LAMIVUDINE ORAL SOLUTION

(LAMIVUDINI SOLUTIO PERORALUM)

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: schmidth@who.int) by 30 September 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.

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Please send any request for permission to:

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/19.785:

LAMIVUDINE ORAL SOLUTION
(LAMIVUDINI SOLUTIO PERORALUM)

<table>
<thead>
<tr>
<th>Description</th>
<th>Date</th>
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<tbody>
<tr>
<td>Drafting of the revision.</td>
<td>January 2019</td>
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<tr>
<td>Discussion at the informal Consultation on Screening Technologies and Pharmacopoeial Specifications for Medicines.</td>
<td>2-3 May 2019</td>
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<tr>
<td>Draft revision sent out for public consultation</td>
<td>August – September 2019</td>
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<tr>
<td>Presentation to the Fifty-fourth WHO Expert Committee on Specifications for Pharmaceutical Preparations.</td>
<td>October 2019</td>
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<td>Further follow-up action as required.</td>
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[Note from the Secretariat. It is proposed to revise the monograph on Lamivudine oral solution as follows:

- revision of the test for related substances; and
- revision of the assay by HPLC.

The proposal is based on information submitted by manufacturers.

Changes from the current monograph are indicated in the text by insert or delete.]
LAMIVUDINE ORAL SOLUTION
(LAMIVUDINI SOLUTIO PERORALUM)

Category. Antiretroviral (Nucleoside/Nucleotide Reverse Transcriptase Inhibitor).

Storage. Lamivudine oral solution should be kept in a well-closed container.

Additional Information. Strength in the current WHO Model List of Essential Medicines (EML): 50 mg per 5 mL (10 mg per mL). Strength in the current WHO EML for children: 50 mg per 5 mL (10 mg per mL).

Requirements

Comply with the monograph for "Liquid preparations for oral use".

Definition. Lamivudine oral solution is a solution of Lamivudine in a suitable vehicle which may be flavoured. It contains not less than 90.0% and not more than 110.0% of the amount of Lamivudine \( \text{C}_8\text{H}_{11}\text{N}_3\text{O}_3\text{S} \) as stated on the label.

Identity tests

Either test A or test 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 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/L) TS as the mobile phase. Apply separately to the plate 10 μl of each of the following two solutions. For solution (A), dilute 5 mL of the oral solution, nominally containing 50 mg of Lamivudine, to 50 mL with methanol R, filter, and use the filtrate. For solution (B), use a solution containing 1.0 mg of lamivudine RS per mL of methanol. After removing the plate from the chromatographic chamber, allow it to dry in a current of cool air and 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.
Spray the plate with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120°C. Examine the chromatogram in daylight. The principal spot obtained with solution A corresponds in position, appearance and intensity to that obtained with solution B.

B. Carry out test B.1 or, where HPLC with a diode array detector is available, test B.2.

B.1 Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions and solutions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to lamivudine in the chromatogram obtained with solution (2).

The absorption spectrum (1.6) of solution (1), when observed between 210 nm and 300 nm, exhibits one maximum at about 280 nm.

B.2 Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time and the UV spectrum of the principal peak in the chromatogram obtained with solution (1) correspond to the retention time and the UV spectrum of the peak due to lamivudine in the chromatogram obtained with solution (2).

pH value (1.13). pH of the oral solution, 5.0 – 7.0.

Related substances. 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 end-capped 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: dissolve 1.0 g sodium octanesulfonate R in 1000.0 mL of water R, add 5.0 mL of phosphoric acid (~680 g/L) TS and mix.
- Mobile phase B: 25 volumes of methanol R and 75 volumes of acetonitrile R.

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<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
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¹ A Hypersil MOS-2 column was found suitable.
Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 277 nm. Maintain the column temperature at 40 °C.

