WHO Drug Information

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Abbreviations and websites

CHMP Committee for Medicinal Products for Human Use (EMA)
EMA European Medicines Agency (www.ema.europa.eu)
EU European Union
FDA U.S. Food and Drug Administration (www.fda.gov)
Health Canada Federal department responsible for health product regulation in Canada (www.hc-sc.gc.ca)
HPRA Health Products Regulatory Authority, Ireland (www.hpra.ie)
HSA Health Sciences Authority, Singapore (www.hsa.gov.sg)
ICDRA International Conference of Drug Regulatory Authorities
ICH International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (www.ich.org)
IGDRP International Generic Drug Regulators Programme (https://www.igdrp.com)
MHLW Ministry of Health, Labour and Welfare, Japan
MHRA Medicines and Healthcare Products Regulatory Agency, United Kingdom (www.mhra.gov.uk)
Medsafe New Zealand Medicines and Medical Devices Safety Authority (www.medsafe.govt.nz)
Ph. Int The International Pharmacopoeia (http://apps.who.int/phint/)
PRAC Pharmacovigilance Risk Assessment Committee (EMA)
PMDA Pharmaceuticals and Medical Devices Agency, Japan (www.pmda.go.jp/english/index.htm)
Swissmedic Swiss Agency for Therapeutic Products (www.swissmedic.ch)
TGA Therapeutic Goods Administration, Australia (www.tga.gov.au)
U.S. United States of America
WHO World Health Organization (www.who.int)
WHO EMP WHO Essential medicines and health products (www.who.int/medicines/en/)
WHO PQT WHO Prequalification team (https://extranet.who.int/prequal/)

Note: The online version of this issue (freely available at www.who.int/medicines/publications/druginformation) has direct clickable hyperlinks to the documents and websites referenced
ENVIRONMENTAL ASPECTS OF MANUFACTURING FOR THE PREVENTION OF ANTIMICROBIAL RESISTANCE

Antimicrobial resistance (AMR) is a global and multisectoral challenge that requires a comprehensive One Health response to bridge human, animal, plant and environmental health. The environment is considered as one driver/reservoir of AMR as the discharge of antimicrobial residues and resistant microbes from health care facilities, household and farms and pharmaceutical manufacturing can potentially increase the risk of AMR infections.

According to research by UN Environment,2 growing antimicrobial resistance (AMR) linked to the discharge of drugs and particular chemicals into the environment is one of the most worrying health threats of today. There is an emerging evidence showing that pharmaceutical plants can act as hotspots that release large amounts of antibiotics into the environment, which is of particular concern in the regions without adequate monitoring and wastewater management capacity.

The Organisation for Economic Cooperation and Development (OECD) in its latest report “Pharmaceutical Residues in Freshwater” (November 2019), highlights the need to better understand the effects of pharmaceutical residues in the environment, and to employ policy instruments across the pharmaceutical life-cycle to mitigate the risks, by improvements in the design, authorization, production, use, solid waste and wastewater treatment. A focus on preventive options early in the pharmaceutical life-cycle, including production, may deliver the most long-term, cost-effective and large-scale benefits3.

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Against a backdrop of rising global concern about AMR, following up on the Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP) request from October 2018, the WHO Secretariat developed a document outlining points for manufacturers and inspectors to consider in preventing AMR. This Points to Consider document addresses also the November 2018 decision of the World Health Organization (WHO) Executive Board to provide technical input to good manufacturing practices guidance on waste and wastewater management from the production of critically important antimicrobials for human medicine.

The ECSPP at its last meeting in October 2019 adopted the text “Environmental aspects of manufacturing for the prevention of antimicrobial resistance” that is going to be published in spring 2020 as an Annex to the ECSPP report in the WHO Technical Report Series (TRS).

The purpose of the document is to leverage on the current WHO Good Manufacturing Practices (GMP) with the following objectives:

- raise awareness among manufacturers, GMP inspectors and inspectorates of the existing GMP guidance that applies to the production of antimicrobials;
- encourage Member States to establish and enforce appropriate requirements on their local pharmaceutical production facilities; and
- consider options for reducing and mitigating the uncontrolled disposal of waste and wastewater containing antimicrobials, with a focus on the role of GMP and inspectors in this.

The first draft of the document (April 2019) prepared by WHO Prequalification (PQ) Team - Inspections and the WHO Secretariat was reviewed by WHO colleagues from the AMR Surveillance, Prevention and Control Department within the AMR Division, and then it was mailed to the Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations inviting their comments. The document was also posted on the WHO website for public consultation (May-June 2019). In addition, it was discussed during several international meetings. All the comments received were consolidated and discussed during the Informal Consultation on Good Practices for Health Products Manufacture and Inspection (Geneva, July 2019).

