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

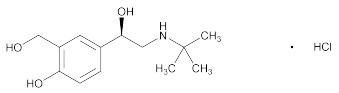
- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 8.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** It contains NMT 166.7 USP Endotoxin Units/mg of leuprolide acetate.

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)

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

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **HEAVY METALS,** *Method I* ⟨**231**⟩**:** NMT 10 ppm

Organic Impurities

- **PROCEDURE 1**

Solution A, Solution B, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.

Standard solution: [NOTE—Prepare a solution containing the following in *Diluent*.]
USP Levalbuterol Hydrochloride RS, 100 µg/mL
USP Levalbuterol Related Compound A RS, 0.05 µg/mL
USP Levalbuterol Related Compound B RS, 0.05 µg/mL
USP Levalbuterol Related Compound C RS, 0.05 µg/mL
USP Levalbuterol Related Compound D RS, 0.05 µg/mL
USP Levalbuterol Related Compound E RS, 0.05 µg/mL
USP Levalbuterol Related Compound F RS, 0.05 µg/mL
USP Levalbuterol Related Compound H RS, 0.05 µg/mL

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	70	30
50	28	72
50.01	0	100
55	0	100
55.01	100	0
70	100	0

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: 45°
Flow rate: 1 mL/min
Injection size: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.9 between levalbuterol and levalbuterol related compound A; NLT 1.5 between levalbuterol related compound B and levalbuterol related compound C
Column efficiency: NLT 4000 for levalbuterol
Tailing factor: NMT 4.0 for levalbuterol
Relative standard deviation: Less than 20% from any of the six related compound peaks

Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Integrate all peaks with an area greater than 0.05% of the area corresponding to the levalbuterol peak.]
Calculate the percentage of each impurity in the portion of Levalbuterol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks
F = relative response factor for each impurity (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levalbuterol related compound A	1.2	1.0	0.1
Levalbuterol related compound H	1.3	1.0	0.15
Levalbuterol related compound B	1.5	1.0	0.10
Levalbuterol related compound C	1.6	1.0	0.15
Levalbuterol related compound D	1.7	3.0	0.05
Levalbuterol related compound E	2.1	1.0	0.1
Levalbuterol related compound F	3.5	1.2	0.10
Any unknown impurity	—	—	0.10
Total unknown impurities	—	—	0.1
Total impurities	—	—	0.5

- **PROCEDURE 2: ENANTIOMERIC PURITY AND CHIRAL IDENTITY**

Mobile phase: Acetonitrile, methanol, acetic acid, and triethylamine (500:500:3:1)
Diluent: *Mobile phase*
System suitability solution A: 0.10 mg/mL of USP Levalbuterol Hydrochloride RS and 0.40 µg/mL of USP Albuterol RS in *Diluent*
System suitability solution B: 1.5 mg/mL of USP Albuterol RS in *Diluent*
Sample solution: 0.8 mg/mL of Levalbuterol Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L63
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Sample: *System suitability solution A*
Suitability requirements
Resolution: NLT 2.0 between levalbuterol and (*S*)-albuterol
Column efficiency: NLT 4000, calculated from either peak
Tailing factor: NMT 2.2 for levalbuterol and (*S*)-albuterol
Relative standard deviation: NMT 20% for (*S*)-albuterol, injected three times

Analysis
Samples: *System suitability solution B* and *Sample solution*
Calculate the percentage of (*S*)-albuterol in the portion of Levalbuterol Hydrochloride taken:

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

r_U = peak response of (*S*)-albuterol

r_T = sum of the peak responses for both levalbuterol and (*S*)-albuterol

Acceptance criteria: NMT 0.2% of (*S*)-albuterol

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic bacterial count is less than 10 cfu/g. The total combined molds and yeasts count is less than 10 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.
- **pH** ⟨**791**⟩**:** 4.5–5.5, in a 10-mg/mL solution
- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Albuterol RS

USP Levalbuterol Hydrochloride RS
(*R*)-α¹-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α,α′-diol hydrochloride.

USP Levalbuterol Related Compound A RS
4-(2-*tert*-Butylamino-ethyl)-2-hydroxymethyl-phenol.

USP Levalbuterol Related Compound B RS
α [{(1,1-Dimethylethyl)amino}methyl]-4-hydroxy-3-methyl-benzenemethanol.

USP Levalbuterol Related Compound C RS
α [{(1,1-Dimethylethyl)amino}methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

USP Levalbuterol Related Compound D RS
5-[2-{(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde.

USP Levalbuterol Related Compound E RS
α [{(1,1-Dimethylethyl)amino}methyl]-3-(ethoxymethyl)-4-hydroxy-benzenemethanol.