Prepare the following solutions using as a diluent a mixture of 80 volumes of water R and 20 volumes of methanol R. For solution (1), transfer a volume of the oral solution, nominally containing 50 mg of Lamivudine, to a 100 mL volumetric flask. Add about 60 mL of diluent and sonicate for about 20 minutes with intermittent shaking. Allow to cool to room temperature, dilute to volume and filter. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3), dilute 5.0 mL of solution (2) to 100.0 mL. For solution (4), dissolve 5 mg of lamivudine for system suitability RS (containing lamivudine and lamivudine impurities A and B) and dilute to 10.0 mL. For solution (5), dissolve 25 mg of cytosine R, 25 mg of uracil R and 25 mg of salicylic acid R and dilute to 50.0 mL. Dilute 1.0 mL of this solution to 100.0 mL. For solution (6), dissolve a suitable amount of each of the excipients stated on the label in 10 mL of a suitable solvent and dilute to 100.0 mL with the diluent.

Inject alternately 20 μl each of solutions (1), (2), (3), (4), (5) and (6).

The test is not valid unless in the chromatogram obtained with solution (4), the resolution factor between the peaks due to lamivudine and impurity B is at least 1.5. Also, the signal-to-noise ratio of the peak due to lamivudine in the chromatogram obtained with solution (3) is at least 10.

Use the chromatogram obtained with solutions (4) and (5) to identify peaks due to impurities in the chromatogram obtained with solution (1), if present. The impurity peaks are eluted at the following relative retention with reference to lamivudine (retention time about 45 minutes): impurity J about 0.12; impurity E about 0.27; impurity G about 0.30; impurity H about 0.31;
Impurity A about 0.63; impurity B about 0.84. Use the chromatogram obtained with solution (6) to identify the peaks due to excipients.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity J is not greater than 1.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (1.5%);
- the area of any peak corresponding to impurity H is not greater than 0.6 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.6%);
- the area of any peak corresponding to either impurities E or G is not greater than 0.3 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.3%);
- the area of any other impurity peak is not greater than 0.2 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.2%);
- the sum of the areas of all impurity peaks is not greater than two times the area of the peak due to lamivudine obtained with solution (2) (2.0%). Disregard any peak with the same retention time as that of any of the peaks in the chromatogram obtained with solution (6) and any peak with an area less than the area of the principal peak obtained with solution (3) (0.05%).

**Assay.** 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 end-capped particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm).³

Use the following conditions for gradient elution:

- **Mobile phase A:** dissolve 1.0 g sodium octanesulfonate R in 1000.0 mL of water R, add 5.0 mL of phosphoric acid (~680 g/L) TS and mix.
- **Mobile phase B:** acetonitrile R.

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<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
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³ An Xterra RP18 column was found suitable.
Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 255 nm. For identity test B.2, use a diode array detector in the range of 210 to 300 nm. Maintain the column temperature at 30 °C.

Prepare the following solutions using as a diluent a mixture of 80 volumes of water R and 20 volumes of methanol R. For solution (1), transfer a volume of the oral solution, nominally containing 100 mg of Lamivudine, to a 200 mL volumetric flask. Add 120 mL of diluent and sonicate for about 20 minutes with frequent shaking. Allow to cool to room temperature, dilute to volume and filter. For solution (2), dissolve 50.0 mg of lamivudine RS and dilute to 100.0 mL.

Inject alternately 20 μl each of solutions (1) and (2). Measure the areas of the peak corresponding to lamivudine in the chromatograms of solutions (1) and (2) and calculate the percentage content of lamivudine (C₈H₁₁N₃O₃S), weight in volume, and in the oral solution, using the declared content of (C₈H₁₁N₃O₃S) in lamivudine RS.

Impurities. The impurities limited by the requirements of this monograph include those listed in the monograph for Lamivudine (excluding impurity D).

Reagent to be established

Phosphoric acid (~680 g/L) TS

Procedure: dilute 680 g of phosphoric acid (~1440 g/L) TS with sufficient water to produce 1000 mL.

Reference substances invoked
Lamivudine RS

Established ICRS.

Lamivudine for system suitability (containing lamivudine and impurities A and B) RS

Established ICRS.

