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Alendronic Acid and Colecalciferol Tablets

General Notices

Sodium Alendronate and Colecalciferol Tablets

Action and use

Bisphosphonate + Vitamin D3 analogue.

DEFINITION

Alendronic Acid and Colecalciferol Tablets contain Sodium Alendronate Trihydrate and Colecalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of alendronic acid, $C_4H_{13}NO_7P_2$

92.5 to 105.0% of the stated amount.

Content of colecalciferol, $C_{27}H_{44}O$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing the equivalent of 70 mg of alendronic acid with 50 mL of *water* for 30 minutes and filter.
(2) 0.2% w/v of *sodium alendronate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose*.
(b) Use the mobile phase as described below.
(c) Apply 2 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in a current of warm air, spray with *ninhydrin solution*, heat at 105° and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 8 volumes of 2.5% v/v *trichloroacetic acid* and 11 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for alendronic acid, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions. *Prepare the solutions immediately before use*.

Solution A A solution containing 1% w/v of *squalane* and 0.01% w/v of *butylated hydroxytoluene* in *ethylene chloride*.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 1 mg of Colecalciferol with 20 mL of *methanol*, centrifuge and filter. Evaporate the filtrate to dryness and dissolve the residue immediately in 0.4 mL of solution A.

(2) 0.25% w/v of *colecalciferol BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 20 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in current of warm air, and spray with *sulfuric acid*.

MOBILE PHASE

0.01% w/v of *butylated hydroxytoluene* in a mixture of equal volumes of *cyclohexane* and *peroxide-free ether*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in size, position and colour to that in the chromatogram obtained with solution (2).

D. In the Assay for colecalciferol, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

For alendronic acid

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.
(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 15 minutes withdraw a sample of the medium and filter (Whatman GF/C is suitable). Use the filtered dissolution medium diluted, if necessary, to produce a solution expected to contain the equivalent of 0.0078% w/v of alendronic acid.
(2) 0.01% w/v of *sodium alendronate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *anion exchange resin* (Waters IC PAK Anion HC is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use a column temperature of 35°.
(e) Use a conductivity detector, maintained at 35°.
(f) Inject 100 µL of each solution.

MOBILE PHASE

0.59% w/v of *succinic acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to alendronic acid is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the total content of alendronic acid, $C_4H_{13}NO_7P_2$ in the medium using the declared content of $C_4H_{12}NNaO_7P_2$ in *sodium alendronate BPCRS* and the peak heights in the chromatograms obtained. Each mg of $C_4H_{12}NNaO_7P_2$ is equivalent to 0.9189 mg of $C_4H_{13}NO_7P_2$.

LIMITS

The amount of alendronic acid released is not less than 75% (Q) of the stated amount.

For colecalciferol

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.
(b) Use 500 mL of a mixture of 3% w/v of *sodium lauryl sulfate* and 0.9% w/v of *sodium chloride*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 15 minutes withdraw a sample of the medium and filter (Whatman GF/C is suitable). Use the filtered dissolution medium diluted with medium, if necessary, to produce a solution expected to contain 0.000014% w/v of colecalciferol.
(2) Dilute 1 volume of a solution containing 0.0014% w/v of *colecalciferol BPCRS* in *methanol* to 100 volumes with the dissolution medium.
(3) Heat an aliquot of solution (2) on a water bath at 50° for 2 hours (generation of pre-colecalciferol).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Phenomenex Synergi-Hydro RP is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use a column temperature of 30°.
(e) Use a detection wavelength of 269 nm.
(f) Inject 150 µL of each solution.

MOBILE PHASE

methanol (96%).

When the chromatograms are recorded under the prescribed conditions the retention times of pre-colecalciferol and colecalciferol are about 11 minutes and 12 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to colecalciferol and pre-colecalciferol is at least 1.2.

DETERMINATION OF CONTENT

Use the sum of the peak areas of colecalciferol and pre-colecalciferol. Calculate the total content of colecalciferol, $C_{27}H_{44}O$, in the medium using the declared content of $C_{27}H_{44}O$, in *colecalciferol BPCRS*.

LIMITS

The amount of colecalciferol released is not less than 75% (Q) of the stated amount.

Related substances

For alendronic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise each solution prior to analysis.

Solution A A solution containing 0.294% w/v of *sodium citrate* and 0.142% w/v of *anhydrous disodium hydrogen orthophosphate*, adjusted to pH 8.0 with *orthophosphoric acid*.

(1) Mix a quantity of the powdered tablets containing the equivalent of 30 mg of alendronic acid with a 2.94% w/v solution of *sodium citrate*, dilute to 50 mL with the same solvent and filter.
(2) 0.00012% w/v of 4-aminobutanoic acid in 2.94% w/v *sodium citrate*.
(3) Dilute 1 volume of solution (1) to 100 volumes with a 2.94% w/v solution of *sodium citrate*, further dilute 1 volume of this solution to 5 volumes with the same solvent.
(4) 0.06% w/v of *sodium alendronate BPCRS* and 0.01% w/v of *4-aminobutanoic acid*(impurity A) in 2.94% w/v *sodium citrate*.
(5) 0.000065% w/v of *sodium alendronate BPCRS* in 2.94% w/v of *sodium citrate*.

