determine the absorbances of the test solutions and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 284 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{20}H_{21}CaN_7O_7$ in each Tablet taken by the formula:

$$(T / D)C(A_U / A_S)$$

in which T is the labeled quantity, in mg, of leucovorin calcium in the Tablet; D is the concentration, in µg per mL, of the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution; C is the concentration, in µg per mL, of USP Leucovorin Calcium RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Chromatographic purity—Calculate the percentage of each peak, other than the leucovorin peak, in the chromatogram obtained from the *Assay preparation* by the formula:

$$100(r_i / r_t)$$

in which r_i is the response of each impurity peak, and r_t is the sum of the responses of all of the peaks: not more than 2.5% of any individual impurity and not more than 4.0% of total impurities is found.

Assay—

Diluting solvent—Prepare a mixture of water and methanol (80:20).

Mobile phase—Prepare a 0.005 M solution of tetrabutylammonium phosphate in *Diluting solvent*. Adjust the pH of this solution to 7.5 by the addition of 50% (w/v) sodium hydroxide solution, filter, and degas.

Standard preparation—Dissolve accurately weighed quantities of USP Leucovorin Calcium RS and USP 10-Formylfolic Acid RS in water to obtain a solution having known concentrations of about 500 µg of USP Leucovorin Calcium RS and 10 µg of USP 10-Formylfolic Acid RS per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of leucovorin, to a 100-mL volumetric flask. Add about 50 mL of water, sonicate for 30 minutes, dilute with water to volume, mix, and filter.

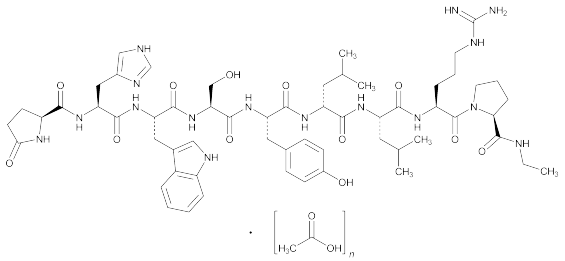
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R,* between leucovorin and 10-formylfolic acid is not less than 1.5; and the relative standard deviation for replicate injections, and retention times is not more than 2.0% for the leucovorin peak.

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 peak area responses. The relative retention times are about 2.3 for 10-formylfolic acid and 1.0 for leucovorin. Calculate the quantity, in mg, of $C_{20}H_{23}N_7O_7$ in the portion of Tablets taken by the formula:

$$(473.45 / 511.50)(L / D)(C)(r_U / r_S)$$

in which 473.45 and 511.50 are molecular weights of leucovorin and anhydrous leucovorin calcium, respectively; L is the labeled amount, in mg, of leucovorin in each Tablet; D is the concentration, in mg per mL, of leucovorin in the *Assay preparation*, based on the labeled quantity per Tablet and the extent of dilution; C is the concentration, in mg per mL, of anhydrous USP Leucovorin Calcium RS in the *Standard preparation;* and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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

- **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:

$$\text{Result} = [(r_U/r_S) \times (C_S/C_U) \times P \times M \times 100]/(100 - AC - WC)$$

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

OTHER COMPONENTS

• **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
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 = weight 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 before 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:

$$\text{Result} = (r_U/r_S) \times (W_S/W_U) \times P \times M \times 0.01$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of leuprolide 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 (%)
M = (100 − *H*)/100, where *H* is equal to the water content of USP Leuprolide Acetate RS

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-Leu^6-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 *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**⟩

Sample solution: 10 mg/mL, in 1% acetic acid

Acceptance criteria: −38.0° to −42.0° expressed on an anhydrous, acetic acid-free basis

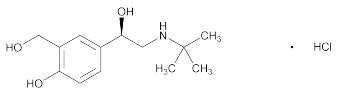
- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 8.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** It contains NMT 166.7 USP Endotoxin Units/mg of leuprolide acetate.

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

Levalbuterol Hydrochloride



$C_{13}H_{21}NO_3 \cdot HCl$ 275.77

(*R*)-α^1-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α,α′-diol hydrochloride [50293-90-8].

DEFINITION

Levalbuterol Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{13}H_{21}NO_3 \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

- **INFRARED ABSORPTION** ⟨**197K**⟩

ASSAY

- **PROCEDURE**

Solution A: 1 in 1000 solution of phosphoric acid in water

Solution B: Acetonitrile, methanol, phosphoric acid, and water (350:350:1:300)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	91.5	8.5
15	91.5	8.5
15.01	0	100
20	0	100
20.01	91.5	8.5
30	91.5	8.5

Diluent: *Solution A*

Standard solution: 100 µg/mL of USP Levalbuterol Hydrochloride RS in *Diluent*

Sample solution: 100 µg/mL of Levalbuterol Hydrochloride in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

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

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: Greater than 5500 theoretical plates

Tailing factor: Less than 2.3

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{13}H_{21}NO_3 \cdot HCl$ in the portion of Levalbuterol Hydrochloride taken:

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

r_U = peak response of levalbuterol hydrochloride from the *Sample solution*

r_S = peak response of levalbuterol hydrochloride from the *Standard solution*

C_S = concentration of USP Levalbuterol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)