetic acid-free basis
Sample solution: 10 mg/mL, in 1% acetic acid
- **Water Determination, *Method Ic* ⟨921⟩:** NMT 8.0%
- **Bacterial Endotoxins Test ⟨85⟩:** It contains NMT 166.7 USP Endotoxin Units/mg of leuprolide acetate.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at a temperature not higher than 30°.
- **USP Reference Standards ⟨11⟩**
USP Endotoxin RS
USP Leuprolide Acetate RS

Levetiracetam



$C_8H_{14}N_2O_2$ 170.21
1-Pyrrolidineacetamide, α-ethyl-2-oxo-, (α*S*)-;
(–)-(*S*)-α-Ethyl-2-oxo-1-pyrrolidineacetamide [102767-28-2].

DEFINITION
Levetiracetam contains NLT 98.0% and NMT 102.0% of $C_8H_{14}N_2O_2$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION
- **A. Infrared Absorption** ⟨**197K**⟩
- **B.** The retention time of the major peak for levetiracetam from the *Sample solution* corresponds to that of the levetiracetam *S*-enantiomer from the *System suitability solution*, as obtained in the test for *Limit of Levetiracetam R-enantiomer*.

ASSAY

Change to read:

- **Procedure**

Buffer: 0.26 g/L of monobasic potassium phosphate in water. Adjust with 2% aqueous potassium hydroxide (w/v) to a pH of 5.5.
Solution A: Acetonitrile and *Buffer* (1:19)
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
20	71	29

System suitability solution: 0.2 mg/mL of USP Levetiracetam RS and 0.08 mg/mL of USP Levetiracetam Related Compound A RS in *Solution A*. Prepare by first dissolving the required amount of USP Levetiracetam RS in a suitable volumetric flask. Add 10% of the flask volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Add the required amount of USP Levetiracetam Related Compound A RS, sonicate to dissolve, dilute with *Solution A* to volume, and mix.
Standard solution: 0.1 mg/mL of USP Levetiracetam RS in *Solution A*
Sample solution: 0.1 mg/mL of Levetiracetam in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 0.9 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times are given in *Impurity Table 1*.]
Suitability requirements
●(IRA 1-Feb-2011)
Relative standard deviation: NMT 1.0%
[NOTE—If system suitability criteria cannot be met, it is recommended that the column temperature be maintained at 20° to stabilize the system.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_8H_{14}N_2O_2$ in the portion of Levetiracetam taken:

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

r_U = peak response of Levetiracetam from the *Sample solution*
r_S = peak response of levetiracetam from the *Standard solution*
C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
F = percentage of levetiracetam *R*-enantiomer from the test for *Limit of Levetiracetam R-enantiomer*

Acceptance criteria: 98.0%–102.0% on the anhydrous and 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 Levetiracetam Related Compound B**

[NOTE—Perform this test only if levetiracetam related compound B is a known process impurity.]
Buffer: 1.22 g of sodium 1-decanesulfonate in 1 L of water containing about 1.3 mL of phosphoric acid. Adjust with 20% (w/v) potassium hydroxide to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (3:17)
System suitability solution: 2 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*
Standard solution: 0.002 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*
Sample solution: 2.0 mg/mL of Levetiracetam in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection size
System suitability: 10 µL
Analysis: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The retention time for levetiracetam related compound B is 9 min.]
Suitability requirements
Tailing factor: NMT 3.0
[NOTE—If a significant tailing of the levetiracetam related compound B peak is observed (greater than 3.0), it is recommended that the column temperature be maintained at 27° to stabilize the system.]
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levetiracetam related compound B in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levetiracetam related compound B from the *Sample solution*
r_S = peak response of levetiracetam related compound B from the *Standard solution*
C_S = concentration of USP Levetiracetam Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levetiracetam related compound B free base, 102.1

M_{r2} = molecular weight of levetiracetam related compound B, 138.6

Acceptance criteria: NMT 0.10%

[NOTE—The amount of levetiracetam related compound B measured is to be included in the total impurities in the test for *Organic Impurities, Procedure 2*.]

- **PROCEDURE 2**

Buffer, Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.005 mg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: 5 mg/mL of Levetiracetam in *Solution A*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Levetiracetam 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 levetiracetam from the *Standard solution*

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

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

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

[NOTE—Disregard any peak with a relative retention time of 0.19 or less.]