Lamivudine oral solution (Lamivudini solutio peroralum)

Category Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

Storage. Lamivudine oral solution should be kept in a well-closed container, protected from light.

Additional Information: Strength in the current WHO Model list of essential medicines: 50 mg per 5 mL (10 mg per mL). Strength in the current WHO Model list of essential medicines for children: 50 mg per 5 mL (10 mg per mL).

Requirements

Complies with the monograph for "Liquid preparations for oral use".

Definition. Lamivudine oral solution is a solution of Lamivudine in a suitable vehicle, which may be flavoured. It contains not less than 90.0% and not more than 110.0% of the amount of lamivudine (C$_{8}$H$_{11}$N$_{3}$O$_{3}$S) stated on the label.

Identity tests

- Either tests A and C or tests B and C may be applied.

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 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the
mobile phase. Apply separately to the plate 10 μl of each of the following 2 solutions. For solution (A), dilute a volume of the oral solution containing 50 mg of Lamivudine to 50 mL with methanol R, filter, and use the filtrate. For solution (B), use 1.0 mg of lamivudine RS per mL of methanol. After removing the plate from the chromatographic chamber, allow it to dry in a current of cool air and 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 silica gel R5 as the coating substance. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120°C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance and intensity to 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 that obtained with solution (2).

C. The absorption spectrum (1.6) of the final solution prepared for Assay method B, when observed between 210 nm and 300 nm, exhibits one maximum at about 280 nm.

**pH value (1.13)**. pH of the oral solution, 5.7–6.3.

**Related substances.** 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. For solution (1), mix a quantity of the oral solution containing 50 mg of Lamivudine with sufficient mobile phase to produce 100 mL and filter. For solution (2), dilute 1.0 mL of solution (1) to 100 mL with mobile phase.

For solution (3) dissolve about 5 mg of lamivudine for system suitability RS (containing lamivudine and lamivudine impurities A and B) in the mobile phase, add 1 mL of solution (CUS) prepared as described below and dilute to 10 mL with the mobile phase. For solution (CUS) dissolve 25 mg of cytosine R (impurity E), 25 mg of uracil R (impurity F) and 25 mg
of salicylic acid R in the mobile phase, dilute to 50 mL with the mobile phase and dilute 1 mL of the resulting solution to 10 mL with the mobile phase. For solution (4) dissolve a suitable amount of each of the excipients (other than any parahydroxybenzoates) stated on the label in 10 mL of a suitable solvent and dilute to 100 mL with the mobile phase.

Inject separately 20 μl each of solutions (1), (2), (3) and (4). Record the chromatograms for about 3 times the retention time of lamivudine in solution (2). For preparations containing parahydroxybenzoates, continue the chromatography for solution (1) for about 9 times the retention time of lamivudine in solution (2) in order to wash these excipients from the column.

Inject separately 20 μL each of solutions (1), (2), (3) and (4). Record the chromatograms for about 3 times the retention time of lamivudine in solution (2). For preparations containing parahydroxybenzoates, continue the chromatography for solution (1) for about 9 times the retention time of lamivudine in solution (2) in order to wash these excipients from the column.

Use the chromatogram obtained with solution (3) to identify the peaks due to impurities E, F, A, B and C. The impurity peaks are eluted at the following relative retention with reference to lamivudine (retention time about 11 to 12 minutes): impurity E (cytosine) about 0.31; impurity F (uracil) about 0.36; impurity A about 0.40; impurity B about 0.9; impurity C (salicylic acid) about 2.6. The test is not valid unless in the chromatogram obtained with solution (3) the resolution factor between the peaks due to lamivudine and impurity B is at least 1.5. The test is also not valid if any of the peaks in the chromatogram obtained with solution (4) corresponds to any of the peaks in the chromatogram obtained with solution (3) or, if interference by excipients has been demonstrated by any other means.

