2.3.55. Limit test for Fluorides. Page 170

Insert at the end

2.3.56. Assay of Calcium Pantothenate

NOTE - The following liquid chromatographic procedures are provided for the estimation of calcium pantothenate as an active pharmaceutical ingredient in pharmaceutical dosage forms. While conducting these procedures, protect solutions from light, with the use of low-actinic glassware, use the appropriate IP Reference Substances.

Assay. Determine by liquid chromatography (2.4.14).

Method A and Method B can be used to determine calcium pantothenate in:

- Oil- and Water-Soluble Vitamins Capsules
- Oil- and Water-Soluble Vitamins Tablets
- Oil- and Water-Soluble Vitamins with Minerals Capsules
- Oil- and Water-Soluble Vitamins with Minerals Tablets
- Water-Soluble Vitamins Capsules
- Water-Soluble Vitamins Tablets
- Water-Soluble Vitamins with Minerals Capsules
- Water-Soluble Vitamins with Minerals Tablets

Method A

NOTE- This procedure involves the extraction of calcium pantothenate from the formulation by the internal standard solution.

Unless specified in the individual monographs, internal standard solution, reference solution, test solution, and reagent solutions are prepared as follows:

Internal standard solution. Dissolve 80 mg of *p-hydroxybenzoic acid* in 3 ml of *ethanol (95 per cent)*. Add 50 ml of *water* and 7.1 g of *dibasic sodium phosphate* and dilute to 1000 ml with *water*, adjusted to pH 6.7 with *orthophosphoric acid*.

Test solution (for capsules). Disperse a quantity of the mixed contents of capsules containing 60 mg of Calcium Pantothenate in 100.0 ml of internal standard solution, centrifuge and use the supernatant liquid.

Test solution (for tablets). Transfer a quantity of the powdered tablets containing 15 mg of Calcium Pantothenate in a centrifuge tube, add 25.0 ml of the internal standard solution, shake vigorously for 10 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.06 per cent w/v of *calcium pantothenate IPRS* in internal standard solution

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a 0.1 per cent v/v solution of *orthophosphoric acid* in *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

The relative retention time with reference to *p*-hydroxybenzoic acid for calcium pantothenate is about 0.5.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{32}CaN_2O_{10}$.

Method B

NOTE - This procedure involves dissolving an accurately weighed quantity of the sample containing 10 mg of Calcium Pantothenate in 10 ml of methanol and dilute to 250.0 ml with water.

Unless specified in the individual monographs, reference solution, test solution, and reagent solutions are prepared as follows:

Test solution (for capsules). Transfer a quantity of the mixed contents of capsules containing 10 mg of Calcium Pantothenate to a 250-ml volumetric flask, add 10 ml of *methanol*, swirl to disperse and dilute to volume with *water*.

Test solution (for tablets). Transfer a quantity of the powdered tablets containing 10 mg of Calcium Pantothenate to a 250-ml volumetric flask, add 10 ml of *methanol*, swirl to disperse and dilute to volume with *water*.

Reference solution (a). A 0.025 per cent w/v solution of *calcium pantothenate IPRS* in *water* (*NOTE – Use the solution within four weeks and store in refrigerator*).

Reference solution (b). Dilute 4.0 ml of reference solution (a) to 25.0 ml with *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50º,
- mobile phase: a mixture of 90 volumes of a buffer solution prepared by dissolving 5 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid* and 10 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 25 µl.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{18}H_{32}CaN_2O_{10}$.

Method C

This procedure can be used to determine calcium pantothenate in:

- Oil- and Water-Soluble Vitamins oral solutions
- Oil- and Water-Soluble Vitamins with minerals oral solutions
- Water-Soluble Vitamins with minerals oral solutions

NOTE - This procedure involves dissolve a quantity of liquid sample in water to obtain a solution containing 0.008 per cent v/v of calcium pantothenate.

Unless specified in the individual monographs, reference solution, system suitability solution, test solution, and reagent solutions are prepared as follows:

Test solution. Dilute a volume of oral solution with the mobile phase to obtain a solution containing 0.008 per cent w/v of Calcium Pantothenate.

Reference solution (a). A 0.008 per cent w/v solution of *calcium pantothenate IPRS* in the mobile phase.

Reference solution (b). A 0.008 per cent w/v solution of *racemic panthenol IPRS* in the mobile phase. Mix equal volumes of the solution with reference solution (a).

Chromatographic system

- a stainless steel column 10 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 97 volumes of *0.2 M sodium dihydrogen phosphate* and 3 volumes of *methanol*, adjusted to pH 3.2 with 1.7 M *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to panthenol and calcium pantothenate is not less than 1.5 in the chromatogram obtained with reference solution (b), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{32}CaN_2O_{10}$.

Method D

This procedure can be used to determine calcium pantothenate in:

- Active pharmaceutical ingredients

NOTE - This method involves the dissolution of the sample directly into water.

Unless specified in the individual monographs, reference solution, system suitability solution, test solution, and reagent solutions are prepared as follows.

Test solution. Dissolve 50 mg of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution (a). A 0.05 per cent w/v solution of *calcium pantothenate IPRS* in *water*.

Reference solution (b). A solution containing 0.01 per cent w/v of *calcium pantothenate IPRS* and 0.05 per cent w/v, each of, *beta alanine IPRS*, *sodium D-pantoate IPRS* and *pantolactone IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50º,
- mobile phase: a mixture of 98 volumes of a buffer solution prepared by dissolving 3.2 g of *sodium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 5.5 with *1 M sodium hydroxide* and 2 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 10 µl.

Name	Relative retention time
Beta alanine	0.3
Pantoic acid	0.6
Pantothenic acid	1.0
Pantolactone	1.9

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to pantothenic acid and pantoic acid is not less than 5.0 in the chromatogram obtained with reference solution (b), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{32}CaN_2O_{10}$.

4.2. GENERAL REAGENTS. Page 890

Inset before **4-Hydroxycoumarin;** Page 918

***p*-hydroxybenzoic acid**: $C_7H_6O_3$= 138.12

Chromatographic grade of commerce.

White crystals; mp, about 216°; contains not less than 97.0 per cent of $C_7H_6O_3$.

ASSAY. Dissolve 0.7 g in 50 ml of *acetone*, add 100 ml of *water*, mix and titrate with *0.5 M sodium hydroxide* determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.06906 g of $C_7H_6O_3$.