Consultation documents

Medicines quality assurance texts

General guidance on “hold-time” studies

This is a revised draft proposal for a new medicines quality assurance guideline (Working document QAS/13.521/Rev.3, August 2014).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; Dr Sabine Kopp, kopps@who.int and Ms Marie Gaspard, gaspardm@who.int.

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1. Introduction and background

Manufacturers should ensure that the products that they manufacture are safe, effective and of the quality required for their intended use. Products should be consistently manufactured to the quality standards appropriate to their intended use and as required by the marketing authorization. Systems should ensure that pharmaceutical products are produced according to validated processes and to defined procedures. Manufacturing processes should be shown to be capable of consistently manufacturing pharmaceutical products of the required quality that comply with their specifications.

Arrangements should exist to ensure that the dispensed raw materials and packaging materials, intermediate products, bulk and finished products are stored under appropriate conditions. Storage should not have any significant negative effect on the processing, stability, safety, efficacy or quality of the materials, intermediate products and bulk products prior to final packing. Good manufacturing practices (GMP) require that a maximum acceptable holding period should be established to ensure that intermediates and bulk product can be held, pending the next processing step, without any significant adverse effect to the quality of the material. Such a holding period should be underwritten by data, but need not be extended to find the edge of failure.

2. Glossary

Bulk product
Any pharmaceutical product which has completed all processing stages up to, but not including, final packaging.

Intermediate
Partly processed product that must undergo further manufacturing steps before it becomes a bulk product.
3. Scope

This guideline focuses primarily on aspects that should be considered in the design of the hold-time studies during the manufacture of solid dosage forms. Many of the principles herein also apply to other dosage forms such as liquids, creams, and ointments. This guideline does not cover aspects for hold times in cleaning validation or the manufacturing of active pharmaceutical ingredients (APIs).

This guideline is intended as a basic guide for use by pharmaceutical manufacturers and GMP inspectors. This document does not intend to prescribe a process for establishing hold times, but reflects aspects that should be considered in the design of the hold-time study.

Manufacturers should gather scientific and justifiable data to demonstrate that the dispensed raw materials and packaging materials, intermediate and bulk products:

- remain of appropriate quality before processing to the next stage;
- meet the acceptance criteria and release specification for the finished product.

4. Aspects to be considered

Hold time can be considered as the established time period for which materials (dispensed raw materials, intermediates and bulk dosage form awaiting final packaging) may be held under specified conditions and will remain within the defined specifications.

Data to justify the hold time can be collected, but not limited to:

- during development on pilot-scale batches,
- during scale up,
- during process validation, or
- as part of an investigation of a deviation that occurred during manufacture.

Hold-time studies establish the time limits for holding the materials at different stages of production to ensure that the quality of the product does not deteriorate significantly during the hold time. The design of the study should reflect the holding time at each stage. Hold times should normally be determined prior to marketing of a product and following any significant changes in processes, equipment, starting and packaging materials and represent actual processing. Hold time studies should be included during process validation (Ref: Process validation guideline).

Manufacturers may use a flow chart to review the manufacturing procedure of a product and then break up the critical stages of manufacturing process on the basis of time duration required for the particular storage and processing stages, typical pauses in the manufacturing campaign, and the potential impact of storage with reference to environmental and storage conditions. An example for a flow chart is given below.

For example, for oral tablets that are coated the following stages may be considered:

- binder preparation to granulation – consider the granulate;
- wet granulation to drying – the dried granulate;
- dried granules to lubrication/blending – the lubricated blend;
- blend to compression;
- compression to coating – the tablet cores;
- coating solution to preparation – the coating solution;
- coating to packing – consider the bulk coated tablets;
- coating to packing in bulk or FDF;
- packing in bulk to FDF.
A written protocol, procedure or programme should be followed which includes the activities to be performed, test parameters and acceptance criteria appropriate to the material or product under test. The protocol and report should generally include the following: a title; reference number; version; date; objective; scope; responsibility; procedure; description of the material/product; sample quantities; sampling method and criteria; acceptance limits; frequency for sampling; sampling locations; pooling of samples; storage conditions; type of container; methods of analysis; results; conclusion; recommendation; signatures and dates. Acceptance criteria are typically more stringent than registered specifications to provide assurance that the material is well within control.

When setting the specifications any known stability trends will need to be taken into account.

For certain products microbiological aspects should also be considered and included where appropriate.

Typically one or more batches of a material, intermediate or product can be used for determining hold times. A risk-based approach can be used to determine the appropriate number of batches, considering inter alia the characteristics of the materials. A representative sample of the batch of material or product subjected to the hold-time study should be held for the defined hold period. The maximum hold period for each category of material should be established on the basis of the study by keeping the material in either
the original or simulated container used in production. The containers used in which hold-time samples are stored should be the same pack as used in production unless the pack is exceptionally large, in which case one that is equivalent (same material of construction and closure system to the production packaging system) may be used. Reducing the size of container when necessary for testing holding time, should be justified. Where head space is important the hold-time samples should represent the maximum possible head space (worst-case scenario) to bulk stored in manufacturing/quarantine. The sample storage environmental conditions should be same as that of the quarantine area/manufacture stage. A sampling plan should be established and followed for taking samples for testing at the different intervals. The required sample amount should be calculated based on the batch size, the intervals and tests to be performed. Results should be compared with the initial baseline data of the control sample. Samples may be pooled for analysis where appropriate, e.g. when the analysis of a composite sample will not miss issues expected in the variation of the product.

Where appropriate, statistical analysis of the data generated should be performed to identify trends and to justify the limits and hold time set.

Batches of finished products made from intermediates or bulk products and subjected to a hold-time study should be considered for long-term stability testing if data show adverse trending or shifting patterns during the intermediate time points up to the end of the shelf-life. The shelf-life of the product – irrespective of hold times – should be measured from the time the active ingredients are mixed with other ingredients. Normally intermediate and bulk products should not be stored beyond the established hold time. All testing of bulk intermediates and product should be performed using validated stability-indicating methods.

The following table provides examples of stages and tests that may be considered.

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### Continued

<table>
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Good review practices guidelines for regulatory authorities

A revised draft guidance text on good review practices for medical products was endorsed by the Regulatory Harmonization Steering Committee (RHSC) of the Asia-Pacific Economic Cooperation (APEC) at its meeting in China in August 2014 for submission to WHO. The document incorporates outcomes and comments from a parallel consultation process through both the WHO Expert Committees on Specifications for Pharmaceutical Preparations and on Biological Standardization.

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; Dr Sabine Kopp, kopps@who.int and Ms Marie Gaspard, gaspardm@who.int.

Due to space limitations it was not possible to include the full proposed text in this issue of WHO Drug Information. An outline is reproduced below.

1. Introduction
   1.1 Document objective
      The objective of the document is to provide high level guidance on good review practice (GRevP) principles and processes, for use across a range of regulatory authority (RA) maturities. It is not intended to provide detailed instruction on how to conduct a scientific review.
      This document is envisioned as one building block in a set of tools and is sufficiently expandable to accommodate additional annexes or ancillary documents in the future.
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Pyranteli embonas
Pyrantel embonate

This is a draft proposal for The International Pharmacopoeia (Working document QAS/14.589, June 2014).

The working document with line numbers and tracked changes is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. It is proposed to revise the monograph on Pyrantel embonate.]
[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

Molecular formula. \( C_{11}H_{14}N_2S_{23}H_{16}O_6 \)

Relative molecular mass. 594.7

Graphic formula.


Other name. Pyrantel pamoate.

Description. A pale yellow or yellow powder.

Solubility. Practically insoluble in water and methanol R; soluble in dimethyl sulfoxide R; slightly soluble in dimethylformamide R.

Category. Anthelmintic.

Storage. Pyrantel embonate should be kept in a well-closed container, protected from light.

Requirements

Definition. Pyrantel embonate contains not less than 98.0 and not more than 102.0 of \( C_{11}H_{14}N_2S_{23}H_{16}O_6 \), calculated with reference to the dried substance.
Identity tests

• Either tests A alone or tests B and C may be applied.

  A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from pyrantel embonate RS or with the reference spectrum of pyrantel embonate.

  B. The absorption spectrum of a 13 μg/mL solution in methanol R, when observed between 230 nm and 360 nm, exhibits 2 maxima at about 288 nm and 300 nm. The ratio of the absorbance at 288 nm to that at 300 nm is about 1.0.

  C. See the test described under “Related substances”, Method A. The principal spots obtained with solution (1) correspond in position, appearance and intensity with those obtained with solution (3).

Chlorides. Dissolve 0.46 g of P yrantel embonate in a mixture of 10 mL of nitric acid (~130 g/L) TS and 30 mL of water R. Heat on a water-bath for 5 min, allow to cool, dilute to 50 mL with water R, mix well and filter. Add 1 mL of nitric acid (~130 g/L) TS to 15 mL of the filtrate and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.36 mg/g.