During the GMP consultation on Good Practices for Health Products Manufacture and Inspection (Geneva, July 2019), some proposals were discussed including:

a. the potential role of GMP inspectors to tackle AMR; and
b. the need to revise the WHO GMP main text in order to specifically address this issue.
The main limitations identified when considering broadening up either the current WHO GMP scope or the role of the inspectors to encompass environmental aspects were the following:

i. GMP inspectors may not be adequately trained for inspecting waste and wastewater management processes on a required level and that the duration of GMP inspections, being limited to no more than 2 to 5 days, depending on the type of site, should not be considerably extended to cover those aspects.

ii. The competence on environmental issues is often with environmental inspectors reporting to a different authority with specific competences and mandate.

iii. There are no WHO or government-developed thresholds (only industry ones) on the acceptable residual limits from antimicrobial production in waste and wastewater to perform a meaningful evaluation.

iv. The knowledge on the waste and wastewater treatment is limited for most GMP inspectors, therefore additional training in this area may be required if they are to verify those aspects.

v. This Point to consider document focuses purely on the contamination of the environment through production and does not attempt to address the many other drivers of AMR and the life-cycle of managing medicines in the environment.

vi. The potential challenges to implementing the points to consider in practice and the importance of ensuring collaboration between product and environment inspections were also acknowledged.

After a careful reflection of the pros/cons and the stated limitations, the document was restructured and the main changes were made to narrow the scope and structure of the initial document by:

- including the relevant text from the GMP guidelines4 relating to environment protection and waste management to prevent AMR in the main body;
- elaborating on recommendations to manufacturers, including explanations on the clauses listed to clarify the expectation of Inspectors from manufacturers in this area;
- focusing on the new target audience, namely pharmaceutical manufacturers and GMP inspectors; and
- amending the scope: it is now drafted as a policy document for use by manufacturers when designing and selecting their waste management processes, when performing self-audits and also for use by both inspectors and manufacturers during GMP inspections of pharmaceutical manufacturing sites.

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The *Point to Consider* document discussed and adopted by the 54th ECSPP last October 2019 will be used in conjunction with a survey of waste and wastewater management practices that will be sent to manufacturers to raise awareness of AMR and to verify the practices currently in use in the industry as well as the application of the recommendations made in the newly-adopted *Points to Consider for manufacturers and inspectors: Environmental aspects of manufacturing for the prevention of antimicrobial resistance*.

The WHO Prequalification (PQ) Team – Inspections is also initiating a phased approach in the verification of the waste and wastewater management practices in use at pharmaceutical manufacturing sites who are active in the production of antimicrobials during onsite inspections in 2020.

Results of the survey will be presented to the 55th ECSPP in 2020.
DRAFT MONOGRAPH ON SOFOSBUVIR TABLETS
(SOFOSBUVIR COMPRESSI)

Draft proposal for inclusion in The International Pharmacopoeia
(November 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 20 February 2020.

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.
DRAFT PROPOSAL FOR INCLUSION IN
THE INTERNATIONAL PHARMACOPOEIA

SOFOSBUVIR TABLETS
(SOFOSBUVIRI COMPRESSIONI)

**Category.** Antiviral (Hepatitis C viral polymerase nucleotide inhibitor)

**Storage.** Sofosbuvir tablets should be kept in a well-closed container not exceeding 30 °C

**Additional information.** Strength in the current WHO Model List of Essential Medicines: 400 mg sofosbuvir

**Requirements**

Comply with the monograph for “Tablets”.

**Definition.** Sofosbuvir tablets contain not less than 90.0 % and not more than 110.0 % of the amount of sofosbuvir (C_{22}H_{29}FN_{3}O_{9}P) stated on the label.

**Identity tests**

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

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. Record the UV spectrum of the principle peak in the chromatograms with a diode array detector in the range of 200 nm to 400 nm. The retention time and the UV spectrum of the principal peak in the chromatogram obtained with solution (1) correspond to the retention time and UV spectrum of the peak due to sofosbuvir in the chromatogram obtained with solution (2).
B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions 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 sofosbuvir in the chromatogram obtained with solution (2).

C. 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 methanol R, 4 volumes of ethyl acetate R and 0.1 volume of ammonia R as the mobile phase.

Apply separately to the plate 10 µL of each of the following solutions in methanol R. For solution (A), sonicate a quantity of the powdered tablets, nominally containing 10 mg of sofosbuvir with 10 mL of methanol, allow to cool to room temperature and filter. For solution (B), use 1 mg of sofosbuvir RS per mL. After removing the plate from the chromatographic chamber, allow it to dry exhaustively 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).

D. To a quantity of the powdered tablets, nominally equivalent to 50 mg of sofosbuvir, add 30 mL of methanol R and sonicate for 10 minutes. Allow to cool to room temperature, dilute to 50 mL with the same solvent and filter. Dilute 1.0 mL of the filtrate to 100.0 mL using methanol R. The absorption spectrum (1.6) of the resulting solution, when observed between 200 and 400 nm, exhibits a maximum at about 260 nm.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms using as dissolution medium 900 mL of dissolution buffer pH 6.8 TS. Rotate the paddle at 75 revolutions per minute. At 15 minutes, withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered solution to cool down to room temperature. Dilute 5.0 mL of the filtrate to 50.0 mL with dissolution medium and use it as solution (1). For solution (2), transfer 44.0 mg of sofosbuvir RS to a 100 mL volumetric flask. Add 4 mL of methanol R and 70 mL of dissolution medium, sonicate for 5 minutes, cool to room temperature and dilute to volume. Dilute 5.0 mL of this solution to 50.0 mL with dissolution medium.

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1 Silica gel on TLC Alu foils from Fluka is suitable.
Measure the absorbance as described under 1.6 Spectrophotometry in the visible and ultraviolet regions of a 1 cm layer of the resulting solutions at the maximum at about 262 nm, using the dissolution medium as the blank.

