DOLUTEGRAVIR, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE TABLETS

(DOLUTEGRAVIRI, LAMIVUDINE ET TENOFOVIRI DISOPROXILI FUMARATI COMpressoI)

Draft proposal for inclusion in The International Pharmacopoeia

(September 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 October 2019.

Working documents are sent out electronically and they will also be placed on the Medicines website for comments under “Current projects”.

http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en

If you wish to receive our draft guidelines, please send your email address to (jonessi@who.int) and your name will be added to our electronic mailing list.
Dolutegravir, Lamivudine and Tenofovir
Disoproxil Fumarate Tablets

(DOLUTEGRAVIRI, LAMIVUDINE ET TENOFOVIRI DISOPROXILI
FUMARATI COMPRESSI)

Category. Antiretroviral (Integrase inhibitor; Nucleoside/Nucleotide reverse transcriptase inhibitor; Nucleoside/Nucleotide reverse transcriptase inhibitor).

Storage. Dolutegravir, lamivudine and tenofovir disoproxil tablets should be kept in a tightly closed container.

Labelling. The designation of the container should state that the active ingredient, dolutegravir, is in sodium form and that the quantity should be indicated in terms of the equivalent amount of dolutegravir. The quantities of the two other active ingredients should be indicated in terms of the amounts of lamivudine and tenofovir disoproxil fumarate.

Additional information. Strength in the current WHO Model List of Essential Medicines: 50 mg Dolutegravir, 300 mg Lamivudine and 300 mg Tenofovir disoproxil fumarate.

Requirements
Comply with the monograph for Tablets.

Definition. Dolutegravir, lamivudine and tenofovir disoproxil tablets contain Dolutegravir sodium, Lamivudine and Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amount of dolutegravir (C\textsubscript{20}H\textsubscript{19}F\textsubscript{2}N\textsubscript{3}O\textsubscript{5}), lamivudine (C\textsubscript{8}H\textsubscript{11}N\textsubscript{3}O\textsubscript{3}S) and tenofovir disoproxil fumarate (C\textsubscript{19}H\textsubscript{30}N\textsubscript{5}O\textsubscript{10}P, C\textsubscript{4}H\textsubscript{4}O\textsubscript{4}) stated on the label.

Manufacture. The manufacturing process and the product packaging are designed and controlled so as to minimize the moisture content of the tablets. They ensure that, if tested, the tablets would comply with a water content limit of not more than 50 mg/g when determined as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the powdered tablets.

Identity test. 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 three principal peaks in the chromatogram obtained with solution (1) correspond to the retention time of the corresponding peaks due to dolutegravir, lamivudine and tenofovir disoproxil fumarate in the chromatograms obtained with solutions (2), (3) and (4).
Dissolution. Carry out the test described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium 900 mL of dissolution buffer, pH 6.8, 0.25% SDS TS and rotating the paddle at 60 revolutions per minute. At 30 minutes, withdraw a sample of 10 mL of the medium through an in-line filter (sample (A)). Add 10 mL of the dissolution medium, maintained at 37.0 °C (+/- 0.5 °C), to each dissolution vessel and continue the dissolution for a further 30 minutes. At 60 minutes withdraw again a sample of 10 mL of the dissolution medium through an in-line filter (sample (B)). Dilute 5.0 mL each of sample (A) and sample (B) to 25.0 mL with diluent (2), described under “Assay”, and use the obtained solution as solution (1) and solution (2).

Measure the concentration of lamivudine and tenofovir disoproxil fumarate in solution (1) and the concentration of dolutegravir in solution (2). Carry out the test as described under 1.14.4 High-performance liquid chromatography using the chromatographic conditions and solutions as described under “Assay”.

For each of the tablets tested, calculate the total amount each of lamivudine, tenofovir disoproxil fumarate and dolutegravir in the medium from the results obtained, using the declared content of dolutegravir sodium (C20H18F2NaO5) in dolutegravir sodium RS, the declared content of lamivudine (C8H11N3O3S) in lamivudine RS and the declared content of tenofovir disoproxil fumarate (C19H30N5O10P.C4H4O4) in tenofovir disoproxil fumarate RS. Each mg of dolutegravir sodium is equivalent to 0.950 mg of dolutegravir.

Evaluate the results as described under 5.5 Dissolution test for solid oral dosage forms, Acceptance criteria. The amount of lamivudine (C8H11N3O3S) and tenofovir disoproxil fumarate (C19H30N5O10P.C4H4O4) released after 30 minutes is not less than 80% (Q) of the amounts declared on the label and the amount of dolutegravir (C20H18F2NaO5) released after 60 minutes is not less than 80% (Q) of the amount declared on the label.

