ALBENDAZOLE
(ALBENDAZOLUM)

Draft proposal for revision for The International Pharmacopoeia
(July 2019)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to Dr Herbert Schmidt, Technical Officer, Medicines Quality Assurance, Technologies Standards and Norms (email: schmidt@who.int) by 31 August 2019.

Working documents are sent out electronically and they will also be placed on the WHO Medicines website (http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en/) for comments under the “Current projects” link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

© World Health Organization 2019
All rights reserved.

This draft is intended for a restricted audience only, i.e. the individuals and organizations having received this draft. The draft may not be reviewed, abstracted, quoted, reproduced, transmitted, distributed, translated or adapted, in part or in whole, in any form or by any means outside these individuals and organizations (including the organizations’ concerned staff and member organizations) without the permission of the World Health Organization. The draft should not be displayed on any website.

Please send any request for permission to:
Dr Sabine Kopp, Group Lead, Medicines Quality Assurance, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland, (email: kopp@who.int).

The designations employed and the presentation of the material in this draft do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this draft. However, the printed material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

This draft does not necessarily represent the decisions or the stated policy of the World Health Organization.
SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/18.755:

ALBENDAZOLE
(ALBENDAZOLUM)

<table>
<thead>
<tr>
<th>Description</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revision of the monograph.</td>
<td>February 2018</td>
</tr>
<tr>
<td>Discussion at the informal Consultation on Screening Technology, Sampling and Specification for Medicines.</td>
<td>2-4 May 2018</td>
</tr>
<tr>
<td>Presentation to the Fifty-third WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP).</td>
<td>October 2018</td>
</tr>
<tr>
<td>Discussion at the informal Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.</td>
<td>2-4 May 2019</td>
</tr>
<tr>
<td>Draft revision sent out for public consultation.</td>
<td>July-August 2019</td>
</tr>
<tr>
<td>Presentation to the Fifty-fourth WHO ECSPP.</td>
<td>October 2019</td>
</tr>
<tr>
<td>Further follow-up action as required.</td>
<td></td>
</tr>
</tbody>
</table>

[Note from the Secretariat. It is proposed to revise the monograph on Albendazole as follows:

- addition of the information that the substance shows polymorphism (with a subsequent change in the identity test by IR (test A);
- addition of a test “Clarity and colour of solution”;
- replacement of the TLC test for related substances with an HPLC method;
- update the style of the monograph; and
- further minor changes in the tests and methods prescribed.

The revision is based on information found in other pharmacopoeias, in particular, the European Pharmacopoeia and on laboratory investigations.

Changes from the current monograph are indicated in the text by insert or delete.]
ALBENDAZOLE
(ALBENDAZOLUM)

**Graphic formula**

![Graphic formula of ALBENDAZOLE](image)

**Molecular formula.** $C_{12}H_{15}N_3O_2S$

**Relative molecular mass.** 265.3

**Chemical name.** Methyl $N$-[5-(propylthio)-2-benzimidazolyl]carbamate; CAS Reg. No. 54965-21-8.

**Description.** A white or almost white powder.

**Solubility.** Practically insoluble in water; soluble in glacial acetic acid R; slightly soluble in acetone R and dichloromethane R, very slightly soluble in ethanol (~750 g/L) TS.

**Category.** Anthelminthic.

**Storage.** Albendazole should be kept in a well-closed container, protected from light.

**Additional information.** Melting temperature, about 210°C, with decomposition Albendazole shows polymorphism.

**Requirements**

Albendazole contains not less than 99.0% and not more than 101.0% of $C_{12}H_{15}N_3O_2S$, calculated with reference to the dried substance.
Identity tests

- Either test A alone or any two of tests B, C, and D 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 albendazole RS or with the reference spectrum of albendazole.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and albendazole RS in a small amount of dichloromethane R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from albendazole RS.

B. See the test described below under "Related substances". The principal spot obtained with solution (1)B corresponds in position, appearance and intensity with that obtained with solution (3)C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Related substances”. Use solution (1) as described thereunder. For solution (2), transfer 25 mg of albendazole RS to a 50 mL volumetric flask. Add 5 mL of sulfuric acid/methanol (1%) TS and immediately dilute to volume with the mobile phase. Inject 5 µL of solutions (1) and (2). The retention time and the size of the principal peak in the chromatogram obtained with solution (1) correspond to the retention time and the size of the peak due to albendazole in the chromatogram obtained with solution (2).