For each of the tablets tested, calculate the total amount of sofosbuvir (C_{20}H_{29}FN_{3}O_{9}P) in the medium. Evaluate the results as described under 5.5 Dissolution test for solid oral dosage forms, Acceptance criteria. The amount of sofosbuvir released is not less than 75% (Q) of the amount declared on the label.

[Note from the Secretariat. It is intended to determine the absorptivity value of sofosbuvir during the establishment of the corresponding International Chemical Reference Standard and to use this value for the calculation of the test result.]

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 octadecylsilyl groups (3.5 μm).^2

Use the following conditions for gradient elution:
As mobile phase A, use a mixture of 21 volumes of 0.05 % phosphoric acid (~1440 g/L) TS, 77 volumes of water R and 2 volumes of acetonitrile R. As mobile phase B, use a mixture of 21 volumes of 0.05 % phosphoric acid (~1440 g/L) TS and 79 volumes of acetonitrile R.

Use the following gradient:

<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 - 2.5</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2.5 - 27.4</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>27.4 - 38</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>38 - 45</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

^2 XSelect® C18 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 260 nm. Maintain the column temperature at 30 °C.

Prepare the following solutions using as diluent a mixture of 50 volumes of mobile phase A and 50 volumes of mobile phase B. Prepare solution (1) as described under “Assay”. 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 50.0 mL. For solution (4), dissolve 1.0 mg of sofosbuvir for peak identification RS (containing sofosbuvir and the impurities A, B, C, D and E) in 5.0 mL. For solution (5), dissolve 25.0 mg of phenol R and dilute to 50.0 mL. Dilute 2.0 mL to of this solution to 100.0 mL. Dilute 5.0 mL of this solution to 50.0 mL.

Inject 20 µL of solutions (3) and (4).

The test is not valid unless the peak-to-valley ratio (Hp/Hv) in the chromatogram obtained with solution (4) is at least x³, where Hp is the height above the extrapolated baseline of the peak due to impurity A and Hv is the height above the extrapolated baseline at the lowest point of the curve separating this impurity from the peak due to sofosbuvir. Also, the test is not valid unless the signal-to-noise of the peak due to sofosbuvir in the chromatogram obtained with solution (3) is at least 10.

Inject alternately 20 µL each of solutions (1), (2) and (5).

Use the chromatograms obtained with solutions (4) and (5) and the relative retentions below to identify the peaks due to impurities D, 1, 2, 3, 4 and 5.

The impurities, if present, are eluted at the following relative retentions with reference to sofosbuvir (retention time about 16 minutes): impurity A about 0.98; impurity B about 1.05; impurity C about 1.41; impurity D about 0.38; impurity E about 0.93, impurity F about 1.15; impurity G about 1.57; impurity 1 about 0.21; impurity 2 about 0.45; impurity 3 about 0.61; impurity 4 about 0.65; impurity 5 about 0.72.

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³ A value for the Hp/Hv has to be determined based on the available sofosbuvir for peak identification RS.
In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 0.5, is not greater than 0.2 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurities 1, 2, 3 or 4 is not greater than 0.2 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurity 5 is not greater than the area of the peak due to phenol in the chromatogram obtained with solution (5) (0.2 %).
- The sum of the corrected area of any peak corresponding to impurity D and the areas of any peak corresponding to impurities 1, 2, 3 or 4 is not greater than the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (1.0 %). Disregard any peak with an area less than the area of the peak due to sofosbuvir in the chromatogram obtained with solution (3) (0.1%).

**Assay.** Carry out the test as described under 1.14.4 *High-performance liquid chromatography* using the conditions given under “Related substances” with the following modifications: As the mobile phase use a mixture of 65 volumes of mobile phase A and 35 volumes of mobile phase B.

Prepare the following solutions using the mobile phase as diluent. For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally containing 125.0 mg of sofosbuvir, to a 250 mL volumetric flask. Add about 200 mL of diluent and sonicate for 10 minutes, cool to room temperature, dilute to volume and filter. For solution (2), dilute 50.0 mg of sofosbuvir RS and dilute to 100.0 mL.

Inject alternatively 20 µL each of solutions (1) and (2). Record the chromatograms for 20 minutes.

Measure the areas of the peaks corresponding to sofosbuvir obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of sofosbuvir (C_{22}H_{29}FN_{3}O_{9}P), using the declared content of C_{22}H_{29}FN_{3}O_{9}P in sofosbuvir RS.
**Impurities.** The impurities limited by the requirements of this monograph include the impurities listed in the monograph for sofosbuvir and the impurities 1, 2, 3, 4 and 5.

1. \((2'R)-2'-deoxy-2'-fluoro-2'-methyluridine\) 5'-(dihydrogen phosphate) (fluorouridine phosphate) (degradation product)

2. \(N\cdot\{(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl\)methoxy\}(hydroxy)phosphoryl\]-L-alanine (uridine alanine phosphate) (degradation product)

3. \((2'R)-2'-deoxy-2'-fluoro-5'-O\cdot\{(hydroxy(phenoxyporphoryl)\)-2'-methyluridine (uridine phenyl phosphate) (degradation product)

4. propan-2-yl \(N\cdot\{(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl\)methoxy\}(hydroxy)phosphoryl\]-L-alanine (uridine isopropyl alanine phosphate) (degradation product)

5. phenol (degradation product).
Reference substances invoked

Sofosbuvir RS

International Chemical Reference Substance (ICRS) to be established.