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.4%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pyridin-2-ol[a]	0.37	1.0	0.025
Levetiracetam acid[b]	0.62	1.2	0.3
Levetiracetam	1.00	—	—
Levetiracetam related compound A[c]	1.25	0.35	0.05
Any individual unspecified impurity	—	1.0	0.05

[a] Not included in the *Total impurities* limit.

[b] *(S)*-2-(2-Oxopyrrolidin-1-yl)butanoic acid. Included in the *Total impurities* limit.

[c] *(S)-N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide. Included in the *Total impurities* limit only if levetiracetam related compound B is a known process impurity.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* ⟨**921**⟩**:** NMT 0.5%

Change to read:

- **LIMIT OF LEVETIRACETAM R-ENANTIOMER**

Mobile phase: *n*-Hexane and •dehydrated• (IRA 1-Feb-2011) alcohol (4:1)

System suitability solution: 0.1 mg/mL of USP Levetiracetam Racemic Mixture RS in *Mobile phase*

Standard solution: 0.05 mg/mL of USP Levetiracetam RS in *Mobile phase*

Sample solution: 10 mg/mL of Levetiracetam in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 10-µm packing L51

Flow rate: 1.0 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for levetiracetam *R*-enantiomer and levetiracetam *S*-enantiomer are 0.55 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *R*- and *S*-enantiomers

[NOTE—If a loss of resolution (less than 4.0) is observed, it is recommended that the column temperature be maintained at 25° to stabilize the system.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levetiracetam *R*-enantiomer in the portion of Levetiracetam taken:

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

r_U = peak response of levetiracetam *R*-enantiomer from the *Sample solution*

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

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

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

Acceptance criteria: NMT 0.8%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Levetiracetam RS

USP Levetiracetam Racemic Mixture RS

A 1:1 mixture of levetiracetam *S*-enatiomer-(2*S*)-2-(2-oxopyrrolidin-1-yl)butanamide and levetiracetam *R*-enantiomer (2*R*)-2-(2-oxopyrrolidin-1-yl)butanamide.

USP Levetiracetam Related Compound A RS

(*S*)-*N*-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.

$C_8H_{14}ClNO_3$ 207.65

USP Levetiracetam Related Compound B RS

(*S*)-2-Aminobutanamide hydrochloride.

$C_4H_{10}N_2O \cdot HCl$ 138.6

Norgestimate and Ethinyl Estradiol Tablets

DEFINITION

Norgestimate and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of norgestimate ($C_{23}H_{31}NO_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

- The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Tetrahydrofuran, methanol, and water (5:2:13)

Internal standard solution: 0.05 mg/mL of dibutyl phthalate in methanol

Standard solution: Dissolve appropriate quantities of USP Ethinyl Estradiol RS and USP Norgestimate RS in a volume of *Internal standard solution* equivalent to 80% of the final volume. Add a volume of water equivalent to 20% of the final volume, and mix to obtain a solution having a known concentration of about 7 µg/mL of ethinyl estradiol and a known concentration of norgestimate similar to that expected in the *Sample solution*. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Add a number of Tablets, equivalent to 0.35 mg of ethinyl estradiol, to a suitable glass container. Add 10 mL of water, and mix with a vortex mixer until the Tablets are completely disintegrated. Add 40 mL of *Internal standard solution,* and mix with a vortex mixer for at least 23 min. Sonicate the sample for at least 5 min, pass an aliquot through a suitable filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 2.1 mL/min
Injection size: 25 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethinyl estradiol, (*Z*)-norgestimate, (*E*)-norgestimate, and dibutyl phthalate are about 0.5, 1.0, 1.2, and 1.5, respectively.]
Suitability requirements
Resolution: NLT 1.5 between (*Z*)-norgestimate and (*E*)-norgestimate
Relative standard deviation: NMT 2.0% for the peak response ratio of ethinyl estradiol, (*Z*)-norgestimate, and (*E*)-norgestimate to dibutyl phthalate

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_{UE}/R_{SE}) \times (C_{SE}/C_{UE}) \times 100$$

R_{UE} = ratio of the peak responses of ethinyl estradiol to dibutyl phthalate from the *Sample solution*
R_{SE} = ratio of the peak responses of ethinyl estradiol to dibutyl phthalate from the *Standard solution*
C_{SE} = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)
C_{UE} = nominal concentration of ethinyl estradiol in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of norgestimate ($C_{23}H_{31}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (C_{SN}/C_{UN}) \times [P_A(R_{UA}/R_{SA}) + P_S(R_{US}/R_{SS})] \times 100$$