Sulfates. Dissolve 0.50 g of P yrantel embonate in 2.5 mL of nitric acid (~130 g/L) TS and dilute to 30 mL with water R. Heat on a water-bath for 5 min, shake for 2 min, cool and filter and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 1 mg/g.

Iron. Ignite 0.66 g of Pyrantel embonate at 800 ± 50 °C for 2 h. Cool and dissolve the residue in 2.5 mL of hydrochloric acid (~70 g/L) with gentle heating for 10 min. Cool and dilute to 40 mL with water R and proceed as described under 2.2.4 Limit test for iron; not more than 75 μg/g.

Sulfated ash. Not more than 1.0 mg/g.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 μg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 10 mg/g.

Related substances

Carry out the operations in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

• Either method A or B may be applied.

  A. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 3 volumes of ethyl acetate R, 1 volume of water R and 1 volume of glacial acetic acid R as the mobile phase.

Prepare the following solutions. For solution (1) dissolve about 100 mg of Pyrantel embonate in 10.0 mL dimethylformamide R (1). For solution (2) dilute 1.0 mL of solution (1) to 100 mL with dimethylformamide R. For solution (3) use 10 mg of pyrantel embonate RS per mL dimethylformamide R. For solution (4) expose a quantity of solution (3) under 2000 l x illumination for 24 hours. In case a suitable device to provide the requested illuminance is not available use 10 mg of pyrantel embonate impurity A RS and 2 mg pyrantel embonate RS per mL dimethylformamide R for solution (4).
Apply separately to the plate 5 μL of each of the solutions (1), (2), (3) and (4).

After application allow the spots to dry for 15 minutes in a current of air. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber allow it to dry for 10 minutes in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

Pyrantel and related substances have the following Rf values: impurity A about 0.2; pyrantel about 0.3; embonic acid about 0.9. The test is not valid unless the chromatogram obtained with solution (4) exhibits three well separated spots.

In the chromatogram obtained with solution (1) any spot, other than the two principal spots, is not more intense than the pyrantel spot in the chromatogram obtained with solution (2) (1.0%). Disregard any spot remaining at the point of application.

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with high purity base particles of silica gel for chromatography R (5 μm)¹.

As the mobile phase use a mixture of 92.8 volumes of acetonitrile R and 7.2 volumes of a solvent mixture composed of 5 volumes of glacial acetic R, 5 volumes of water R and 2 volumes of diethylamine R.

Prepare the following solutions. For solution (1) transfer about 72 mg of Pyrantel embonate, accurately weighed, to a 100 mL volumetric flask. Add 7 mL of a mixture composed of 5 volumes of glacial acetic R, 5 volumes of water R and 2 volumes of diethylamine R. Shake and dilute to volume with acetonitrile R, mix and filter. For solution (2), dilute 1.0 mL of the solution (1) to 100.0 mL with mobile phase. For solution (3) expose 10 mL of solution (1) under 2000 lx illumination for 24 hours. In case a suitable device to provide the requested illuminance is not available transfer 10 mg of pyrantel embonate impurity A RS to a 10.0 mL flask, add 8 mL of solution (1) and make up to volume with dimethylformamide R to obtain solution (3).

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 288 nm.

Inject separately 20 μL each of solution (1), (2) and (3) and record the chromatograms for 4 times the retention time of pyrantel.

In the chromatogram obtained with solution (3) the following peaks are eluted at the following relative retention with reference to pyrantel (retention time about 14 minutes): embonic acid about 0.5; impurity A about 1.3. The test is not valid unless the resolution factor between the pyrantel peak and the impurity A peak is at least 4.0.

In the chromatogram obtained with solution (1) the area of any impurity peak is not greater than the area of the pyrantel peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak obtained with solution (2) (0.1%).

Assay
Perform the assay in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

¹ Shim-pack HRS-SIL column (25 cm x 4.6 mm, 5 μm) has been found suitable.
Dissolve about 0.450 g of Pyrantel embonate, accurately weighed, in 10 mL of acetic anhydride R and 50 mL of glacial acetic acid R. Heat at 50 °C and stir for 10 minutes. Allow to cool and titrate the suspension with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically. Carry out a blank titration.

Each ml of perchloric acid (0.1 mol/L) VS is equivalent to 59.47 mg of pyrantel embonate C_{11}H_{14}N_2S.C_{23}H_{16}O_6.

**Impurities**

A. 1-methyl-2-[(1E)-2-(thiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidine.
Pyranteli compressi
Pyrantel tablets

This is a draft proposal for *The International Pharmacopoeia* (Working document QAS/14.588, June 2014).

The working document with line numbers and tracked changes is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en). Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. It is proposed to revise the monograph on Pyrantel embonate tablets.]

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**Category.** Anthelminthic.

**Storage.** Pyrantel tablets should be kept in a tight, lightly-closed container, protected from light.

**Labelling.** The designation on the container of Pyrantel tablets should state that the active ingredient is in the embonate form, and the quantity should be indicated in terms of equivalent amount of pyrantel.

**Additional information.** Strength in the current WHO Model List of Essential Medicines: 250 mg of pyrantel (as embonate or pamoate).

**Requirements**

Comply with the monograph for *Tablets*.

**Definition.** Pyrantel tablets contain not less than 90.0% and not more than 110.0% of the amount of pyrantel \((\text{C}_{11}\text{H}_{14}\text{N}_{2}\text{S})\) stated on the label.

**Identity tests**

- Either test A alone, or any two of tests B, C and D may be applied.

To a quantity of the powdered tablets containing the equivalent of about 20 mg of pyrantel add a mixture of 10 mL of dichloromethane R, 10 mL of methanol R and about 1 mL of ammonia (~260g/L) TS, shake and filter. Evaporate the filtrate to dryness on a water-bath, dissolve in a small volume of methanol R (about 3 mL) by heating on a water-bath and then allowing the solution to cool. Separate the crystals, dry at 80 °C for 2 hours and use the dried crystals for the “Identity tests” A and C.

**A.** Carry out the examination with the dried crystals as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from pyrantel embonate RS or with the reference spectrum of pyrantel embonate.

**B.** See the test described below under “Related substances”, Method A. The principal spots obtained with solution (1) correspond in position, appearance and intensity with those obtained with solution (3).
C. The absorption spectrum (1.6) of a 13 μg/mL solution of the dried crystals in methanol R, when observed between 230 nm and 360 nm, exhibits 2 maxima at about 288 nm and 300 nm. The ratio of the absorbance at 288 nm to that at 300 nm is about 1.0. See the test described under “Assay”. The retention times of the principal peaks in the chromatogram obtained from solution (1) are similar to those obtained from solution (2).

D. See the test described under “Assay”. The retention times of the principal peaks in the chromatogram obtained from solution (1) are similar to those obtained from solution (2).

**Dissolution**

Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms using as the dissolution medium, 900 mL of a solution prepared by dissolving 1.0 g of sodium dodecyl sulphate R and 7 mL hydrochloric acid (~420g/L) TS in 1000 mL of water. Rotate the paddle at 75 revolutions per minute. At 60 minutes withdraw a sample of 10 mL of the medium through an in-line filter. Transfer 1.0 mL of the clear filtrate to a 50 mL volumetric flask and dilute to volume with hydrochloric acid/methanol (0.1 mol/L) VS (solution (1)). For solution (2) transfer about 20 mg of pyrantel embonate RS (equivalent to about 7.0 mg of pyrantel), accurately weighed, into a 25 mL volumetric flask. Add about 10 mL of dimethylformamide R, shake to dissolve and dilute to volume with hydrochloric acid/methanol (0.1 mol/L) VS. Transfer 1.0 mL of this solution to a 50 mL volumetric flask and dilute to volume with hydrochloric acid/methanol (0.1 mol/L) VS. Measure the absorbance (1.6) of the samples at a wavelength of 316 nm, using hydrochloric acid/methanol (0.1 mol/L) VS as the blank.

For each of the tablets tested calculate the total amount of pyrantel (C₁₁H₁₄N₂S) in the medium from the absorbances obtained using the declared content of C₁₁H₁₄N₂S.C₂₃H₁₆O₆ in pyrantel embonate RS. Each mg of pyrantel embonate C₁₁H₁₄N₂S.C₂₃H₁₆O₆ is equivalent to 0.3469 mg of pyrantel C₁₁H₁₄N₂S. Use the requirements as described under 5.5 Dissolution test for solid oral dosage forms. Acceptance criteria to evaluate the results: the amount in solution is not less than 70% (Q) of the amount declared on the label.

**Related substances**

Carry out the operations in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

- Either method A or B may be applied.

A. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 3 volumes of ethyl acetate R, 1 volume of water R and 1 volume of glacial acetic acid R as the mobile phase.