Sofosbuvir for peak identification RS (containing sofosbuvir and the impurities A, B, C, D and E)

International Chemical Reference Substance (ICRS) to be established.

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DRAFT MONOGRAPH ON SOFOSBUVIR
(SOFOSBUVIRUM)

Draft proposal for inclusion in The International Pharmacopoeia
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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.
DRAFT PROPOSAL FOR INCLUSION IN
THE INTERNATIONAL PHARMACOPOEIA

SOFOSBUVIR

(SOFOSBUVIRUM)

**Molecular formula.** $\text{C}_{22}\text{H}_{29}\text{FN}_{3}\text{O}_{9}\text{P}$

**Relative molecular mass.** 529.5

**Graphic formula.**

![Graphic formula image]

**Chemical name.** Propan-2-yl $N$-[[(S)-[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy]phenoxyphosphoryl]-L-alaninate; CAS Reg. No. 1190307-88-0.

**Description.** A white to off-white powder.

**Solubility.** Slightly soluble in water R, freely soluble in dehydrated ethanol R and acetone R, soluble in 2-propanol R and insoluble in heptane R.

**Category.** Antiviral (Hepatitis C viral polymerase nucleotide inhibitor)

**Storage.** Sofosbuvir should be kept in a well-closed container and stored at a temperature below 30 °C.

**Additional information.** Sofosbuvir may exhibit polymorphism.
**Definition.** Sofosbuvir contains not less than 97.5 % and not more than 102.0 % of C\textsubscript{22}H\textsubscript{29}FN\textsubscript{3}O\textsubscript{9}P with reference to the anhydrous substance.

**Identity tests**

- Either test 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 sofosbuvir RS or with the reference spectrum of sofosbuvir. If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and sofosbuvir RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from sofosbuvir RS.

  **B.** Carry out the test as described under 1.14.4 *High-performance liquid chromatography* using the conditions 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 sofosbuvir in the chromatogram obtained with solution (2).

  **C.** Carry out the test as described under 1.14.1 *Thin-layer chromatography*, using silica gel R\textsuperscript{2} as the coating substance and a mixture of 6 volumes of dichloromethane R, 1 volume of methanol R, 4 volumes of ethyl acetate R and 0.1 volume of ammonia (\textasciitilde 260 g/L) TS as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions in methanol R. For solution (A), use 1 mg of the test substance per mL. For solution (B), use 1 mg of sofosbuvir RS per mL. After removing the plate from the chromatographic chamber, allow it to dry 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 the spot due to sofosbuvir in the chromatogram obtained with solution (B).

\footnote{Silica gel on TLC alu foils from Fluka are suitable.}
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.

Water. Carry out the test as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.200 g of the substance; the water content is not more than 10 mg/g.

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 octadecylsilyl groups (3.5 µm). Use the following conditions for gradient elution: As mobile phase A, use a mixture of 21 volumes of 0.05 % phosphoric acid (~1440 g/L) TS, 77 volumes of water R and 2 volumes of acetonitrile R. As mobile phase B, use a mixture of 21 volumes of 0.05 % phosphoric acid (~1440 g/L) TS and 79 volumes of acetonitrile R.

Use the following gradient:

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<thead>
<tr>
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<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>0 - 2.5</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2.5 - 27.4</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>27.4 - 38</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>38 - 45</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm and, for impurities F and G, at 205 nm. Maintain the column temperature at 30 °C.

Prepare the following solutions using as diluent a mixture of 50 volumes of mobile phase A and 50 volumes of mobile phase B. For solution (1), dissolve 50.0 mg of the test substance and dilute to 100.0 mL. 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), dilute 1.0 mg of sofosbuvir for peak identification RS (containing sofosbuvir and the impurities A, B, C, D and E) in 5.0 mL. For solution (5), dissolve 30.0 mg of pentafluorophenol R and dilute to 100.0 mL. Dilute 5.0 mL of this solution to 100.0 mL.

5 XSelect C18 was found suitable.
Inject 20 µL each of solution (3) and (4). The test is not valid unless in the chromatogram obtained with solution (4) the peak-to-valley ratio (Hp/Hv) is at least x\(^6\), where Hp is the height above the extrapolated baseline of the peak due to impurity A and Hv is the height above the extrapolated baseline at the lowest point of the curve separating this peak from the peak due to sofosbuvir. Also, the test is not valid unless in the chromatogram obtained with solution (3) the signal-to-noise of the peak due to sofosbuvir is at least 10.

Inject alternately 20 µL each of solutions (1), (2) and (5).

Use the chromatograms obtained with solutions (4) and (5) and the relative retentions below to identify the impurity peaks.

The impurities, if present, are eluted at the following relative retentions with reference to sofosbuvir (retention time about 16 minutes): impurity A about 0.98; impurity B about 1.05; impurity C about 1.41; impurity D about 0.38; impurity E about 0.93, impurity F about 1.13; impurity G about 1.57.