C_{SN} = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
C_{UN} = nominal concentration of norgestimate in the *Sample solution* (mg/mL)
P_A = fraction of (*E*)-norgestimate in the USP Norgestimate RS
R_{UA} = ratio of the peak responses of (*E*)-norgestimate to dibutyl phthalate from the *Sample solution*
R_{SA} = ratio of the peak responses of (*E*)-norgestimate to dibutyl phthalate from the *Standard solution*
P_S = fraction of (*Z*)-norgestimate in the USP Norgestimate RS
R_{US} = ratio of the peak responses of (*Z*)-norgestimate to dibutyl phthalate from the *Sample solution*
R_{SS} = ratio of the peak responses of (*Z*)-norgestimate to dibutyl phthalate from the *Standard solution*

Calculate the ratio of the content of (*Z*)-norgestimate to ethinyl estradiol in the portion of Tablets taken, for use in the test for *Organic Impurities*:

$$C_Z/C_E = [(C_{SN} \times P_S) \times (R_{US}/R_{SS})]/[C_{SE}(R_{UE}/R_{SE})]$$

The terms are as defined above.

Acceptance criteria: 90.0%–110.0% of ethinyl estradiol ($C_{20}H_{24}O_2$); 90.0%–110.0% of norgestimate ($C_{23}H_{31}NO_3$)

PERFORMANCE TESTS

- **DISINTEGRATION ⟨701⟩:** 15 min
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

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

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: 254 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 50 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethinyl estradiol, (*Z*)-norgestimate, and (*E*)-norgestimate are about 0.5, 1.0, and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.5 between (*Z*)-norgestimate and (*E*)-norgestimate
Relative standard deviation: NMT 2.0% for the •(*Z*)-norgestimate and (*E*)-norgestimate•(IRA 1-Feb-2011) peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of any impurity having a relative retention time of about 0.2 or 0.4, relative to the (*Z*)-norgestimate peak, and detected at 254 nm in the portion of Tablets taken:

$$\text{Result} = (r_U/r_Z) \times (C_Z/C_E) \times F \times 100$$

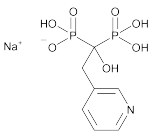
r_U = peak response for each impurity
r_Z = peak response for (*Z*)-norgestimate
C_Z/C_E = ratio of (*Z*)-norgestimate to ethinyl estradiol as defined in the *Assay*
F = relative response factor of these impurities, 1.54

Acceptance criteria: The sum of the impurities having relative retention times of about 0.2 and 0.4 is NMT 4.0%.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨**11**⟩
  USP Ethinyl Estradiol RS
  USP Norgestimate RS

Risedronate Sodium



$C_7H_{10}NNaO_7P_2$	305.09
$C_7H_{10}NNaO_7P_2 \cdot H_2O$	323.12
$C_7H_{10}NNaO_7P_2 \cdot 2.5\ H_2O$	350.13

Phosphonic acid, [1-hydroxy-2-(3-pyridinyl)ethylidene]bis-, monosodium salt;
Sodium trihydrogen [1-hydroxy-2-(3-pyridyl)ethylidene]diphosphonate;
Hemi-pentahydrate [329003-65-8].
Monohydrate [353228-19-0].