Derivatisation procedure Transfer 5 mL of solutions (1) to (5) separately into screw-cap centrifuge tubes, add 5 mL of a 1.91% w/v solution of sodium tetrahydroborate, 10 mL of a 0.2% w/v solution of *(9-fluorenyl)methyl chloroformate* in *acetonitrile*, shake for 1 minute and allow to stand at room temperature for 30 minutes, add 20 mL of *dichloromethane* and shake vigorously for 1 minute; centrifuge and use the aqueous layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.1 mm) packed with *styrene-divinylbenzene copolymer* (10 µm) (Hamilton PRP-1 is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use a column temperature of 45°.
(e) Use a detection wavelength of 266 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 15 volumes of *acetonitrile* and 85 volumes of solution A.

Mobile phase B 30 volumes of solution A and 70 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	100→50	0→50	linear gradient
15-25	50→0	50→100	linear gradient
25-27	0→100	100→0	linear gradient
27-32	100	0	re-equilibration

The retention time of the peaks due to alendronic acid and 4-aminobutanoic acid, as their derivatives, are about 4 minutes and about 9 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the two principal peaks is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminobutanoic acid is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of all impurities is not more than 0.5%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Related substances

For colecalciferol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 0.56 mg of Colecalciferol with 5 mL of *water*. Add 25 mL of *ethanol*, stir for 30 minutes, add sufficient *methanol* to produce 50 mL and centrifuge.
(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.
(3) Heat 10 mL of a 0.00112% w/v solution of *colecalciferol BPCRS* in *methanol* in a capped vessel to 50° for two hours (generation of pre-colecalciferol).
(4) Dilute 1 volume of solution (2) to 10 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Supelco Discovery HS is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 35°.
(e) Use an auto-sampler temperature of 5°.
(f) Use a detection wavelength of 266 nm.
(g) Inject 20 µL of each solution.
(h) For solution (1), allow the chromatography to proceed for 2.5 times the retention time of colecalciferol

MOBILE PHASE

methanol (96%).

The retention time of the peaks due to pre-colecalciferol and colecalciferol are about 12 and 13 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the sum of the areas of the peaks due to pre-colecalciferol and colecalciferol in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the sum of the areas of the peaks due to pre-colecalciferol and colecalciferol in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak due to pre-colecalciferol and any peak with an area less than the sum of the areas of the peaks due to pre-colecalciferol and colecalciferol in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

For colecalciferol

For tablets containing less than 2 mg and/or less than 2% w/w of colecalciferol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse one whole tablet in 5 mL of *water*, add 25 mL of *ethanol* and stir for 30 minutes, add sufficient *methanol* to produce 0.00014% w/v of Colecalciferol and filter (Whatman GF/C is suitable).
(2) 0.00014% w/v of *colecalciferol BPCRS* in *methanol*.
(3) Heat 10 mL solution of a 0.00112% w/v of *colecalciferol BPCRS* in *methanol* in a capped vessel to 50° for two hours (generation of pre-colecalciferol).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances (for colecalciferol) may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.0.

DETERMINATION OF CONTENT

Combine the peak areas of colecalciferol and pre-colecalciferol. Calculate the total content of colecalciferol, $C_{27}H_{44}O$, in the tablets using the declared content of $C_{27}H_{44}O$, in *colecalciferol BPCRS*.

ASSAY

For alendronic acid

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing the equivalent of 0.1 g of alendronic acid with 200 mL of *water* for 30 minutes with the aid of ultrasound and occasional shaking, add sufficient *water* to produce 250 mL and centrifuge.
(2) 0.052% w/v of *sodium alendronate BPCRS* in *water*.
(3) 0.02% w/v of *sodium alendronate BPCRS* and 0.01% w/v of *sodium dihydrogen orthophosphate* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.1 mm) packed with *styrene-divinylbenzene copolymer for chromatography* (10 µm) (Hamilton PRP-X100 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.6 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 240 nm.
(f) Inject 100 µL of each solution.
(g) The method is validated to measure the drop in absorbance due to alendronic acid and an inverted peak will be observed.

MOBILE PHASE

0.007M *nitric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of the inverted peak due to alendronic acid is about 3.5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of alendronic acid, $C_4H_{13}NO_7P_2$ in the tablets using the declared content of $C_4H_{12}NNaO_7P_2$ in *sodium alendronate BPCRS* and the peak depth in the chromatograms obtained. Each mg of $C_4H_{12}NNaO_7P_2$ is equivalent to 0.9189 mg of $C_4H_{13}NO_7P_2$.

For colecalciferol

Use the average of the individual results obtained in the test for Uniformity of content.

LABELLING

The quantity of sodium alendronate is stated in terms of the equivalent amount of alendronic acid.

The quantity of Colecalciferol is expressed in terms of both mg and IU.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Colecalciferol and impurities A and D listed under *Sodium Alendronate Trihydrate*.