Prepare the following solutions. For solution (1) add to a quantity of the powdered tablets equivalent of about 35 mg of pyrantel a mixture of 10 mL of dichloromethane R, 10 mL of methanol R and about 1 mL of ammonia (~260 g/L) TS, shake and filter. Evaporate the filtrate to dryness on a water-bath and dissolve the dried residue in 10.0 mL dimethylformamide R. For solution (2) dilute 1.0 mL of solution (1) to 100 mL with dimethylformamide R. For solution (3) use 10 mg of pyrantel embonate RS (equivalent to about 3.5 mg of pyrantel) per mL dimethylformamide R. For solution (4) expose a quantity of solution (3) under 2000 lux illumination for 24 hours. In case a suitable device to provide the requested illuminance is not available use 10 mg of pyrantel embonate impurity A RS and 2 mg pyrantel embonate RS (equivalent to about 0.7 mg of pyrantel) per mL dimethylformamide R for solution (4).

Apply separately to the plate 5 μl of each of the solutions (1), (2), (3) and (4).

After application allow the spots to dry for 15 minutes in a current of air. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber allow it
to dry in a current of air for 10 minutes. Examine the chromatogram in ultraviolet light (254 nm).

Pyrantel and related substances have the following Rf values: impurity A about 0.2; pyrantel about 0.3; embonic acid about 0.9. The test is not valid unless the chromatogram obtained with solution (4) exhibits three well-separated spots.

In the chromatogram obtained with solution (1) any spot, other than the two principal spots, is not more intense than the pyrantel spot in the chromatogram obtained with solution (2) (1.0%). Disregard any spot remaining at the point of application.

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets and transfer a quantity containing the equivalent of about 25 mg of pyrantel into a 100 mL volumetric flask. Add 7 mL of a mixture composed of 5 volumes of glacial acetic R, 5 volumes of water R and 2 volumes of diethylamine R. Shake and dilute to volume with acetonitrile R, mix and filter. For solution (2) dilute 1.0 mL of the solution (1) to 100.0 mL with mobile phase. For solution (3) expose 10 mL of solution (1) under 2000 I x illumination for 24 hours. In case a suitable device to provide the requested illuminance is not available transfer 10 mg of pyrantel embonate impurity A RS to a 10.0 mL flask, add 8 mL of solution (1) and make up to volume with dimethylformamide R to obtain solution (3).

Inject separately 20 μL each of solution (1), (2) and (3) and record the chromatograms for 4 times the retention time of pyrantel.

In the chromatogram obtained with solution (3) the following peaks are eluted at the following relative retention with reference to pyrantel (retention time about 14 minutes): embonic acid about 0.5; impurity A about 1.3. The test is not valid unless the resolution factor between the pyrantel peak and the impurity A peak is at least 4.0.

In the chromatogram obtained with solution (1): the area of any impurity peak is not greater than the area of the pyrantel peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak obtained with solution (2) (0.1%).

Assay
The operations described below must be carried out in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with high-purity base particles of silica gel for chromatography R (5 μm).\(^1\)

As the mobile phase use a mixture of 92.8 volumes of acetonitrile R and 7.2 volumes of a solvent mixture composed of 5 volumes of glacial acetic R, 5 volumes of water R and 2 volumes of diethylamine R.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the tablets containing the equivalent of about 7.0 mg of pyrantel, accurately weighed, into a 50 mL volumetric flask. Add about 30 mL of mobile phase, shake for 10 minutes and dilute with mobile phase to volume, mix and filter. Transfer 2.0 mL of the clear filtrate to a 10 mL volumetric flask, dilute with mobile phase to volume and mix. For solution (2) prepare a solution of 0.40 mg of pyrantel embonate RS (equivalent to about 0.14 mg of pyrantel) per mL.

\(^1\) Shim-pack HRS-SIL column (25 cm×4.6 mm, 5 μm) has been found suitable
mobile phase. Transfer 2.0 mL of this solution to a 10 mL volumetric flask, dilute with mobile phase to volume, and mix to obtain a standard preparation having a known concentration of 80 μg of pyrantel embonate RS (equivalent to about 28 μg of pyrantel) per mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 288 nm.

Inject separately 20 μL each of solution (1) and (2) and record the chromatograms.

In the chromatogram obtained with solution (2) the peak due to embonic acid is eluted at a relative retention time of about 0.5 with reference to pyrantel (retention time about 14 minutes).

Measure the areas of the peak responses due to pyrantel obtained in the chromatograms from solution (1) and solution (2), and calculate the content of pyrantel \( \text{C}_{11}\text{H}_{14}\text{N}_2\text{S} \) in the tablets, using the declared content of \( \text{C}_{11}\text{H}_{14}\text{N}_2\text{S}\cdot\text{C}_{23}\text{H}_{16}\text{O}_6 \) in pyrantel embonate RS. Each mg of pyrantel embonate \( \text{C}_{11}\text{H}_{14}\text{N}_2\text{S}\cdot\text{C}_{23}\text{H}_{16}\text{O}_6 \) is equivalent to 0.3469 mg of pyrantel \( \text{C}_{11}\text{H}_{14}\text{N}_2\text{S} \).

**Impurities**

![Structural formula of impurity](image)

A. 1-methyl-2-[(1E)-2-(thiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidine.

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**Pyranteli compressi manducabili**

Pyrantel chewable tablets

This is a draft proposal for *The International Pharmacopoeia* (Working document QAS/14.587, June 2014).

The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/). Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

[Note from the Secretariat. It is proposed to revise the monograph on Pyrantel chewable tablets with a view to include a dissolution test.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

**Category.** Anthelminthic.

**Storage.** Pyrantel chewable tablets should be kept in a tightly closed container, protected from light.

**Labelling.** The designation on the container of Pyrantel chewable tablets should state that the active ingredient is in the embonate form and the quantity should be indicated in terms of
equivalent amount of pyrantel and should state that the tablets may be chewed or swallowed whole.

**Additional information.** Strength in the current WHO Model list of essential medicines: 250 mg of pyrantel (as embonate or pamoate).

**Requirements**

Comply with the monograph for Tablets.

Definition. Pyrantel chewable tablets contain Pyrantel embonate in a suitable basis that may contain suitable flavouring agents. They contain not less than 90.0% and not more than 110.0% of the amount of pyrantel \( (C_{11}H_{14}N_2S) \) stated on the label.

**Identity tests**

- Either tests A alone, or any two of tests B, C, D and E may be applied.

To a quantity of the powdered tablets containing the equivalent of about 20 mg of pyrantel add a mixture of 10 mL of dichloromethane R, 10 mL of methanol R and about 1 mL of ammonia \((\sim260 \text{ g/L}) \) TS, shake and filter. Evaporate the filtrate to dryness on a water-bath, dissolve in a small volume of methanol R (about 3 mL) by heating on a water-bath and then allowing the solution to cool. Separate the crystals, dry at 80 °C for 2 hours and use the dried crystals for "Identity tests A, C and D".

A. Carry out the examination with the dried crystals as described under 1.7. **Spectrophotometry in the infrared region.** The infrared absorption spectrum is concordant with the spectrum obtained from pyrantel embonate RS or with the reference spectrum of pyrantel embonate.

B. See the test described under “Related substances”, Method A. The principal spots obtained with solution (1) correspond in position, appearance and intensity with those obtained with solution (3).

C. The absorption spectrum \((1.6)\) of a 13 μg/mL solution of the dried crystals in methanol R, when observed between 230 nm and 360 nm, exhibits 2 maxima at about 288 nm and 300 nm. The ratio of the absorbance at about 288 nm to that at about 300 nm is about 1.0.

D. Dissolve about 5 mg of the dried crystals in 1 mL of hydrochloric acid \((\sim70 \text{ g/L}) \) TS and add 1 mL of formaldehyde/sulfuric acid TS; a violet-red colour is produced.

E. See the test described under “Assay”. The retention times of the principal peaks in the chromatogram obtained from solution (1) are similar to those obtained from solution (2).

**Dissolution**

Carry out the test as described under 5.5 **Dissolution test for solid oral dosage forms** using as the dissolution medium, 900 mL of a solution prepared by dissolving 1.0 g of sodium dodecyl sulphate R and 7 mL hydrochloric acid \((\sim420\text{ g/L}) \) TS in 1000 mL of water. Rotate the paddle at 75 revolutions per minute. At 60 minutes withdraw a sample of 10 mL of the medium through an in-line filter. Transfer 1.0 mL of the clear filtrate to a 50 mL volumetric flask and dilute to volume with hydrochloric acid/methanol \((0.1 \text{ mol/L}) \) VS (solution (1)). For solution (2) transfer about 20 mg of pyrantel embonate RS (equivalent to about 7.0 mg of pyrantel), accurately weighed, into a 25 mL volumetric flask. Add about 10 mL of dimethylformamide R, shake to dissolve and dilute to volume with hydrochloric acid/methanol \((0.1 \text{ mol/L}) \) VS. Transfer 1.0 mL of this solution to a 50 mL volumetric flask and dilute to volume with hydrochloric acid/methanol \((0.1 \text{ mol/L}) \) VS . Measure the absorbance \((1.6)\) of the samples at a wavelength of 316 nm, using hydrochloric acid/methanol \((0.1 \text{ mol/L}) \) VS as the blank.
For each of the tablets tested, calculate the total amount of pyrantel (C\textsubscript{11}H\textsubscript{14}N\textsubscript{2}S) in the medium from the absorbances obtained using the declared content of C\textsubscript{11}H\textsubscript{14}N\textsubscript{2}S\textsubscript{C}_{23}H_{16}O_{6} in pyrantel embonate RS. Each mg of pyrantel embonate C\textsubscript{11}H\textsubscript{14}N\textsubscript{2}S\textsubscript{C}_{23}H_{16}O_{6} is equivalent to 0.3469 mg of pyrantel C\textsubscript{11}H\textsubscript{14}N\textsubscript{2}S. Use the requirements as described under 5.5 Dissolution test for solid oral dosage forms, “Acceptance criteria” to evaluate the results: the amount in solution is not less than 65 % (Q) of the amount declared on the label.