DEFINITION

Risedronate Sodium contains one or two and one-half molecules of hydration. The monohydrate form contains NLT 98.0% and NMT 102.0% of $C_7H_{10}NNaO_7P_2$, calculated on the dried basis. The hemi-pentahydrate form contains NLT 98.0% and NMT 102.0% of $C_7H_{10}NNaO_7P_2$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197⟩:** The spectra of trifluorovinyl chloride polymer and mineral oil dispersions of it, separately prepared from a test specimen, exhibit maxima in the regions of 4000 to 1350 cm^{-1} and 1350 to 450 cm^{-1}, respectively, only at the same wavelengths as those of similar preparations of USP Risedronate Sodium RS. [NOTE—If a difference appears in the infrared spectra of the analyte and the standard, dissolve equal portions of the test specimen and the USP Reference Standard in equal volumes of water containing about 50 mg/mL of potassium bromide. Evaporate the solutions to dryness at 105° for 120 min. Repeat the test on the residues.]
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩**:** It meets the requirements of the flame test.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: 1.8 g/L of edetate disodium in water. Adjust with 1 N sodium hydroxide to a pH of 9.5 ± 0.1.
Standard solution: Dissolve USP Risedronate Sodium RS and USP Risedronate Related Compound A RS in *Mobile phase* to obtain a solution containing 1.0 mg/mL of anhydrous risedronate sodium and 0.1 mg/mL of risedronate related compound A.
Sample solution: 1.1 mg/mL of Risedronate Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 nm
Column: 4.0-mm × 25-cm; 10-µm packing L48
Flow rate: 0.8 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.3 between risedronate and risedronate related compound A
Tailing factor: NMT •1.6• (IRA 1-Feb-2011) for the risedronate peak
Relative standard deviation: NMT 1.0% for the risedronate peak from three replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of risedronate sodium ($C_7H_{10}NNaO_7P_2$) in the portion of Risedronate Sodium 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 Risedronate Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the dried basis for the monohydrate form or on the anhydrous basis for the hemi-pentahydrate form

IMPURITIES

- **HEAVY METALS**

Lead nitrate solution: Add 1 mL of nitric acid to 100 mL of water. Dissolve 100 mg of lead nitrate in it, and then dilute with water to 1000 mL.
Sodium bicarbonate solution: Transfer 0.840 g of sodium bicarbonate to a 1000-mL volumetric flask containing about 950 mL of water. Dissolve in and dilute with water to volume. Adjust with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, as necessary, to a pH of 4.40 ± 0.02.
Hydrogen sulfide solution: Transfer 200 mL of *Sodium bicarbonate solution* to a suitable conical flask, and bubble hydrogen sulfide gas through the solution until it turns a strip of *Lead Acetate Test Paper* black (see *Reagents, Indicators, and Solution—Indicators and Test Papers*).
Standard solutions: Transfer 500 mg of Risedronate Sodium to each of three separate beakers. Add 41 mL of water to each beaker, and stir to dissolve. Adjust with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, as necessary, to a pH of 4.40 ± 0.02. Label the first beaker as *Standard solution 1*. Add 200 µL of *Lead nitrate solution* to the second beaker (*Standard solution 2*) and 400 µL to the third beaker (*Standard solution 3*). These solutions contain the equivalent of 0, 12.5, and 25 µg of lead (representing 0, 10, and 20 ppm, respectively).
Sample solution: Transfer 1.75 g of Risedronate Sodium to a suitable beaker. Add 41 mL of water, and stir to dissolve. Adjust with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, as necessary, to a pH of 4.40 ± 0.02.
Analysis: Add 7 mL of *Hydrogen sulfide solution* to each of the beakers containing the *Standard solutions* and the *Sample solution.* Allow the solutions to stand for at least 5 min. Add 60 µL of 1 N hydrochloric acid to each of the beakers containing the *Standard solutions,* and add 200 µL of 1 N hydrochloric acid to the beaker containing the *Sample solution,* and stir. Transfer the solutions into 50-mL color-comparison tubes, and view downward over a white surface: the color of the solution obtained from the *Sample solution* is not darker than that of the solution from *Standard solution 3*.
Acceptance criteria: NMT 20 ppm

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—Perform both *Procedure 1* and *Procedure 2*.]
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.
Diluted standard solution: Dilute a portion of the *Standard solution* with *Mobile phase* to obtain a solution containing 5 µg/mL of anhydrous risedronate sodium and about 0.5 µg/mL of risedronate related compound A.
System suitability
Samples: *Standard solution* and *Diluted standard solution*
Suitability requirements
Resolution: NLT 2.3 between risedronate related compound A and risedronate, *Standard solution*
Tailing factor: NMT •1.6• (IRA 1-Feb-2011) for the risedronate peak, *Standard solution*

Relative standard deviation: NMT 1.0% for the risedronate peak from three replicate injections, *Standard solution*; NMT 15% for the risedronate related compound A peak from three replicate injections, *Diluted standard solution*

Analysis

Samples: *Sample solution* and *Diluted standard solution*

Calculate the percentage of each impurity in the portion of Risedronate Sodium 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 risedronate from the *Diluted standard solution*
C_S = concentration of USP Risedronate Sodium RS in the *Diluted standard solution* (mg/mL)
C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Table 1

Name	Relative Response Factor	Relative Retention Time
3-Pyridyl acetic acid	1.65	●0.22● (IRA 1-Feb-2011)
2-Pyridinyl isomer (USP Risedronate Related Compound A RS)	1.0	●0.84● (IRA 1-Feb-2011)
Risedronate sodium	—	1.0

Acceptance criteria

Any individual impurity: NMT 0.10%

[NOTE—Disregard ●the peak due to sodium ion, eluting at about 1.6 min, and● (IRA 1-Feb-2011) any peak observed in the blank. The reporting level for impurities is 0.05%.]