Tests for related substances

A. Lamivudine- and tenofovir disoproxil-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 octadecylsilyl groups (5 µm).12

Use the following conditions for gradient elution:

- mobile phase A: acetate buffer pH 4.2; and
- mobile phase B: acetonitrile R.

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12 An Inertsil ODS-3v column was found suitable.
Prepare the acetate buffer pH 4.2 by dissolving 9.64 g of ammonium acetate R in 900 mL of water R, adjust the pH to 4.2 (+/- 0.05) with glacial acetic acid R and dilute to 1000 mL with water R.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2–17</td>
<td>100 to 95</td>
<td>0 to 5</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>17–47</td>
<td>95 to 60</td>
<td>5 to 40</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>47–62</td>
<td>60 to 25</td>
<td>40 to 75</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>62–63</td>
<td>25 to 100</td>
<td>75 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>63–75</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm. Maintain the column temperature at 25 °C and the autosampler temperature at 6 °C.

Prepare the following solutions using water R as diluent.

For solution (1), transfer a quantity of the powdered tablets, nominally containing 225 mg of Tenofovir disoproxil fumarate, to a 250 mL volumetric flask. Add about 175 mL of diluent and sonicate at room temperature for about 30 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 10.0 mL of solution (2) to 100.0 mL.

For solution (4), dissolve about 1 mg of tenofovir disoproxil for system suitability (containing tenofovir disoproxil and the impurities I and H) in 2 mL of water R.

For solution (5), dissolve 10 mg of tenofovir disoproxil fumarate RS in 10 mL of water R.

Heat the solution carefully in a boiling water-bath for 20 minutes.
For solution (6), use a solution containing 0.2 mg of fumaric acid R per mL of water R.
For solution (7), 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 (8), dissolve 25 mg of cytosine R and 25 mg of uracil R and dilute to 50.0 mL. Dilute 1.0 mL of this solution to 100.0 mL.

For solution (9), 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 10 µL each of solutions (1), (2), (3), (4), (5), (6), (7), (8) and (9).

Use the chromatogram obtained with solution (4) to identify the peak due to the tenofovir disoproxil impurity I in the chromatogram obtained with solution (1), if present.

Use the chromatogram obtained with solution (5) to identify the peak due to the tenofovir disoproxil impurity A in the chromatogram obtained with solution (1), if present.

Use the chromatogram obtained with solution (6) to identify the peak due to the fumarate in the chromatogram obtained with solution (1), if present. The peak due to fumarate is eluted at about 2.8 minutes and may appear as single or split peaks.

Use the chromatogram obtained with solution (8) to identify the peaks due to lamivudine impurities E (cytosine) and F (uracil) in the chromatogram obtained with solution (1), if present.
Use the chromatogram obtained with solution (9) to identify the peaks due to excipients. The impurities, if present, are eluted at the following relative retentions with reference to tenofovir disoproxil (retention time about 48 minutes):

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Relative retention</th>
<th>Impurity Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenofovir disoproxil impurity N</td>
<td>0.33</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity A</td>
<td>0.63</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity F</td>
<td>0.73</td>
<td>Degradation</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity E</td>
<td>0.76</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity B</td>
<td>0.80 and 0.81</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity C</td>
<td>0.88</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity D</td>
<td>0.90</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity M</td>
<td>0.94</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity L</td>
<td>0.97</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity I</td>
<td>0.98</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity H</td>
<td>1.01</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Tenofovir disoproxil impurity J</td>
<td>1.19</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Lamivudine impurity E</td>
<td>0.09</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Lamivudine impurity F</td>
<td>0.11</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Lamivudine impurity A</td>
<td>0.15 and 0.17</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Lamivudine impurity G</td>
<td>0.20</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Lamivudine impurity H</td>
<td>0.21</td>
<td>Synthesis/Degradation</td>
</tr>
<tr>
<td>Lamivudine impurity B</td>
<td>0.38</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>0.39</td>
<td>-</td>
</tr>
<tr>
<td>Lamivudine impurity J</td>
<td>0.45</td>
<td>Degradant</td>
</tr>
<tr>
<td>Lamivudine impurity C</td>
<td>0.54</td>
<td>Synthesis</td>
</tr>
</tbody>
</table>
The test is not valid unless:

- in the chromatogram obtained with solution (7), the resolution between the peaks due to lamivudine impurity B and lamivudine is at least 1.5;
- in the chromatogram obtained with solution (3), the signal-to-noise ratios of the peaks due to lamivudine and due to tenofovir disoproxil are at least 20;
- in the chromatogram obtained with solution (4), the resolution between the peaks due impurity I and tenofovir disoproxil is at least 1.5 and the resolution between the peaks due to tenofovir disoproxil and impurity H is at least 1.2;

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to tenofovir impurity A, when multiplied by a correction factor of 0.79, is not greater than three times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) (3.0%);
- the area of any peak corresponding to either tenofovir impurity F, tenofovir impurity I or tenofovir impurity J, is not greater than 0.75 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) (0.75%);
- the area of any peak corresponding to tenofovir impurity M, when multiplied by a correction factor of 0.53, is not greater than two times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (3) (0.2%);
- the area of any peak corresponding to tenofovir impurity E is not greater than two times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (3) (0.2%);
- the area of any peak corresponding to lamivudine impurity E, when multiplied by a correction factor of 0.61, is not greater than two times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.2%);
- the area of any peak corresponding to lamivudine impurity F, when multiplied by a correction factor of 0.48, is not greater than two times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.2%);
- the area of any peak corresponding to either lamivudine impurity G, lamivudine impurity H or lamivudine impurity J, is not greater than two times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.2%).
• Determine the sum of the areas of any peaks corresponding to lamivudine impurity G, lamivudine impurity H and lamivudine impurity J and the corrected areas of any peaks corresponding to lamivudine impurity E and lamivudine impurity F using the area of the peak due to lamivudine in the chromatogram obtained with solution (2) as a reference. Disregard any peak with an area or a corrected area of less than 0.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.05%). Determine the sum of the areas of any peaks corresponding to tenofovir impurity F, tenofovir impurity E, tenofovir impurity I and tenofovir impurity J and the corrected areas of any peaks corresponding to tenofovir impurity M and tenofovir impurity A using the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) as a reference. Disregard any peak with an area or a corrected area of less than 0.5 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (3) (0.05%). The sum of the lamivudine and tenofovir disoproxil related impurities is not greater than 5.0%.

B. Dolutegravir-related substances

Perform the test 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 (15 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded pentafluorophenyl and octadecylsilyl groups (5 µm).13

Use the following conditions for gradient elution:
• mobile phase A: 0.186 g disodium edetate R in 1000 mL of water R adjusted to pH 2.0 with phosphoric acid (~20 g/L) TS;
• mobile phase B: 90 volumes of methanol R and 10 volumes of tetrahydrofuran R.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>60</td>
<td>40</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2–32</td>
<td>60 to 50</td>
<td>40 to 50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>32–37</td>
<td>50</td>
<td>50</td>
<td>Isocratic</td>
</tr>
<tr>
<td>37–42</td>
<td>50 to 30</td>
<td>50 to 70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>42–52</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>52–53</td>
<td>30 to 60</td>
<td>70 to 40</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>53–60</td>
<td>60</td>
<td>40</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

13 An ACE 5 C18-PFP column was found suitable.
Operate at a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet
spectrophotometer set at a wavelength of 320 nm. Maintain the column temperature at
45 °C.

Prepare the following solutions using as the diluent a mixture of 60 volumes of water R
and 40 volumes of acetonitrile R.

For solution (1), transfer a quantity of the powdered tablets, nominally equivalent to
87.5 mg dolutegravir, to a 250 mL volumetric flask. Add about 180 mL diluent and
sonicate for five minutes, cool to room temperature, dilute to volume and filter.

For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 10.0 mL of this solution
to 50.0 mL.

For solution (3), use a solution containing 0.5 mg of dolutegravir sodium for system
suitability RS (containing dolutegravir sodium and impurity E) per mL.
For solution (4), use a solution containing 1 mg of dolutegravir sodium for peak
identification RS (containing dolutegravir sodium and the dolutegravir impurities A, B
and D) per mL.

Inject alternately 30 µL each of solutions (1), (2), (3) and (4).

Use the chromatogram obtained with solution (3) to identify the peak due to the impurity
E. Use the chromatogram obtained with solution (4) to identify the peaks due to the
impurities B and D.