In the chromatogram obtained with solution (1):
- the area of any peak corresponding to either impurity A or B is not greater than 0.15 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.15 %);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 1.5, is not greater than 0.15 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.15 %);
- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 0.5, is not greater than 0.15 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.15 %);
- the area of any peak corresponding to impurity E is not greater than 0.3 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.3 %);
- the area of any peak corresponding to either impurities F or G, recorded at 205 nm, is not greater than the area of the peak due to pentafluorophenol in the chromatogram obtained with solution (5) and recorded at 205 nm (0.15%);
- the area of any other impurity peak is not greater than 0.1 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.10%).

The sum of the corrected areas of any peak corresponding to impurities C and D and the areas of all other impurity peaks, recorded at 260 nm, is not greater than the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (1.0 %).

Disregard any peak with an area less than the area of the peak due to sofosbuvir in the chromatogram obtained with solution (3) (0.05 %).

\(^6\) A value for the Hp/Hv has to be determined based on the available sofosbuvir for peak identification RS.
**Assay.** Carry out the test as described under 1.14.4 *High-performance liquid chromatography* using the conditions given under “Related substances” with the following modifications:
As the mobile phase use a mixture of 65 volumes of mobile phase A and 35 volumes of mobile phase B.

Prepare the following solutions using mobile phase as diluent. For solution (1), dissolve 50.0 mg of the test substance and dilute to 100.0 mL. For solution (2), dissolve 50.0 mg of sofosbuvir RS and dilute to 100.0 mL.

Inject alternately 20 µL each of solutions (1) and (2). Record the chromatograms for 20 minutes.

Measure the areas of the peaks corresponding to sofosbuvir obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of sofosbuvir (C\(_{22}\)H\(_{29}\)FN\(_{3}\)O\(_{9}\)P), using the declared content of C\(_{22}\)H\(_{30}\)FN\(_{3}\)O\(_{4}\)P in sofosbuvir RS.

**Impurities**

A. Propan-2-yl N-\([(R)-[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy]phenoxyphosphoryl]-l-alaninate (Rp isomer) (process related impurity).

B. Propan-2-yl N-\([(S)-[(2R,3R,4R,5R)-4-chloro-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-hydroxy-4-methyloxolan-2-yl]methoxy]phenoxyphosphoryl]-l-alaninate (chloro analogue) (process related impurity).

D. 1-[(2R,3R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyloxolan-2-yl]pyrimidine-2,4(1H,3H)-dione (fluorouridine) (process related impurity and degradation product).

E. Ethyl N-[(S)-[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy]phenoxyphosphoryl]-L-alaninate (ethyl analogue) (process related impurity).

F. Pentafluorophenol (process related impurity).

Reference substances invoked

**Sofosbuvir RS**

*International Chemical Reference Substance (ICRS) to be established.*

**Sofosbuvir for peak identification RS (containing sofosbuvir and the impurities A, B, C, D and E)**

*International Chemical Reference Substance (ICRS) to be established.*

**Sofosbuvir Reference Spectrum**

Reference spectrum of sofosbuvir to be established.

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DOLUTEGRAVIR SODIUM
(DOLUTEGRAVIR NATRICUM)
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 SODIUM

DOLUTEGRAVIR NATRICUM

Molecular formula. \( C_{20}H_{18}F_{2}N_{3}NaO_{5} \)

Relative molecular mass. 441.37

Graphic formula.

\[
\begin{array}{c}
\text{CH}_3 \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{F} \\
\text{F} \\
\text{O} \\
\text{Na}^+ \\
\end{array}
\]

Chemical name. \((4R,12aS)-N-[(2,4-Difluorophenyl)methyl]-3,4,6,8,12,12a-hexahydro-7-hydroxy-4-methyl-6,8-dioxo-2H-pyrido[1′,2′:4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide sodium salt; CAS Reg. No. 1051375-19-9.\)

Description. A white to pale yellow powder.

Solubility. Slightly soluble in water R, and very slightly soluble in methanol R.

Category. Antiretroviral (integrase inhibitor).

Storage. Dolutegravir sodium should be kept in a tightly closed container.

Additional information. Dolutegravir sodium may exhibit polymorphism.

Definition. Dolutegravir sodium contains not less than 97.0% and not more than 102.0% (“Assay”, method A) or not less than 99.0% and not more than 101.0% (“Assay”, method B) of \( C_{20}H_{18}F_{2}N_{3}NaO_{5} \), calculated with reference to the anhydrous substance.
Identity tests

Either tests A and E or tests D and E together with any one of tests B or 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 dolutegravir sodium RS or with the reference spectrum of dolutegravir sodium. If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the substance to be examined and dolutegravir sodium RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from dolutegravir sodium RS.

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

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

C.1 Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6, or similar, as the coating substance and a mixture of 72 volumes of ethyl acetate R, 14 volumes of water R and 14 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 μL of each of the following two solutions in a mixture of 96 volumes of methanol R and 4 volumes of glacial acetic acid R containing (A) 1 mg of the substance to be examined per mL and (B) 1 mg of dolutegravir sodium RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

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

C.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under C.1 but using silica gel R5 as the coating substance. After drying the plate, spray with basic potassium permanganate (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).
D. The absorption spectrum (1.6) of a 10 µg per mL solution of the substance to be examined in methanol R, when observed between 220 nm and 400 nm, exhibits maxima at about 258 nm and 321 nm.