USP Levalbuterol Related Compound F RS
α [{(1,1-Dimethylethyl)amino}methyl]-4-(phenylmethoxy)-1,3-benzenedimethanol.

USP Levalbuterol Related Compound H RS
4-[2-(*tert*-Butylamino)-1-methoxyethyl]-2-(hydroxymethyl)phenol.

$C_{14}H_{23}NO_3$ 253.34

Levalbuterol Inhalation Solution

» Levalbuterol Inhalation Solution is a sterile, aqueous solution of Levalbuterol Hydrochloride, prepared with Sodium Chloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levalbuterol hydrochloride ($C_{13}H_{21}NO_3 \cdot HCl$).

Packaging and storage—Preserve in low-density polyethylene single-use ampuls, with a multilayer foil overwrap. Store at controlled room temperature.

Labeling—The outer label indicates the dose and that the ampuls should be discarded if the solution is not colorless.

USP Reference standards ⟨11⟩**—**

USP Albuterol RS

USP Levalbuterol Hydrochloride RS
(*R*)-α¹-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α,α′-diol hydrochloride.

USP Levalbuterol Related Compound A RS
4-(2-*tert*-Butylamino-ethyl)-2-hydroxymethyl-phenol.

USP Levalbuterol Related Compound B RS
α [{(1,1-Dimethylethyl)amino}methyl]-4-hydroxy-3-methyl-benzenemethanol.

USP Levalbuterol Related Compound C RS
α [{(1,1-Dimethylethyl)amino}methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

USP Levalbuterol Related Compound D RS
5-[2-{(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde.

USP Levalbuterol Related Compound E RS
α [{(1,1-Dimethylethyl)amino}methyl]-3-(ethoxymethyl)-4-hydroxy-benzenemethanol.

USP Levalbuterol Related Compound F RS
α [{(1,1-Dimethylethyl)amino}methyl]-4-(phenylmethoxy)-1,3-benzenedimethanol.

USP Levalbuterol Related Compound G RS
α [{(1,1-Dimethylethyl)amino}methyl]-4,5-dihydroxy-1,3-benzenedimethanol.

Identification—

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

Color ⟨631⟩**:** not more than 20 APHA platinum cobalt units.

Sterility ⟨71⟩**:** meets the requirements.

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

pH ⟨791⟩**:** between 3.3 and 4.5.

Particulate matter ⟨788⟩**:** not more than 250 particles greater than or equal to 10 μm; not more than 25 particles greater than or equal to 25 μm; not more than 2 particles greater than or equal to 100 μm; and not more than 1 particle greater than or equal to 300 μm.

Osmolality ⟨785⟩**:** between 280 and 320 mOsmol per kg.

Related compounds—

Mobile phase and *Chromatographic system*—Proceed as directed for *Related compounds* under *Levalbuterol Hydrochloride*.

Diluent—Dissolve about 9.0 ± 0.05 g of sodium chloride in 950 mL of water. Adjust with dilute sulfuric acid to a pH of 4.0, and dilute with water to 1000 mL. Mix, and pass through 0.45-μm filter.

Standard solution—Dissolve accurately weighed quantities of USP Levalbuterol Hydrochloride RS, USP Levalbuterol Related Compound A RS, USP Levalbuterol Related Compound B RS, USP Levalbuterol Related Compound C RS, USP Levalbuterol Related Compound D RS, USP Levalbuterol Related Compound E RS, USP Levalbuterol Related Compound F RS, and USP Levalbuterol Related Compound G RS in *Diluent* to obtain a solution having known concentrations of about 0.05 μg per mL of each related compound and 100 μg per mL of USP Levalbuterol Hydrochloride RS.

Test solution—Use the *Assay preparation,* prepared as directed in the *Assay.*

Procedure—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Determine the area of the levalbuterol peak, and integrate all the peaks with an area greater than 0.05% of the area corresponding to levalbuterol hydrochloride. Calculate the percentage of each impurity in the portion of Inhalation Solution taken by the formula:

$$100(r_i / r_s)(1/F)$$

in which F is the relative response factor for each impurity and is equal to 1.0 for related compounds A, B, C, E, and G and all unknown peaks, 3.0 for related compound D, and 1.2 for related compound F; r_i is the peak response for each impurity obtained from the *Test solution;* and r_s is the sum of the responses of all the peaks: not more than 0.10% of related compound G is found; not more than 0.08% of related compound D in each ampul is found (total content of related compound D should not be more than 1.0 μg per ampul); not more than 0.25% of total unknown impurities is found; not more than 0.10% of any unknown impurity is found; and not more than 0.70% of total impurities is found.