Alendronic Acid and Colecalciferol Tablets

Details for the public consultation of this monograph are as follows:

EAG/Panel/Working Party	**Medicinal Chemicals**
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Notes:	New monograph If limits are too restrictive, please provide batch/stability data to demonstrate that an increase is required. Please also provide comment on Identification C.

Action and use

Bisphosphonate; treatment of osteoporosis + Vitamin D analogue (Vitamin D3).

DEFINITION

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

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

Content of alendronic acid, $C_4H_{13}NO_7H_{13}P_2$

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 a quantity of the powdered tablets containing the equivalent of 70 mg of alendronic acid with 50 mL of *water* for 30 minutes with the aid of ultrasound and occasional shaking, 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 current of warm air, spray with *ninhydrin solution*, heat 100° to 105° and examine.

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 position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for alendronic acid, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).
C. Shake a quantity of the tablets containing 280 µg of Colecalciferol with 2 mL of ethanol-free chloroform and centrifuge the mixture. To 1 mL of the supernatant liquid add 9 mL of antimony trichloride solution. The light absorption of the resulting solution, Appendix II B, exhibits a maximum at 500 nm.
D. In the Assay for colecalciferol, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal 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) 0.008% w/v solution of *sodium alendronate BPCRS* in water.

CHROMATOGRAPHIC CONDITIONS

(a) Use a 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*.

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 *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 volumes of a solution containing 0.0014% w/v of *colecalciferol BPCRS* in *methanol* to 100 volumes with the medium.
(3) Heat a volume of solution (2) on a water-bath at 50° for 2 hours.

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%).

SYSTEM SUITABILITY

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

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 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 derivatised before use.

Solution A 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 solution of *4-aminobutanoic acid BPCRS* in a 2.94% w/v solution of*sodium citrate*.
(3) 0.00013% w/v solution of *sodium alendronate BPCRS* in a 2.94% w/v solution of*sodium citrate*.
(4) 0.06% w/v of *sodium alendronate BPCRS* and 0.01% w/v of *4-aminobutanoic acid BPCRS* in a 2.94% w/v solution of*sodium citrate*.
(5) 0.000065% w/v solution of *sodium alendronate BPCRS* in a 2.94% w/v solution 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 borate*, 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 85 volumes of solution A and 15 volumes of *acetonitrile*.

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	0	isocratic
15-25	100→50	0→50	linear gradient
25-27	50→0	50→100	linear gradient
27-32	0→100	100→0	linear gradient

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
32-40	100	0	re-equilibration

The retention time of the peaks due to alendronic acid and 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 (3), 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 *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 the area of the principal peak in the chromatogram obtained with solution (5) (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 (in situ preparation 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 *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.

MOBILE PHASE

methanol (96%).

The retention time of the peaks due to pre-colecalciferol and colecalciferol are 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 area of the principal peak 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 principal peak 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 area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

For colecalciferol

Tablets containing less than 2 mg and/or less than 2% w/w of Colecalciferol comply with the requirements stated under Tablets using the following method of analysis. 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 of a 0.00112% w/v of *colecalciferol BPCRS* in *methanol* in a capped vessel to 50° for two hours (in situ preparation of pre-colecalciferol).

CHROMATOGRAPHIC CONDITIONS

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

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 100mg 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) 0.04% 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 phosphate* 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.

MOBILE PHASE

0.007M *nitric acid*.

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 total 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 heights in the chromatograms obtained. Each mg of $C_4H_{12}NNaO_7P_2$, is equivalent to 0.9189 mg of $C_4H_{13}NO_7P_2$.

For colecalciferol

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

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of colecalciferol and the equivalent amount of alendronic acid.

IMPURITIES

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