E. The test substance yields reaction A described under 2.1 General identification tests as characteristic of sodium.

**Sulfated ash (2.3).** Not more than 1.0 mg/g. Use a platin crucible for the determination.

**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.

**Water.** Determine, as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.3000 g of the substance and a mixture of 90 volumes of methanol R and 10 volumes of glacial acetic acid R as the solvent; the water content is not more than 10 mg/g.

**Impurity A (dolutegravir enantiomer) and impurity B (dolutegravir diastereomer).**

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

Carry out test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with cellulose tris (4-chloro-3-methylphenyl carbamate) (5 µm). As the mobile phase, use a mixture of 980 volumes of acetonitrile R, 40 volumes of water R and 2 volumes of phosphoric acid (~1440 g/L) TS. Operate at a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 258 nm. Maintain the column temperature at 25 °C. Prepare the following solutions using as the diluent a mixture of 50 volumes of acetonitrile R and 50 volumes of water R. For solution (1), dissolve 50.0 mg of the substance to be examined in 50.0 mL. For solution (2), dilute 5.0 mL of solution (1) to 100.0 mL. Dilute 3.0 mL of this solution to 100.0 mL. For solution (3), use a solution containing 1 mg of dolutegravir sodium for peak identification RS (containing dolutegravir sodium and the impurities A, B and D) per mL.

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7 A Lux Cellulose-4 column was found suitable.
Inject 15 µL of solution (3). Record the chromatogram for about 45 minutes.

The impurities are eluted at the following relative retentions with reference to dolutegravir (retention time about 22 minutes): impurity A about 0.75, impurity D about 1.25 and impurity B about 1.35.

The test is not valid unless the resolution factor between the peaks due to impurity D and due to impurity B is at least 1.5.

Inject alternately 15 µL of solutions (1) and (2).

In the chromatogram obtained with solution (1):
• the area of any peak corresponding to either impurity A or B is not greater than the area of the peak due to dolutegravir in the chromatogram obtained with solution (2) (0.15%).

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 groups (5 µm).

Use the following conditions for gradient elution:
• mobile phase A: 0.186 g of disodium edetate R in 1000 mL water R adjusted to pH 3.0 with phosphoric acid (~20g/L) TS; and
• 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>0–1</td>
<td>60</td>
<td>40</td>
<td>Isocratic</td>
</tr>
<tr>
<td>1–30</td>
<td>60 to 50</td>
<td>40 to 50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–40</td>
<td>50 to 30</td>
<td>50 to 70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>40–55</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>55–57</td>
<td>30 to 60</td>
<td>70 to 40</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>57–65</td>
<td>60</td>
<td>40</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

8 A Kinetex F5 column or an Ascentis Express F5 column were found suitable.
Operate at a flow rate of 0.8 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 258 nm. Maintain the column temperature at 25 °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), dissolve 35.0 mg of the substance to be examined and dilute to 50.0 mL. 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 50.0 mL. For solution (4), use a solution containing 0.5 mg of dolutegravir sodium for system suitability RS (containing dolutegravir sodium and impurity E) per mL. For solution (5), use a solution containing 1 mg of dolutegravir sodium for peak identification RS (containing dolutegravir sodium and the impurities A, B and D) per mL.

Inject alternately 10 µL each of solutions (1), (2), (3), (4) and (5).

Use the chromatogram obtained with solution (4) and the chromatogram supplied with dolutegravir sodium for system suitability RS to identify the peak due to impurity E. Use the chromatogram obtained with solution (5) and the chromatogram supplied with dolutegravir sodium for peak identification RS to identify the peak due to the impurity D.

The impurities, if present, are eluted at the following relative retentions with reference to dolutegravir (retention time about 27 minutes): impurity C about 0.65; impurity F about 0.72; impurity D about 0.77; impurities E about 0.86.

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution factor between the peaks due to impurity E and due to dolutegravir is at least 3. Also, the test is not valid unless in the chromatogram obtained with solution (3) the peak due to dolutegravir is obtained with a signal-to-noise ratio of at least 20.

In the chromatogram obtained with solution (1):
- the area of any peak corresponding to either impurities C, D, E or F is not greater than 1.5 times the area of the peak due to dolutegravir obtained with solution (3) (0.15%);
- the area of any other impurity peak is not greater than the area of the peak due to dolutegravir obtained with solution (3) (0.10%);
- the sum of the areas of all impurity peaks is not greater than the area of the peak due to dolutegravir obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the peak due to dolutegravir obtained with solution (3) (0.05%).
**Assay.** Perform the assay in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

- Either method A or method B may be applied.
  
  **A.** Carry out 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 groups (5 µm).9

  Use the following mobile phase: Dissolve 0.186 g of disodium edetate R in 1000 mL water R and adjust to pH 3.0 with phosphoric acid (~20g/L) TS. Mix 450 volumes of this solution with 550 volumes of methanol R.

  Operate at a flow rate of 1.0 mL/minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 258 nm. Maintain the column at a temperature of 30 °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), dissolve 50.0 mg of the substance to be examined and dilute to 100.0 mL. Dilute 5.0 mL of this solution to 50.0 mL. For solution (2), dissolve 50.0 mg of dolutegravir sodium RS and dilute to 100.0 mL. Dilute 5.0 mL of this solution to 50.0 mL.