The impurities, if present, are eluted at the following relative retentions with reference to
dolutegravir (retention time about 27 minutes):

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Relative retention</th>
<th>Impurity Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolutegravir impurity C</td>
<td>0.67</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Dolutegravir impurity F</td>
<td>0.70</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Dolutegravir impurity D</td>
<td>0.77</td>
<td>Synthesis</td>
</tr>
<tr>
<td>Dolutegravir impurity E</td>
<td>0.89</td>
<td>Synthesis</td>
</tr>
</tbody>
</table>

The test is not valid unless in the chromatogram obtained with solution (3) the resolution
factor between the peaks due to impurity E and dolutegravir is at least 3.

In the chromatogram obtained with solution (1):
- the area of any peak corresponding to either dolutegravir impurities C, D, E or F is
  not greater than the area of the peak due to dolutegravir in the chromatogram
  obtained with solution (2) (0.2%).
Assay. Perform the test 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 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).\(^\text{14}\)

Use the following conditions for gradient elution:

- mobile phase A: A solution of 0.186 g of disodium edetate R in a mixture of 1 volume of trifluoroacetic acid R in 1000 volumes of water R; and
- mobile phase B: Acetonitrile R.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10.0</td>
<td>98 to 50</td>
<td>2 to 50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>10.0 to 12.0</td>
<td>50</td>
<td>50</td>
<td>Isocratic</td>
</tr>
<tr>
<td>12.0–12.5</td>
<td>50 to 98</td>
<td>50 to 2</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>12.5–18.0</td>
<td>98</td>
<td>2</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate at a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm. Maintain the column temperature at 30 °C.

Prepare a phosphate buffer pH 3.0 by dissolving 12.3 g of sodium dihydrogen phosphate R in 900 mL of water R. Adjust the pH to 3.0 (+/- 0.05) with phosphoric acid (~105 g/L) TS, mix and dilute to 1000 mL with water R.

Prepare the following diluents. For diluent (1), mix 60 volumes of water R and 40 volumes of acetonitrile R. For diluent (2), mix 90 volumes of the phosphate buffer pH 3.0 with 10 volumes of acetonitrile R.

\(^{14}\) An Inertsil C8-3 column was found suitable.
Prepare the following solution. For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally equivalent to 340.0 mg of lamivudine, to a 500 mL volumetric flask. Add about 400 mL of diluent (1) and sonicate for about 10 minutes with intermittent shaking. Allow to cool to room temperature, dilute to volume with diluent (1) and filter. Dilute 5.0 mL of this solution to 50.0 mL with diluent (2). For solution (2), dissolve 28.0 mg of dolutegravir sodium RS in diluent (1) and dilute to 250.0 mL with the same solvent.

Dilute 5.0 mL of this solution to 50.0 mL with diluent (2). For solution (3), dissolve 68.0 mg of lamivudine RS in diluent (1) and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with diluent (2). For solution (4), dissolve 68.0 mg of tenofovir disoproxil fumarate RS in diluent (1) and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with diluent (2).

Inject alternately 25 µL each of solutions (1), (2), (3) and (4).

Measure the areas of the peaks corresponding to dolutegravir, lamivudine and tenofovir disoproxil obtained in the chromatograms of solutions (1), (2), (3) and (4) and calculate the percentage content of dolutegravir (C20H18F2N3O5), lamivudine (C8H11N3O3S) and tenofovir disoproxil fumarate (C19H30N5O10P.C4H4O4) in the tablets using the declared content of dolutegravir sodium (C20H18F2NaO5) in dolutegravir sodium RS, the declared content of lamivudine (C8H11N3O3S) in lamivudine RS and the declared content of tenofovir disoproxil fumarate (C19H30N5O10P.C4H4O4) in tenofovir disoproxil fumarate RS. Each mg of dolutegravir sodium is equivalent to 0.950 mg of dolutegravir.

**Impurities.** The impurities limited by the requirements of this monograph include those listed in the monographs on Dolutegravir sodium, Lamivudine and Tenofovir disoproxil fumarate, excluding dolutegravir impurity A and B.
Reference substances invoked

Tenofovir disoproxil for system suitability RS (containing tenofovir disoproxil and the impurities I and H)
International Chemical Reference Substance to be established.

Lamivudine for system suitability RS (containing lamivudine and lamivudine impurities A and B)
Established International Chemical Reference Substance.

Tenofovir disoproxil fumarate RS
Established International Chemical Reference Substance.

Lamivudine RS
Established International Chemical Reference Substance.

Dolutegravir RS
International Chemical Reference Substance to be established.

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