C. Ignite about 0.1 g; fumes are evolved, staining lead acetate paper R black. 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 6 volumes of dichloromethane R, 1 volume of ether R and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following 2 solutions in a mixture of 9 volumes of dichloromethane R and 1 volume of glacial acetic acid R containing (A) 1.0 mg of the test substance per mL and (B) 1.0 mg of albendazole RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air and examine the chromatogram in ultraviolet light (254 nm). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and
intensity with the spot due to albendazole in the chromatogram obtained with solution (B).

D. Add about 0.1 g of the test substance to 3 mL of sulfuric acid (~100 g/L) TS and warm to dissolve. Add about 1 mL of potassium iodobismuthate/acetic acid TS; a reddish brown precipitate is produced.

**Sulfated ash.** Not more than 4.0 mg/g.

**Loss on drying.** Dry at 105 °C for 4 hours; it loses not more than 5.0 mg/g.

**Clarity and colour of solution.** Dissolve 0.10 g of the test substance in a mixture of 10 volumes of anhydrous formic acid R and 90 volumes of dichloromethane R and dilute to 10 mL with the same mixture of solvents. This solution is clear and not more intensely coloured than reference solution BY₆, when compared as described under 1.11.2 Degree of coloration of liquids, Method II.

**Heavy metals.** Use 1.0 g of the test substance 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 B; not more than 10 μg/g.

**Related substances.** Prepare the solutions immediately before use.

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 end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 μm).¹

As the mobile phase, use a solution prepared as follows: dissolve 1.67 g of ammonium dihydrogen phosphate R in 1000 mL of water R. Mix 300 mL of this solution with 700 mL of 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.

¹ A Symmetry C18 column was found suitable.
Prepare the following solutions. For solution (1), transfer 25.0 mg of the test substance to a 50 mL volumetric flask. Add 5 mL of sulfuric acid/methanol (1%) TS and immediately dilute to volume with the mobile phase. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL with the mobile phase. For solution (3), dilute 1.0 mL of solution (2) to 10.0 mL with the mobile phase. For solution (4), dissolve 5 mg of albendazole for system suitability RS (containing albendazole and the impurities B, C, E, F and H) in 1 mL of sulfuric acid/methanol (1%) TS and dilute to 10 mL with mobile phase. For solution (5), dilute 1 mL of sulfuric acid/methanol (1%) TS to 10 mL with the mobile phase. Use 1 mL of this solution to dissolve the content of a vial of albendazole impurity mixture RS (containing the impurities A and D).

Inject separately 20 µL each of solutions (1), (2), (3), (4) and (5). Record the chromatogram for about twice the retention time albendazole (retention time about 11 minutes).

Use the chromatogram obtained with solution (3) and the chromatogram supplied with albendazole for system suitability RS to identify the peaks due to the impurities B, C, E, F and H. Use the chromatogram obtained with solution (4) and the chromatogram supplied with albendazole impurity mixture RS to identify the peaks due to the impurities A and D. The impurities are eluted at the following relative retention with reference to albendazole: impurity D about 0.35, impurity B and C about 0.40, impurity E about 0.45, impurity A about 0.48, impurity F about 0.57 and impurity H about 0.66.

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution factor between the peak due to impurities B and C (impurities B and C co-elute) and the peak due to impurity E is at least 1.5. Also, the test is not valid unless in the chromatogram obtained with solution (3), the peak due to albendazole is detected with a signal-to-noise ratio of at least 20.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 1.7, is not greater than four times the area of the peak due to albendazole in the chromatogram obtained with solution (3) (0.4%);
- the sum of the areas of any peaks corresponding to impurities B and C (impurities B and C co-elute), when multiplied by a correction factor of 1.4, is not greater than four times
the area of the peak due to albendazole in the chromatogram obtained with solution (3) (0.4%);

- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 1.9, is not greater than two times the area of the peak due to albendazole in the chromatogram obtained with solution (3) (0.2%);