**Related substances**

Carry out the operations in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

- Either method A or B may be applied.

**A.** Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 3 volumes of ethyl acetate R, 1 volume of water R and 1 volume of glacial acetic acid R as the mobile phase.

Prepare the following solutions. For solution (1) add to a quantity of the powdered tablets equivalent of about 35 mg of pyrantel a mixture of 10 mL of dichloromethane R, 10 mL of methanol R and about 1 mL of ammonia (\sim 260 g/L) TS, shake and filter. Evaporate the filtrate to dryness on a water-bath and dissolve the dried residue in 10.0 mL dimethylformamide R. For solution (2) dilute 1.0 mL of solution (1) to 100 mL with dimethylformamide R. For solution (3) use 10 mg of pyrantel embonate RS (equivalent to about 3.5 mg of pyrantel) per mL dimethylformamide R. For solution (4) expose a quantity of solution (3) under 2000 L x illumination for 24 hours. In case a suitable device to provide the requested illuminance is not available use 10 mg of pyrantel embonate impurity A RS and 2 mg pyrantel embonate RS (equivalent to about 0.7 mg of pyrantel) per mL dimethylformamide R for solution (4).

Apply separately to the plate 5 \mu L of each of the solutions (1), (2), (3) and (4).

After application allow the spots to dry for 15 minutes in a current of air. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber allow it to dry in a current of air for 10 minutes. Examine the chromatogram in ultraviolet light (254 nm).

Pyrantel and related substances have the following Rf values: impurity A about 0.2; pyrantel about 0.3; embonic acid about 0.9. The test is not valid unless the chromatogram obtained with solution (4) exhibits three well-separated spots.

In the chromatogram obtained with solution (1) any spot, other than the two principal spots, is not more intense than the pyrantel spot in the chromatogram obtained with solution (2) (1.0%). Disregard any spot remaining at the point of application.

**B.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets and transfer a quantity containing the equivalent of about 25 mg of pyrantel into a 100 mL volumetric flask. Add 7 mL of a mixture composed of 5 volumes of glacial acetic R, 5 volumes of water R and 2 volumes of diethylamine R. Shake and dilute to volume with acetonitrile R, mix and filter. For solution (2) dilute 1.0 mL of the solution (1) to 100.0 mL with mobile phase. For solution (3) expose 10 mL of solution (1) under 2000 L x illumination for 24 hours. In case a suitable device to provide the requested illuminance is not available transfer 10 mg of pyrantel embonate impurity A RS to a 10.0 mL flask, add 8 mL of solution (1) and make up to volume with dimethylformamide R to obtain solution (3).
Inject separately 20 μL each of solution (1), (2) and (3) and record the chromatograms for 4 times the retention time of pyrantel.

In the chromatogram obtained with solution (3) the following peaks are eluted at the following relative retention with reference to pyrantel (retention time about 14 minutes): embonic acid about 0.5; impurity A about 1.3. The test is not valid unless the resolution factor between the pyrantel peak and the impurity A peak is at least 4.0.

In the chromatogram obtained with solution (1) the area of any impurity peak is not greater than the area of the pyrantel peak obtained with solution (2) (1.0%).

**Assay**

Carry out the operations in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with high-purity base particles of silica gel for chromatography R (5 μm).\(^1\)

As the mobile phase use a mixture of 92.8 volumes of acetonitrile R and 7.2 volumes of a solvent mixture composed of 5 volumes of glacial acetic R, 5 volumes of water R and 2 volumes of diethylamine R.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the chewable tablets containing the equivalent of about 7.0 mg of pyrantel, accurately weighed, into a 50 mL volumetric flask. Add about 30 mL of mobile phase, shake for 10 minutes and dilute with mobile phase to volume, mix and filter. Transfer 2.0 mL of the clear filtrate to a 10 mL volumetric flask, dilute with mobile phase to volume and mix. For solution (2) prepare a solution of 0.40 mg of pyrantel embonate RS (equivalent to about 0.14 mg of pyrantel) per mL mobile phase. Transfer 2.0 mL of this solution to a 10 mL volumetric flask, dilute with mobile phase to volume and mix to obtain a standard preparation having a known concentration of 80 μg of pyrantel embonate RS (equivalent to about 28 μg of pyrantel) per mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 288 nm.

Inject separately 20 μL each of solution (1) and (2) and record the chromatograms.

In the chromatogram obtained with solution (2) the peak due to embonic acid is eluted at a relative retention time of about 0.5 with reference to pyrantel (retention time about 14 minutes).

Measure the areas of the peak responses due to pyrantel obtained in the chromatograms from solution (1) and solution (2), and calculate the content of pyrantel (C\(_{11}\)H\(_{14}\)N\(_2\)S) in the chewable tablets, using the declared content of C\(_{11}\)H\(_{14}\)N\(_2\)S,C\(_{23}\)H\(_{16}\)O\(_6\) in pyrantel embonate RS. Each mg of pyrantel embonate C\(_{11}\)H\(_{14}\)N\(_2\)S,C\(_{23}\)H\(_{16}\)O\(_6\) is equivalent to 0.3469 mg of pyrantel C\(_{11}\)H\(_{14}\)N\(_2\)S.

**Impurities**

\[\text{A. 1-methyl-2-[(1E)-2-(thiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidin}e.\]

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\(^1\) Shim-pack HRS-SIL column (25 cm×4.6 mm, 5 μm) has been found suitable.
Dexamethasoni natrii phosphas
Dexamethasone sodium phosphate

This is a draft proposal for The International Pharmacopoeia (Working document QAS/14.579, June 2014).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. It is proposed to revise the monograph on Dexamethasone sodium phosphate in The International Pharmacopoeia.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

C_{22}H_{28}FNa_2O_8P

Relative molecular mass. 516.4


Description. A white or almost white, crystalline powder.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/L) TS; practically insoluble in ether R and methylene chloride R.

Category. Adrenal hormone.

Storage. Dexamethasone sodium phosphate should be kept in a tightly closed container, protected from light.

Additional information. Dexamethasone sodium phosphate is very hygroscopic. Even in the absence of light it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Dexamethasone sodium phosphate may exhibit polymorphism.
Requirements

Definition. Dexamethasone sodium phosphate contains not less than 97.0% and not more than 102% of $C_{22}H_{28}FNa_2O_8P$, calculated with reference to the anhydrous and ethanol-free substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R1 as the coating substance and a freshly prepared mixture of 3 volumes of 1-butanol R, 1 volume of acetic anhydride R and 1 volume of water as the mobile phase. Apply separately to the plate 5 μL of each of 4 solutions in methanol R containing (A) 1 mg of the test substance per ml, (B) 1 mg of dexamethasone sodium phosphate RS per ml, (C) a mixture of equal volumes of solutions (A) and (B), and (D) equal volumes of solution (A) and a solution of 1 mg of prednisolone sodium phosphate RS per ml of methanol R. After removing the plate from the chromatographic chamber allow it to dry in air until the solvents have evaporated, spray it with sulfuric acid/ethanol (20%) TS, heat it at 120°C for 10 minutes, allow it to cool and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B). The principal spot obtained with solution (C) appears as a single compact spot whereas the chromatogram of solution (D) shows 2 closely running spots.

B. Dissolve 10.0 mg of the test substance in 5 ml of water R and dilute to 100.9 ml with dehydrated ethanol R. Transfer 2.0 ml of this solution to a glass-stoppered tube, add 10.0 ml of phenylhydrazine/sulfuric acid TS, mix and heat in a water-bath at 60°C for 20 minutes. Cool immediately. The absorbance (1.6) measured at the absorption maximum at about 419 nm is at least 0.20.