In the chromatogram obtained with solution (1), the area of any peak, other than the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%). The area of not more than one such peak is greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%). The area of not more than two such peaks is greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%). The sum of the areas of all peaks, other than the principal peak, is not greater than 3 times the area of the principal peak obtained with solution (2) (3.0%). Disregard any peak with the same retention time as that of any of the peaks in the chromatogram obtained with solution (4), any peak with a relative retention with reference to lamivudine greater than 2.0 (corresponding to parahydroxybenzoates) and any peak with an area less than 0.05 times the area of the principal peak obtained with solution (2) (0.05%).

**Assay**
Either method A or 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 x 4.6 mm) packed with base deactivated particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). As the mobile phase, use a mixture of 5 volumes of methanol R and 95 volumes of buffer 3.8 (a 1.9 g/l solution of ammonium acetate R previously adjusted to pH 3.8 with glacial acetic acid R).

Prepare the following solutions in the mobile phase. For solution (1), mix an accurately weighed quantity of the oral solution containing about 50 mg of Lamivudine with sufficient mobile phase to produce 100 mL and dilute 10 mL to 25 mL with mobile phase. Filter a portion of this solution through a 0.45-μm filter, discarding the first few mL of the filtrate. For solution (2), use 0.2 mg of lamivudine RS per mL. For solution (3) dissolve about 5 mg of lamivudine for system suitability RS (containing lamivudine and lamivudine impurities A and B) in the mobile phase, add 1 mL of solution (CUS) prepared as described below and dilute to 10 mL with the mobile phase. For solution (CUS) dissolve 25 mg of cytosine R, 25 mg of uracil R and 25 mg of salicylic acid R in the mobile phase, dilute to 50 mL with the mobile phase and dilute 1 mL of the resulting solution to 10 mL with the mobile phase. For solution (4) dissolve a suitable amount of each of the excipients (other than any parahydroxybenzoates) stated on the label in 10 mL of a suitable solvent and dilute to 100 mL with the mobile phase.

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of about 277 nm. Maintain the temperature of the column at 35°C.

Inject separately 20 μl each of solutions (3) and (4). Record the chromatograms for about 3 times the retention time of lamivudine in solution (2). In the chromatogram obtained with solution (3) the impurity peaks are eluted at the following relative retention with reference to lamivudine (retention time about 11 to 12 minutes): impurity E (cytosine) about 0.31; impurity F (uracil) about 0.36; impurity A about 0.40; impurity B about 0.9; impurity C (salicylic acid) about 2.6. The assay is not valid unless in the chromatogram obtained with solution (3) the resolution factor between the peaks due to lamivudine and impurity B is at least 1.5. The assay
is also not valid if any of the peaks in the chromatogram obtained with solution (4) corresponds
to the peak due to lamivudine in the chromatogram obtained with solution (3).

Inject separately 20 μl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms of solutions (1) and
(2). Determine the weight per mL (1.3.1) of the oral solution and calculate the percentage
content of lamivudine (C₈H₁₁N₃O₃S) weight in volume in the oral solution.

B. Dilute an accurately weighed quantity of the oral solution containing about 20 mg of
Lamivudine to 50 mL with water R. Add 1 mL of sulfuric acid (0.1 mol/l) VS and extract with
two 30 mL quantities of diethyl ether R. Wash the combined ether extracts with 20 mL of water,
combine the aqueous solutions and remove the residual ether using a current of nitrogen. Add
sufficient water R to produce 200 mL and dilute 5 mL to 50 mL with sulfuric acid (0.1 mol/l)
VS. Measure the absorbance (1.6) of the resulting solution in a 1 cm layer at the maximum at
about 280 nm against a solvent cell containing the blank. For the blank, use a solution prepared
by diluting 1 mL of sulfuric acid (0.1 mol/l) VS to 200 mL with water R and further dilute 5
mL of this solution to 50 mL with sulfuric acid (0.1 mol/l) VS. Determine the weight per mL
(1.3.1) of the oral solution and calculate the content of lamivudine (C₈H₁₁N₃O₃S) weight in
volume in the oral solution, using the absorptivity value of 60.7 (Aₐ₁cm = 607).

**Impurities.** The impurities limited by the requirements of this monograph include those listed
in the monograph for Lamivudine.