  Inject alternately 20 µL each of solutions (1) and (2). Record the chromatograms for about 20 minutes.

  Measure the areas of the peaks corresponding to dolutegravir obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of dolutegravir sodium (C₂₀H₁₈F₂N₃NaO₅) using the declared content of C₂₀H₁₈F₂N₃NaO₅ in dolutegravir sodium RS.

  **B.** Dissolve about 0.300 g of the substance to be examined in 30 mL of anhydrous acetic acid R and titrate with perchloric acid (0.1 mol/L) VS as described under *2.6 Non-aqueous titration, Method A*. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 44.14 mg of C₂₀H₁₈F₂N₃NaO₅.

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9 A Kinetex F5 column or an Ascentis Express F5 column were found suitable
Impurities

A. (4S,12aR)-N-[(2,4-Difluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido[1’,2’:4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide (dolutegravir enantiomer) (synthesis-related impurity).

B. (4R,12aR)-N-[(2,4-difluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido[1’,2’:4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide (dolutegravir diastereomer) (synthesis-related impurity).


E. (4R, 12αS)-N-[(4-fluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12α-
hexahydro-2H-pyrido[1′, 2′:4,5]pyrazino-[2,1-b][1,3] oxazine-9-carboxamide, 4-Fluoro
dolutegravir (synthesis-related impurity).

F. (4R, 12αS)-N-[(2,6-difluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-
3,4,6,8,12,12α-hexahydro-2H-pyrido[1′, 2′:4,5]pyrazino-[2,1-b][1,3] oxazine-9-
carboxamide, 2,6-Difluoro dolutegravir (synthesis-related impurity).

Reference substances invoked

Dolutegravir sodium RS.
International Chemical Reference Substance (ICRS) to be established.

Dolutegravir sodium for peak identification RS (containing dolutegravir sodium and
impurities A, B and D)
ICRS to be established.

Dolutegravir sodium for system suitability RS (containing dolutegravir sodium and
impurity E)
ICRS to be established.

***
DOLUTEGRAVIR TABLETS

DOLUTEGRAVIR COMPRESSI

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.

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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 TABLETS

DOLUTEGRAVIR COMPRESSI

Category. Antiretroviral (integrase inhibitor).

Storage. Dolutegravir tablets should be kept in a well-closed container.

Labelling. The designation of the container should state that the active ingredient is the sodium salt and the quantity should be indicated in terms of the equivalent amount of dolutegravir.


Requirements. Comply with the monograph for Tablets.

Definition. Dolutegravir tablets contain Dolutegravir sodium. They contain not less than 90.0% and not more than 110.0% of the amount of dolutegravir (C_{20}H_{19}F_{2}N_{3}O_{5}) stated on the label.

Identity tests

- Either test A or test B may be applied.
  A. Carry out test A.1, or where a diode array detector is available, test A.2.
  A.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 dolutegravir in the chromatogram obtained with solution (2).

  To a quantity of the powdered tablets, nominally equivalent to 10 mg dolutegravir, add 40 mL methanol R, sonicate for five minutes, allow to cool to room temperature, dilute to 50 mL and filter. Dilute 1 mL of the filtrate to 20 mL with methanol R. The absorption spectrum (I.6) of the resulting solution, when observed between 220 nm and 400 nm, exhibits maxima at about 258 nm und 321 nm.
A.2 Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions and solutions given under “Assay”. Record the UV spectrum of the principle peak in the chromatograms with a diode array detector in the range of 220 and 400 nm. 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 spectrum of the peak due to dolutegravir in the chromatogram obtained with solution (2).

B. Carry out test B.1 or, where UV detection is not available, test B.2.
B.1 Carry out test as described under 1.14.1 Thin-layer chromatography using silica gel R6, or similar, as the coating substance and a mixture of 72 volumes of ethyl acetate R, 14 volumes of water R and 14 volumes of glacial acetic acid R as the mobile phase. Prepare as a solvent solution a mixture of 96 volumes of methanol R and 4 volumes of glacial acetic acid R. Apply separately to the plate 5 μL of each of the following two solutions. For solution (A), shake a quantity of the powdered tablets containing 10 mg of dolutegravir with 10 mL of the solvent solution and filter. For solution (B), use a solution containing 1 mg of dolutegravir sodium RS per mL solvent solution. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

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

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under text A.1 but using silica gel R5 as the coating substance. After drying the plate spray with basic potassium permanganate (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).

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 2.5 g of sodium dodecyl sulfate R in 1000 mL dissolution buffer pH 6.8. Rotate the paddle at 50 revolutions per minute. At 20 minutes withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered solution to cool down to room temperature and dilute 5.0 mL of to 10.0 mL with dissolution medium. Use this solution as solution (1). For solution (2), dissolve a suitable amount of dolutegravir sodium RS in dissolution medium and dilute to a suitable volume with the same solvent.
Measure the absorbance as described under 1.6 Spectrophotometry in the visible and ultraviolet regions of a 1 cm layer of the resulting solutions at the maximum at about 258 nm, using the dissolution medium as the blank.

For each of the tablets tested, calculate the amount of dolutegravir (C₂₀H₁₉F₂N₃O₅) in the medium. 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 dolutegravir in solution for each tablet is not less than 80 (Q) of the amount declared on the label.