C. Heat carefully 0.04 g of the test substance with 2 ml of sulfuric acid (~1760 g/L) TS until white fumes are evolved, add drop by drop nitric acid (~1000 g/L) TS until oxidation is complete and cool. Add 2 ml of water, heat until white fumes are again evolved, cool, add 10 ml of water and neutralize with ammonia (~100 g/L) TS using pH-indicator paper R. Keep half of the solution for test D. The remaining solution yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

D. The solution prepared in test C yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Specific optical rotation. Use a 10 mg/mL solution of the test substance in water R and calculate with reference to the anhydrous and ethanol-free substance; $[\alpha]_{D}^{20^\circ} = +75$ to +83.

Clarity of solution. A solution of 0.10 g of the test substance in 10 ml of carbon-dioxide-free water R is clear.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.3 g of the substance. The sum of the contents of water and ethanol (described below), both calculated in mg/g, is not more than 130 mg/g.

Ethanol. Carry out the test as described under 1.14.5 Gas chromatography with the apparatus equipped with an injection system for the performance of static head-space chromatography. Use a fused-silica capillary or wide bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with macrogol 20 000 R (film thickness: 0.25 μm).
As a detector use a flame ionization detector.

Use nitrogen for chromatography R or helium for chromatography R as the carrier gas at an appropriate pressure and a split ratio 1:5 with a linear velocity of about 35 cm/sec.

The following head-space injection conditions may be used:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Equilibration temperature (°C)</td>
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</tr>
<tr>
<td>Equilibration time (min)</td>
<td>60</td>
</tr>
<tr>
<td>Transfer line temperature (°C)</td>
<td>85</td>
</tr>
<tr>
<td>Pressurization time (s)</td>
<td>30</td>
</tr>
<tr>
<td>Injection volume (ml)</td>
<td>1</td>
</tr>
</tbody>
</table>

Maintain the temperature of the column at 30°C for 7 minutes then raise the temperature at a rate of 35°C per minute to 180°C and maintain for 10 minutes, maintaining the temperature of the injection port at 140°C and that of the flame ionization detector at 250°C.

Test solution. Dissolve 0.200 g of the test substance in water R and dilute to 20.0 ml with the same solvent. Introduce 5.0 ml of this solution and 1.0 ml of water R into a headspace vial. Prepare two more vials.

Reference solutions. Add 0.100 g of ethanol R to water R and dilute to 200.0 ml with the same solvent. Transfer respectively 2.0 ml, 4.0 ml and 6.0 ml in separate headspace injection vials and bring the volume to 6.0 ml with water R if necessary.

Blank solution. Introduce 6.0 ml of water R into a headspace vial.

Analyse the blank solution and then alternatively three times the test solution and the three reference solutions.

The test is not valid unless the relative standard deviation on the areas of the peaks obtained from the test solutions is not more than 5%.

Calculate the ethanol content by using the results obtained with the test solution and with the reference solutions; the ethanol content is not more than 30 mg/g.

**pH value** (1.13). pH of a 10 mg/mL solution in carbon-dioxide-free water R, 7.5–9.5.

**Related substances**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (12.5 cm × 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically-bonded octylsilyl gel groups (5 µm) and end-capped.

Prepare solution (A) by dissolving 7.0 g of ammonium acetate R in 1000 ml of water R.

The mobile phase for the gradient elution consists of a mixture of mobile phase A and mobile phase B using the following conditions:

- Mobile phase A: Mix 30 volumes of solution (A) with 35 volumes of water R, adjust to pH 3.8 then add 35 volumes of methanol R.
- Mobile phase B: 30 volumes of solution (A) adjusted to pH 4.0 with glacial acetic acid R and 70 volumes of methanol R.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3.5</td>
<td>90</td>
<td>10</td>
<td>Isocratic</td>
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<td>90–60</td>
<td>10–40</td>
<td>Linear gradient</td>
</tr>
<tr>
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<td>40–95</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>34.5–50</td>
<td>5</td>
<td>95</td>
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<td>50–55</td>
<td>5–90</td>
<td>95–10</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>55–65</td>
<td>90</td>
<td>10</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow of 1.0 ml/min. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column temperature at 30°C.

Prepare the following solutions in mobile phase A. For solution (1) use 1.0 mg of the test substance per ml. For solution (2) use a solution containing 20 µg of betamethasone sodium phosphate RS per ml and 20 µg of dexamethasone sodium phosphate RS per ml. For solution (3) mix equal volumes of solution (2) and a solution containing 20 µg of dexamethasone RS per ml. For solution (4) dilute a suitable volume of solution (1) to obtain a concentration of 10 µg of dexamethasone sodium phosphate per ml.

Inject 20 µL of solution (2). The test is not valid unless the resolution between the peaks due to dexamethasone phosphate (retention time about 22 min) and betamethasone phosphate (with a relative retention time of about 0.95) is at least 2.0.

Inject alternatively 20 µL each of solutions (1), (3) and (4). In the chromatogram obtained with solution (3) the following peaks are eluted at the following relative retention with reference to dexamethasone phosphate (retention time about 22 min): impurity B (betamethasone phosphate): about 0.95; impurity A (dexamethasone): about 1.37. The chromatogram obtained with solution (1) may show the following impurities at the following relative retention with reference to dexamethasone phosphate: impurity C: about 0.5; impurity D: about 0.6; impurity E: about 0.8; impurity F: about 0.92; impurity B: about 0.95; impurity H: about 1.19; impurity A: about 1.37; impurity G: about 1.41.

In the chromatogram obtained with solution (1):

– the area of any peak corresponding to impurity A, when multiplied by a correction factor of 0.75, is not greater than 0.5 times the area of the principal peak obtained with solution (4) (0.5%);

– the area of any peak corresponding to impurity G is not greater than 0.3 times the area of the principal peak obtained with solution (4) (0.3 %);

– the area of any peak corresponding to each impurity B, C, D, E or F is not greater than 0.2 the area of the principal peak obtained with solution (4) (0.2%);

– the area of any other peak, other than the principle peak, is not greater than 0.1 the area of the principal peak obtained with solution (4) (0.1%);

– the sum of the corrected area of any peak corresponding to impurity A and the areas of all other peaks, other than the principal peak, is not greater than the area of the principal peak obtained with the solution (4) (1.0 %).

Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (4) (0.05%).
Assay
Dissolve about 0.2 g, accurately weighed, in sufficient water to produce 200 ml. Dilute 5 ml to 250 ml with water and measure the absorbance of this solution (1.6) in a 1 cm layer at the maximum at about 241 nm. Calculate the content of $\text{C}_{22}\text{H}_{28}\text{FNa}_2\text{O}_8\text{P}$, using the absorptivity value of 29.7 ($A_{1\text{cm}} = 297$).

Additional requirements for Dexamethasone sodium phosphate for parenteral use
Complies with the monograph for Parenteral preparations.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 31.3 IU of endotoxin per mg.

Impurities
A. 9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione (dexamethasone),

B. 9-fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl dihydrogen phosphate (betamethasone phosphate),

C, D, E, F. for each impurity, one or more diastereoisomer(s) of (9-fluoro-11β,17a-dihydroxy-16-methyl-3,17-dioxo-D-homo-androsta-1,4-dien-17a-yl)methyl dihydrogen phosphate (undefined stereochemistry at C-16 and C-17a), or
(9-fluoro-11β,17-dihydroxy-16α-methyl-3,17a-dioxo-D-homo-androsta-1,4-dien-17-yl)methyl dihydrogen phosphate (undefined stereochemistry at C-17),

G. 9-fluoro-11β,17-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid,


ICRS referred to:

- betamethasone sodium phosphate RS (already established as an ICRS)
- dexamethasone RS (already established as an ICRS)
- dexamethasone sodium phosphate RS (already established as an ICRS)
- prednisolone sodium phosphate RS (already established as an ICRS)

Test solutions to be added

Sulfuric acid/ethanol (10%) TS

Cool separately 20 ml of sulfuric acid (~1760 g/L) TS and 60 ml of ethanol (~750 g/L) TS to about -5°C. Carefully add the acid to the ethanol, keeping the solution as cool as possible, mix gently and dilute to 100 ml with ethanol.

Note: Sulfuric acid/ethanol (20%) TS must be freshly prepared.
Dexamethasoni phosphatis injectio
Dexamethasone phosphate injection

This is a draft proposal for The International Pharmacopoeia (Working document QAS/14.580, June 2014).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidtth@who.int.

[Note from the Secretariat. To prevent oxidative decomposition of dexamethasone phosphate in aqueous solution sodium metabisulfite is used as an antioxidant. Dijkstra and Dekker1 reported the addition of bisulfite at the C-1 of the corticosteroid. Comments are in particular sought regarding an appropriate limit for this adduct (impurity I) (see also test for related substances).]

Description. A clear, colourless solution

Category. Adrenal hormone.

Storage. Dexamethasone phosphate injection should be kept in a tightly closed container, protected from light. It should not be allowed to freeze.

Labelling. The designation on the container should state the amount of active ingredient as the equivalent quantity of Dexamethasone phosphate in a suitable dose volume.