- the area of any peak corresponding to impurity E, when multiplied by a correction factor of 1.4, is not greater than three times the area of the peak due to albendazole in the chromatogram obtained with solution (3) (0.3%);

- the area of any peak corresponding to impurity F is not greater than 0.5 times the area of the peak due to albendazole in the chromatogram obtained with solution (2) (0.5%);

- the area of any peak corresponding to impurity H, when multiplied by a correction factor of 1.7, is not greater than 0.6 times the area of the peak due to albendazole in the chromatogram obtained with solution (2) (0.6%);

- the area of any other impurity peak is not greater than the area of the peak due to albendazole in the chromatogram obtained with solution (3) (0.10%);

- the sum of the corrected areas of any peaks corresponding to impurities A, B/C, D and E and the areas of all other impurity peaks is not greater than 1.3 times the area of the peak due to albendazole in the chromatogram obtained with solution (2) (1.3%). Disregard any peak with an area less than 0.5 times the area of the peak due to albendazole in the chromatogram obtained with solution (3) (0.05%).

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 6 volumes of dichloromethane R, 1 volume of ether R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 μl of each of 5 solutions in a mixture of 9 volumes of dichloromethane R and 1 volume of anhydrous formic acid R containing (A) 10.0 mg of Albendazole per mL, (B) 1.0 mg of Albendazole per mL, (C) 1.0 mg of albendazole RS per mL, (D) 0.05 mg of albendazole RS per mL, and (E) 0.025 mg of albendazole RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, and examine the chromatogram in ultraviolet light (254 nm).
Any spot obtained with solution A, other than the principal spot, is not more intense than the principal spot obtained with solution D (0.5%), and only one spot may be more intense than the principal spot obtained with solution E (0.25%).

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached.

Dissolve 0.250 g in 3 mL of anhydrous formic acid R, add 40 mL of glacial acetic acid R. Then add 0.2 mL of 1-naphtholbenzoin/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, until a green colour is obtained determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Carry out a blank determination and make any necessary correction.

Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 26.53 mg of C_{12}H_{15}N_{3}O_{2}S.

**Impurities**

A. 5-(propylsulfanyl)-1H-benzimidazol-2-amine (degradation product).

B. Methyl N-[5-(propylsulfinyl)-1H-benzimidazol-2-yl]carbamate (degradation product).

C. Methyl N-[5-(propylsulfonyl)-1H-benzimidazol-2-yl]carbamate (degradation product).
D. (2-amino-1H-benzimidazol-5-yl)propyl-$\lambda^6$-sulfanedione (degradation product).

E. Methyl N-(1H-benzimidazol-2-yl)carbamate (degradation product, synthesis related impurity).

F. Methyl N-[5-(methylsulfanyl)-1H-benzimidazol-2-yl]carbamate (degradation product, synthesis related impurity).

G. Methyl N-(5-chloro-1H-benzimidazol-2-yl)carbamate.

H. Methyl N-[5-[(2-methyl-4-oxopentan-2-y1)sulfanyl]-1H-benzimidazol-2-yl]carbamate (degradation product).
I. Methyl N-(5-propoxy-1H-benzimidazol-2-yl)carbamate.

J. Methyl N-(4,6-dichloro-1H-benzimidazol-2-yl)carbamate.

K. Methyl N-[5-(butylsulfanyl)-1H-benzimidazol-2-yl]carbamate.

L. Methyl N-[5-[(propan-2-yl)sulfanyl]-1H-benzimidazol-2-yl]carbamate.

Reagents to be added

Sulfuric acid/methanol (1%) TS

Procedure: cool separately 10 mL of sulfuric acid (~1760 g/L) TS and 900 mL of methanol R to about -5 °C. Very carefully add the acid to the methanol keeping the solution as cool as possible and mix gently.

Reference substances to be established
Albendazole for system suitability RS (containing albendazole and the impurities B, C, E, F and H)

It is intended to refer to the corresponding reference substance established for the European Pharmacopoeia.

Albendazole impurity mixture RS (containing the impurities A and D)

It is intended to refer to the corresponding reference substance established for the European Pharmacopoeia.

***