- **ORGANIC IMPURITIES, PROCEDURE 2**

Mobile phase: Transfer 16.15 g of dibasic potassium phosphate and 0.46 g of edetate disodium to a 1-L beaker, and dissolve in about 400 mL of water. Add 1 vial of commercially available tetrabutylammonium dihydrogen phosphate buffered solution in methanol[1] and 1 mL of hydrochloric acid. Adjust with 1 N sodium hydroxide or 1 N hydrochloric acid, as necessary, to a pH of 7.5 ± 0.1, and dilute with water to 480 mL. Add 20 mL of methanol, mix well, pass the solution through a nylon filter of 0.45-µm pore size, and degas.

Diluent: Transfer 0.46 g of edetate disodium to a 1-L beaker, and dissolve in 500 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5 ± 0.1.

Standard solution: 5 µg/mL of USP Risedronate Related Compound B RS in *Diluent*

Diluted standard solution: 0.5 µg/mL of USP Risedronate Related Compound B RS in *Diluent* from the *Standard solution*

Sample solution: 2 mg/mL of Risedronate Sodium, in *Diluent*, using sonication if necessary

Chromatographic system

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

Mode: LC

Detector: UV 263 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution* and *Diluted standard solution*

Suitability requirements

Capacity factor: Greater than 2, *Standard solution*

Tailing factor: Less than 1.5, *Standard solution*

Relative standard deviation: NMT 5% from three replicate injections, *Standard solution*

Relative standard deviation: NMT 10% from three replicate injections, *Diluted standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Disregard any peak eluting before risedronate related compound B. The risedronate peak elutes unretained at the void volume.]

Calculate the percentage of each impurity in the portion of Risedronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of risedronate related compound B from the *Standard solution*
C_S = concentration of USP Risedronate Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Risedronate Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of risedronate related compound B as a free acid, 530.20
M_{r2} = molecular weight of risedronate related compound B as a tetrahydrate disodium salt, 646.22

Acceptance criteria

Risedronate related compound B: NMT 0.10%

Individual impurities: NMT 0.10%

Total impurities: NMT 0.50%, *Procedure 1* and *Procedure 2* being combined

[NOTE—Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.]

SPECIFIC TESTS

Change to read:

- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩ (where it is labeled as a hemi-pentahydrate)**:** 11.9%–13.9%. ●Perform the test by direct introduction of solid sample into the titrator. Alternatively, *Method 1a* may be used.● (IRA 1-Feb-2011)
- **LOSS ON DRYING** ⟨**731**⟩ (where it is labeled as a monohydrate)**:** (See *Thermal Analysis* ⟨891⟩.) Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument, using 7–15 mg of Risedronate Sodium. Heat the specimen under test at a rate of 10°/min in a stream of nitrogen at a flow rate of about 40 mL/min. Record the thermogram from ambient temperature to 250°.

Acceptance criteria: It loses between 5.5% and 7.5% of its weight.

ADDITIONAL REQUIREMENTS

- **LABELING:** Label to indicate whether it is the monohydrate or the hemi-pentahydrate form.
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Risedronate Sodium RS

USP Risedronate Related Compound A RS

2-Pyridinyl isomer [1-hydroxy-2-(2-pyridinyl)ethylidene]bis(phosphonic acid) monohydrate.

$C_7H_{11}NO_7P_2$ 283.12

USP Risedronate Related Compound B RS

Cyclic dimer, disodium tetrahydrate salt, [3,6-bis[(3-pyridinyl)methyl]-2,5-dihydroxy-2,5-dioxido-1,4,2,5-dioxadiphosphorinane-3,6-diyl]bis[phosphonic acid] disodium tetrahydrate salt.

$C_{14}H_{16}N_2O_{12}P_4Na_2 \cdot 4H_2O$ 646.22

[1] Available from Waters Corp. as Part #85101 (PIC A).