Additional information. Strength in the current WHO Model list of essential medicines for dexamethasone: 4 mg/mL (as disodium phosphate salt) in 1 ml ampoule. Strength in the current WHO Model list of essential medicines for children: 4 mg/mL (as disodium phosphate salt) in 1 ml ampoule.

4 mg of dexamethasone phosphate is approximately equivalent to 4.37 mg of dexamethasone sodium phosphate.

Requirements

Complies with the monograph for Parenteral Preparations.

Definition. Dexamethasone sodium phosphate injection is a sterile solution of Dexamethasone sodium phosphate in water for injections. It contains not less than 90.0% and not more than 110.0% of the amount of Dexamethasone phosphate C22H30FO8P stated on the label.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R2 as the coating substance and a mixture of 60 volumes of 1-butanol R, 20 volumes of acetic acid (~300 g/L) TS and 20 volumes of water R. Apply separately to the plate 5 µL of the following 3 solutions in methanol R. For solution (A) dilute a volume of the injection

to obtain a solution containing 1.0 mg of dexamethasone phosphate per ml. For solution (B) use dexamethasone sodium phosphate RS to obtain a solution containing 1.0 mg of dexamethasone phosphate per ml. For solution (C) use dexamethasone sodium phosphate RS and prednisolone sodium phosphate RS to obtain a solution containing 1.0 mg of dexamethasone phosphate and 1.0 mg of prednisolone phosphate per ml. After removing the plate from the chromatographic chamber allow it to dry in air and heat at 110°C for 10 minutes. Spray the hot plate with sulfuric acid/ethanol (20%) TS and heat the plate at 120°C for 10 minutes, allow it to cool and examine the chromatogram in daylight and in ultraviolet light (365 nm).

The test is not valid unless the chromatogram obtained with solution (C) shows 2 spots which may, however, not be completely separated.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

B. See the test described under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

pH value (1.13). pH of the injection, 7.0–8.5.

Related substances

Carry out the test as described under 1.14.4 High-performance liquid chromatography using the chromatographic conditions given under “Assay”.

Prepare the following solutions in mobile phase A. For solution (1) dilute a volume of the injection to obtain a concentration equivalent to 1 mg of dexamethasone sodium phosphate per ml. For solution (2) use a solution containing 20 µg of betamethasone sodium phosphate RS per ml and 20 µg of dexamethasone sodium phosphate RS per ml. For solution (3) mix equal volumes of solution (2) and a solution containing 20 µg of dexamethasone RS per ml. For solution (4) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 10 µg of dexamethasone sodium phosphate per ml. Inject 20 µL of solution (2). The test is not valid unless the resolution between the peaks due to dexamethasone phosphate (retention time about 22 min) and betamethasone sodium phosphate (with a relative retention time of about 0.95) is not less than 2.0.

Inject alternatively 20 µL each of solutions (1), (3) and (4). In the chromatogram obtained with solution (3) the following peaks are eluted at the following relative retention with reference to dexamethasone phosphate (retention time about 22 min): impurity B (betamethasone phosphate): about 0.95; impurity A (dexamethasone): about 1.37. The chromatogram obtained with solution (1) may show the following impurities at the following relative retention with reference to dexamethasone phosphate: impurity I: about 0.13; impurity C: about 0.5; impurity D: about 0.6; impurity E: about 0.8; impurity F: about 0.92; impurity B: about 0.95; impurity H: about 1.19; impurity A: about 1.37; impurity G: about 1.41.

In the chromatogram obtained with solution (1):

– the area of any peak corresponding to impurity A, when multiplied by a correction factor of 0.75, is not greater than 0.5 times the area of the principal peak obtained with solution (4) (0.5%);

– the area of any peak corresponding to impurity I is not greater than X [to be determined] times the area of the principal peak obtained with solution (4) (X %) [to be determined].
[Note from the Secretariat. To prevent oxidative decomposition of dexamethasone phosphate in aqueous solution sodium metabisulfite is used as an antioxidant. Dijkstra and Dekker reported the addition of bisulfite at the C-1 of the corticosteroid. Comments are in particular sought regarding an appropriate limit for this adduct (impurity I).]

**Assay**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (12.5 cm × 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically-bonded octylsilyl gel groups (5 µm) and end-capped.

Prepare solution (A) by dissolving 7.0 g of ammonium acetate R in 1000 ml of water R.

The mobile phase for the gradient elution consists of a mixture of mobile phase A and mobile phase B using the following conditions:

- **Mobile phase A:** Mix 30 volumes of solution (A) with 35 volumes of water R, adjust to pH 3.8 then add 35 volumes of methanol R.
- **Mobile phase B:** 30 volumes of solution (A) adjusted to pH 4.0 with glacial acetic acid R and 70 volumes of methanol R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%v/v)</th>
<th>Mobile phase B (%v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3.5</td>
<td>90</td>
<td>10</td>
<td>Isocratic</td>
</tr>
<tr>
<td>3.5–23.5</td>
<td>90–60</td>
<td>10–40</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>23.5–34.5</td>
<td>60–5</td>
<td>40–95</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>34.5–50</td>
<td>5</td>
<td>95</td>
<td>Isocratic</td>
</tr>
<tr>
<td>50–55</td>
<td>5–90</td>
<td>95–10</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>55–65</td>
<td>90</td>
<td>10</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow of 1.0 ml/min. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column temperature at 30°C.

Prepare the following solutions in mobile phase A. For solution (1) dilute a volume of the injection to obtain a concentration equivalent to 80 µg dexamethasone phosphate per ml (approximately equivalent to 87 µg dexamethasone sodium phosphate). For solution (2) use a solution containing 87 µg of dexamethasone sodium phosphate RS per ml. For solution (3) use a solution containing 20 µg of betamethasone sodium phosphate RS per ml and 20 µg of dexamethasone sodium phosphate RS per ml.

Inject 20 µL of solution (3). The test is not valid unless the resolution between the peaks due to dexamethasone phosphate (retention time about 22 min) and betamethasone phosphate (with a relative retention time of about 0.95) is at least 2.0.

Inject alternatively 20 µL each of solutions (1) and (2). Measure the areas of the peak responses corresponding to dexamethasone phosphate and calculate the content of dexamethasone phosphate, C_{22}H_{30}FO_{8}P, in the injection using the declared content of C_{22}H_{30}FO_{8}P in dexamethasone sodium phosphate RS.

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains less than 34.2 IU of endotoxin per mg dexamethasone phosphate.
Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph for Dexamethasone sodium phosphate and the following:

I. Dexamethasone bisulfite adduct [chemical name and formula to be added]

ICRS referred to:

betamethasone sodium phosphate RS
(already established as an ICRS)

dexamethasone RS
(already established as an ICRS)

dexamethasone sodium phosphate RS
(already established as an ICRS)

prednisolone sodium phosphate RS
(already established as an ICRS)

Test solutions to be added

Sulfuric acid/ethanol (20%) TS

Cool separately 20 ml of sulfuric acid (~1760 g/L) TS and 60 ml of ethanol (~750 g/L) TS to about -5°C. Carefully add the acid to the ethanol keeping the solution as cool as possible, mix gently and dilute to 100 ml with ethanol.

Note: Sulfuric acid/ethanol (20%) TS must be freshly prepared.

***
Atazanaviri sulfas
Atazanavir sulfate

This is a revised draft proposal for The International Pharmacopoeia (Working document QAS/13.566/Rev.1, June 2014).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidtth@who.int.

Molecular formula. $C_{38}H_{52}N_{6}O_{7} \cdot H_2O_4S$

Relative molecular mass. 802.9

Chemical name.
Dimethyl (3S,8S,9S,12S)-9-benzyl-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-6-[[4-(pyridin-2-yl)phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioate monosulfate (3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioic acid 1,14-dimethyl ester, sulfate (1:1) ; CAS 229975-97-7

Description. A white to a pale yellow crystalline powder.

Solubility. Freely soluble in methanol, practically insoluble in water.

Category. Antiretroviral (protease inhibitor).

Storage. Atazanavir sulfate should be kept in a tightly closed container.

Additional information. Atazanavir sulfate is slightly hygroscopic and may exhibit polymorphism.

Requirements
Atazanavir sulfate contains not less than 99.0% and not more than 101.0% of $C_{38}H_{52}N_{6}O_{7} \cdot H_2SO_4$ calculated on the dried basis.

[Note from the Secretariat. Comments are being sought in particular on the suitability of the proposed content limits.]

Identity tests
Either test A and D, or test B, C and D should be performed.
A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from atazanavir sulfate RS or with the reference spectrum of atazanavir sulfate. If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and atazanavir sulfate RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from atazanavir sulfate RS.

B. Carry out test B.1, or where UV detection is not available, test B.2.

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 9.5 volumes of dichloromethane R and 0.5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 10 μl of each of 2 solutions in methanol R containing (A) 1 mg of the test substance per mL and (B) 1 mg of atazanavir sulfate RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of air.

Examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described under test B.1, but using a plate containing silica gel R5 as the coating substance.

Spray the plate with potassium permanganate, basic (~5 g/L) TS. Examine the chromatogram in daylight. The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

C. The absorption spectrum of a 10 µg/mL solution in methanol R, when observed between 230 nm and 340 nm, exhibits two maxima at about 250 nm and 280 nm.

D. A 20 mg/mL solution yields Reaction A described under 2.1 General identification tests as characteristic of sulfates.

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to method A; not more than 20 µg/g.

**Sulfated ash (2.3).** Not more than 1.0 mg/g.

**Loss on drying.** Dry for 3 hours at 105 °C; it loses not more than 10.0 mg/g.

**Specific optical rotation.** Use a 10 mg/mL solution in equal volumes of methanol R and water R at 22°C calculated with reference to the anhydrous substance; the optical rotation is between -40° and -44°.

**Related substances**

Carry out the test as described under 1.14.4 High–performance liquid chromatography using a column (150 mm x 4.6 mm) packed with end-capped, base-deactivated particles of silica gel the surface of which has been modified with chemically-bonded octylsilyl groups (5 μm).\(^1\) Use the following conditions for gradient elution:

- Mobile phase A: 0.02 M phosphate buffer pH 3.5, acetonitrile R (70:30 v/v).
- Mobile phase B: 0.02 M phosphate buffer pH 3.5, acetonitrile R. (30:70 v/v)

\(^1\) An Inertsil C8 column has been found suitable.
Prepare the phosphate buffer pH 3.5 by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 3.5 by adding phosphoric acid (~105 g/L) TS and dilute to 1000 mL with water R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2–10</td>
<td>100–75</td>
<td>0–25</td>
<td>Linear gradient</td>
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<td>75–50</td>
<td>25–50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–45</td>
<td>50–0</td>
<td>50–100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>45–50</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>50–52</td>
<td>0–100</td>
<td>100–0</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>52–60</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

Prepare the following solutions using as diluent a mixture of equal volumes of water R and acetonitrile R. For solution (1) use 1 mg of the test substance per mL. For solution (2) dilute a suitable volume of solution (1) with the diluent to obtain a concentration equivalent to 5 μg of Atazanavir sulfate per mL. For solution (3) mix 1 mL of solution (1) with 4.5 mL of water R and 0.5 mL of sodium hydroxide (10 g/L) TS and heat the mixture in a water-bath at 85°C for 15 minutes.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of 30°C.

Inject 20 μL of solution (3). The test is not valid unless the resolution between the peak due to atazanavir (retention time about 22 minutes) and the peak with a relative retention of about 1.2 is at least 4.

Inject alternatively 20 μL each of solutions (1) and (2).

In the chromatograms obtained with test solution (1) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Assay**

Dissolve 0.300 g, accurately weighed, in 30 mL of methanol R and by sonication for 10 minutes. Then add 30 mL of water and titrate with sodium hydroxide (0.1 mol/L), VS, determining the end-point potentiometrically. Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 40.145 mg of C_{36}H_{52}N_{6}O_{7}•H_{2}SO_{4}.

***
Atazanavir capsules

This is a revised draft proposal for *The International Pharmacopoeia* (Working document QAS/13.567/Rev.1, June 2014).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

Category. Antiretroviral (Protease Inhibitor).

Storage. Atazanavir capsules should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg, 150 mg, 300 mg of atazanavir (as sulfate). Strength in the current WHO Model List of essential medicines for children: 100 mg, 150 mg, 300 mg of atazanavir (as sulfate).

Each mg of atazanavir (C₃₈H₅₂N₆O₇) is equivalent to 1.139 mg of atazanavir sulfate (C₃₈H₅₂N₆O₇•H₂SO₄).

Requirements

Comply with the monograph for Capsules.

Definition

Atazanavir capsules contain atazanavir sulfate. They contain not less than 90.0% and not more than 110.0% of the amount of atazanavir, C₃₈H₅₂N₆O₇, stated on the label.

Identity tests

A. Carry out test A.1, or where UV detection is not available, test A.2.

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 9.5 volumes of dichloromethane R and 0.5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 10 µL of each of the following 2 solutions in methanol R. For solution (A) disperse a quantity of the contents of the capsules containing about 20 mg of atazanavir in 10 mL of methanol R, sonicate for 10 minutes, allow to cool to room temperature, dilute to 20 mL, filter and use the filtrate. For solution (B) use 1.1 mg of atazanavir sulfate RS per mL.

After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or a current of air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution (A) corresponds in position, appearance, and intensity to that obtained with solution (B).

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using a plate containing silica gel R5 as the coating substance. Spray with potassium permanganate, basic (~5 g/L) TS. Examine the chromatogram in daylight.
The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

B. Disperse a quantity of the contents of the capsules containing about 20 mg of atazanavir in 10 mL of methanol R, sonicate for 10 min, allow to cool to room temperature, dilute to 20 mL and filter. Dilute 1 mL of the filtrate to 100 mL with methanol R. The absorption spectrum of the resulting solution, when observed between 230 and 340 nm, exhibits two maxima at about 250 nm and 280 nm.

C. To a quantity of the contents of the capsules equivalent to 0.2 g of atazanavir add 10 mL of a mixture of 1 volume of water R and 1 volume of acetonitrile R, shake and filter. The filtrate yields Reaction A described under 2.1 General identification tests as characteristic of sulfates.

**Dissolution**

Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 mL of dissolution buffer pH 2.5 TS, and rotating the paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. Measure the absorbance of a 1 cm layer of the resulting solution, suitably diluted if necessary, at the maximum at about 250 nm. Determine the content of atazanavir \((C_{38}H_{52}N_{6}O_{7})\) in the medium from the absorbance obtained using an absorptivity value of 15.9 \((A_{1cm} = 159)\). The amount in solution for each capsule is not less than 75\% (Q) of the amount stated on the label.

**Related substances**

Carry out the test as described under 1.14.4 High–performance liquid chromatography, using a stainless steel column (150 mm x 4.6 mm) packed with end-capped base deactivated particles of silica gel the surface of which has been modified with chemically bonded octylsilyl groups (5 \(\mu\)m). Use the following conditions for gradient elution:

- Mobile phase A: 0.02 M phosphate buffer pH 3.5, acetonitrile R (70:30 v/v).
- Mobile phase B: 0.02 M phosphate buffer pH 3.5, acetonitrile R (30:70 v/v).

Prepare the phosphate buffer pH 3.5 by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 3.5 by adding phosphoric acid (~105 g/L) and dilute to 1000 mL with water R.

<table>
<thead>
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<th>Comments</th>
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<tr>
<td>52–60</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

Prepare the following solutions using as diluent a mixture of equal volumes of acetonitrile R and water R. For solution (1) weigh and mix the contents of 20 capsules. Transfer a quantity of the mixed contents equivalent to 20 mg of atazanavir into a 20 mL volumetric flask. Add about 10 mL of the diluent, sonicate for 10 minutes, allow to cool to room temperature, make up to volume and filter. For solution (2) dilute a suitable volume of solution (1) with the diluent to obtain a concentration of 10 \(\mu\)g of atazanavir per mL. For solution (3) mix 1 mL of solution (1)

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1 Value subject to confirmation.
2 An Inertsil C8 column has been found suitable.
with 4.5 mL of water R and 0.5 mL of sodium hydroxide (10 g/L) TS and heat the mixture in a water bath at 85°C for 15 min.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of 30°C.

Inject 20 µL of solution (3). The test is not valid unless the resolution between the peak due to atazanavir (retention time about 22 minutes) and the peak with a relative retention of about 1.2 is at least 4.

Inject alternatively 20 µL each of solutions (1) and (2).

In the chromatograms obtained with test solution (1) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (2) (2.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

**Assay**

Either test A or test B may be applied.

**A** Carry out the test as described under **1.14.4 High–performance liquid chromatography**, using a stainless steel column (150 mm x 4.6 mm) packed with end-capped base deactivated particles of silica gel the surface of which has been modified with chemically bonded octylsilyl groups (5 µm).³

As the mobile phase, use a solution prepared as follows: 60 volumes of acetonitrile R and 40 volumes of 0.02 M phosphate buffer pH 3.5. Prepare the phosphate buffer pH 3.5 according to the procedure described in the related substances test.

Prepare the following solutions using as diluent a mixture of equal volumes of acetonitrile R and water R. For solution (1) weigh and mix the contents of 20 capsules. Transfer a quantity equivalent to 20.0 mg of atazanavir, accurately weighed, into a 20 mL volumetric flask. Add about 10 mL of the diluent, sonicate for about 10 minutes, allow to cool to room temperature and make up to volume. Filter a portion of this solution, discarding the first few mL. Dilute 1.0 mL of the filtrate to 10.0 mL with the diluent. For solution (2) use 0.11 mg of atazanavir sulfate RS per mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of 30°C.

Inject alternatively 20 µL each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of atazanavir.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of atazanavir, \( C_{38}H_{52}N_{6}O_{7} \), using the declared content of \( C_{38}H_{52}N_{6}O_{7} \) in atazanavir sulfate RS.

**B.** Weigh and mix the contents of 20 capsules. Transfer a quantity equivalent to 20 mg of atazanavir, accurately weighed, to a 20 mL volumetric flask. Add about 10 mL of methanol R, sonicate for about 10 minutes, allow to cool to room temperature and make up to volume. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtrate. Dilute 1.0 mL of the filtrate to 10.0 mL with methanol R. Measure the absorbance of this solution in a 1 cm layer at the maximum at about 250 nm against a solvent cell containing methanol R. Calculate the content of \( C_{38}H_{52}N_{6}O_{7} \), using an absorptivity value of 15.9 \( A_{1\text{cm}} = 159 \).⁴

³ An Inertsil C₈ column has been found suitable.

⁴ Value subject to confirmation.
Albendazoli compressi
Albendazole chewable tablets

This is a revised draft proposal for The International Pharmacopoeia (Working document QAS/14.592, June 2014).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. It is proposed to revise the monograph with a view to include a dissolution test and acceptance criterion.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

Category. Anthelmintic.

Storage. Albendazole chewable tablets should be kept in a tightly closed container.

Labelling. The designation on the container should state that the tablets may be chewed, swallowed whole or crushed and mixed with food or liquid, and the tablets should be crushed before being given to a young child.

Additional information. Strengths in the current WHO Model list of essential medicines: 400 mg. Strengths in the current WHO Model list of essential medicines for children: 400 mg.

Requirements

Comply with the monograph for Tablets.

Definition. Albendazole chewable tablets contain Albendazole in a suitable basis that may contain suitable flavouring agents. They contain not less than 90.0% and not more than 110.0% of the amount of Albendazole (C₁₂H₁₅N₃O₂S) stated on the label.

Identity tests

• Any two of tests A, B and C may be applied

A. Carry out the test as described under 1.14.1 Thin-layer chromatography using the chromatographic conditions given under “Related substances”, Test B. Apply separately to the plate 10 μL each of the following solutions in a mixture of 9 volumes of dichloromethane R and 1 volume of glacial acetic acid R. For solution (A) shake a quantity of the powdered tablets containing about 2.5 mg of Albendazole with 25 mL, filter and use the filtrate. For solution (B) use 0.1 mg of albendazole RS per mL. For solution (C) use 0.1 mg of albendazole RS and 0.1 mg of oxibendazole R per mL. After removing the plate from the chromatographic chamber allow the plate to dry in a current of warm air and examine the chromatogram under ultraviolet light (254 nm). The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).
B. See the test described below under “Assay”, Method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to the retention time of the peak due to albendazole obtained with solution (3).

C. See the test described under “Assay”, Method B. The absorption spectrum \((1.6)\) of the test solution, when observed between 220 and 340 nm, exhibits maxima at about 231 nm and at 308 nm; the absorbance at 308 nm is about 0.59.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms using 900 mL of hydrochloric acid \((0.1 \text{ mol/L})\) VS as the dissolution medium and rotating the paddle at 75 revolutions per minute. At 30 minutes withdraw a sample of about 15 mL of the dissolution medium through an in-line filter. Cool the filtered sample to room temperature. Transfer 1.0 mL of the clear filtrate to a 50 mL volumetric flask and dilute to volume with sodium hydroxide \((\sim 4 \text{ g/L})\) TS. Measure the absorbance \((1.6)\) of a 1 cm layer of the resulting solution at the maximum at about 308 nm.

For each of the six tablets tested calculate the total amount of Albendazole \((\text{C12H15N3O2S})\) in the medium, using the absorptivity value of 74.2 \((A_{10\text{cm}} = 742)\). The amount in solution for each tablet is not less than 80% \((Q)\) of the amount declared on the label.

Related substances
• Either method A or method B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography using the conditions given below under “Assay”, Method A.

Prepare the following solutions.
Solvent mixture: dilute 1 volume of sulfuric acid R with 99 volumes of methanol R.

For solution (1) transfer a quantity of the powdered tablets containing about 25 mg of Albendazole to a 50 mL volumetric flask. Add 5 mL of the solvent mixture and 20 mL of methanol R and shake to dissolve for about 15 minutes. Dilute to volume with methanol R.

For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL with methanol R. For solution (3) dissolve about 20 mg of albendazole RS and about 20 mg of oxibendazole R in 5 mL of solvent mixture and dilute to 100.0 mL with methanol R.

Inject separately 20 µL each of solutions (1), (2) and (3). Record the chromatogram for about 25 minutes.

In the chromatogram obtained with solution (3) the peak due to oxibendazole is eluted at a retention time of about 9.9 min and the peak due to albendazole at a retention time of about 13.6 minutes. The test is not valid unless the resolution factor between the peak due to oxibendazole and the peak due to albendazole is at least 3.0.

In the chromatogram obtained with solution (1):
• the area of any peak, other than the principal peak, is not greater than the area of the peak due to albendazole in the chromatogram obtained with solution (2) \((1.0\%)\);
• the area of not more than one such peak is greater than 0.75 times the area of the peak due to albendazole in the chromatogram obtained with solution (2) \((0.75\%)\).

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R5 as the coating substance and a mixture of dichloromethane R, glacial acetic acid R and ether R \((30:7:3 \text{ v/v})\) as the mobile phase. Apply separately to the plate 10 µL each of the following solutions in a mixture of 9 volumes of dichloromethane R and 1 volume of glacial acetic acid R. For solution (A) shake a quantity of the powdered tablets containing about 250 mg of Albendazole with 25 mL, filter and use the filtrate. For solution (B) use 0.1 mg of albendazole RS per mL. For solution (C) use 0.075 mg of albendazole RS per mL. For
solution (D) use 0.1 mg albendazole RS and 0.1 mg oxibendazole R per mL. After removing
the plate from the chromatographic chamber allow the plate to dry in a current of warm air.
Examine the chromatogram in ultraviolet light (254 nm). The test is not valid unless the
chromatogram obtained with solution (D) shows two clearly separated spots.

In the chromatogram obtained with solution (A) any spot, other than the principal spot, is
not more intense than the principal spot obtained with solution (B) (1.0%) and not more
than one spot is more intense than the principal spot obtained with solution (C) (0.75%).

Assay

• Either method A or method B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography
using a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl base-
deactivated silica gel for chromatography R (5 µm).

As the mobile phase use a solution prepared as follows: dissolve 1.67 g of monobasic
ammonium phosphate R in 1000 mL of water R, mix and filter. Mix 300 mL of this solution
with 700 mL of methanol R. Make adjustments if necessary.

Prepare the following solutions.

Solvent mixture: dilute 1 volume of sulfuric acid R with 99 volumes of methanol R.

For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets
containing about 100 mg of Albendazole, accurately weighed, to a 50 mL volumetric flask.
Add 5 mL of the solvent mixture and 20 mL of methanol R and shake for about 15 minutes.
Dilute to volume with methanol R, mix and filter, discarding the first 15 mL of the filtrate.
Dilute 5.0 mL of this solution to 50.0 mL with methanol R. For solution (2) transfer 25.0 mg
of Albendazole RS to a 25 mL volumetric flask, add 5 mL of the solvent mixture and 15 mL
of methanol R and shake to dissolve. Dilute to volume with methanol R. For solution (3)
dilute 2.0 mL of solution (2) to 10.0 mL with methanol R. For solution (4) dissolve about 20
mg of oxibendazole R in 5 mL of solvent mixture in a 100 mL volumetric flask, add 20 mL of
solution (2), mix and dilute to volume with methanol R.

Operate with a flow rate of 0.7 mL per minute. As a detector use an ultraviolet
spectrophotometer set at a wavelength of 254 nm.

Inject separately 20 µL each of solutions (1), (3) and (4). The test is not valid unless in the
chromatogram obtained with solution (4) the resolution factor between the peaks due to
albendazole and due to oxibendazole is at least 3.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions
(1) and (3) and calculate the content of Albendazole \(\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_2\text{S}\) in the tablets using the
declared content of \(\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_2\text{S}\) in albendazole RS

B. Weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing
about 20 mg of Albendazole, accurately weighed, to a 50 mL volumetric flask, add 30 mL
of hydrochloric acid/methanol (0.01 mol/L) VS, shake for 15 minutes and dilute to volume
with the same solvent. Mix and filter, discarding the first 10 mL of the filtrate. Transfer 1.0
mL of the subsequent filtrate to a 50 mL volumetric flask and dilute to volume with sodium
hydroxide (−4 g/L) TS. Measure the absorbance of the resulting solution at the maximum
at about 308 nm. Calculate the content of Albendazole (C12H15N3O2S), using the
absorptivity value of 74.2 (\(A_{1\text{cm}} = 742\)).

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