[Note from the Secretariat. It is intended to determine the absorptivity value of dolutegravir during the establishment of dolutegravir sodium RS and to use this value for the calculation of the test result.]

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 groups (5 µm).¹⁰

Use the following conditions for gradient elution:

- mobile phase A: 0.186 g disodium edetate R in 1000 mL water R adjusted to pH 3.0 with phosphoric acid (~20 g/L) TS; and
- 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>
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<tbody>
<tr>
<td>0–1</td>
<td>60</td>
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<td>Isocratic</td>
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<td>Linear gradient</td>
</tr>
<tr>
<td>30–40</td>
<td>50 to 30</td>
<td>50 to 70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>40–55</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>55–57</td>
<td>30 to 60</td>
<td>70 to 40</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>57–65</td>
<td>60</td>
<td>40</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

¹⁰ A Kinetex F5 column or an Ascentis Express F5 column were found suitable.
Operate at a flow rate of 0.8 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 258 nm. Maintain the column temperature at 25 °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 70.0 mg dolutegravir, to a 100 mL volumetric flask. Add about 70 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 impurities A, B and D) per mL.

Inject alternately 10 µL of solutions (1), (2), (3) and (4).

Use the chromatogram obtained with solution (3) and the chromatogram supplied with dolutegravir sodium for system suitability RS to identify the peak due to the impurity E. Use the chromatogram obtained with solution (4) and the chromatogram supplied with dolutegravir sodium for peak identification RS to identify the peak due to the impurity D.

The impurities, if present, are eluted at the following relative retentions with reference to dolutegravir (retention time about 27 minutes): impurity C about 0.65; impurity F about 0.72; impurity D about 0.77; impurities E about 0.86.

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. Also, the test is not valid unless in the chromatogram obtained with solution (2) the peak due to dolutegravir is obtained with a signal-to-noise ratio of at least 20.

In the chromatogram obtained with solution (1):
- the area of any impurity peak is not greater than the area of the peak due to dolutegravir in the chromatogram obtained with solution (2) (0.2%).
- The sum of the areas of all impurity peaks is not greater than 5 times the area of the peak due to dolutegravir in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the peak due to dolutegravir in the chromatogram obtained with solution (2) (0.1%).
**Assay.** Perform the test in subdued light and without any prolonged interruptions, preferably using low-actinic glassware. Carry out 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 pentafluorophenyl groups (5 µm).  

Use the following mobile phase: Dissolve 0.186 g of disodium edetate R in 1000 mL water R and adjust to pH 3.0 with phosphoric acid (~20g/L) TS. Mix 450 volumes of this solution with 550 volumes of methanol R.

Operate at a flow rate of 1.0 mL/minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 258 nm. For identity test A.2 use a diode array detector in the range of 220 nm to 400 nm. Maintain the column at a temperature of 30 °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), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally equivalent to 100.0 mg of dolutegravir, to a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate for five minutes, cool to room temperature and make up to volume with diluent. Filter and dilute 5.0 mL of the filtrate to 100.0 mL. For solution (2), dissolve 55.0 mg of dolutegravir sodium RS and dilute to 50.0 mL. Dilute 5.0 mL of this solution to 100.0 mL.

Inject alternately 20 µL each of solutions (1) and (2). Record the chromatogram for about 20 min.

Measure the areas of the peaks corresponding to dolutegravir obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of dolutegravir (C₂₀H₁₈F₂N₃O₅) in the tablets using the declared content of C₂₀H₁₈F₂N₃NaO₅ in dolutegravir sodium 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 monograph on Dolutegravir sodium, excluding impurity A and B.

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11 A Kinetex F5 column or an Ascentis Express F5 column were found suitable.
Reference substances invoked

**Dolutegravir sodium RS.**

International Chemical Reference Substance (ICRS) to be established.

**Dolutegravir sodium for peak identification RS** (containing dolutegravir sodium and impurities A, B and D)

ICRS to be established.

**Dolutegravir sodium for system suitability RS** (containing dolutegravir sodium and impurity E)

ICRS to be established.

***
DOLUTEGRAVIR, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE TABLETS

(DOLUTEGRAVIRI, LAMIVUDINE ET TENOFOVIRI DISOPROXILI FUMARATI COMPRESSI)

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 Tenofovirii 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 ($C_{20}H_{19}F_2N_3O_5$), lamivudine ($C_8H_{11}N_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P$, $C_6H_{24}O_4$) 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 (C\textsubscript{20}H\textsubscript{33}F\textsubscript{2}NaO\textsubscript{3}) in dolutegravir sodium RS, the declared content of lamivudine (C\textsubscript{8}H\textsubscript{11}N\textsubscript{3}O\textsubscript{3}S) in lamivudine RS and the declared content of 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}) 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 (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}) released after 30 minutes is not less than 80% (Q) of the amounts declared on the label and the amount of dolutegravir (C\textsubscript{20}H\textsubscript{33}F\textsubscript{2}NaO\textsubscript{3}) 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).\textsuperscript{12}

Use the following conditions for gradient elution:

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

\textsuperscript{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</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>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>Degradaent</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 octadecysilyl 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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TENOFOVIR DISOPROXIL FUMARATE

(TENOFOVIRI DISOPROXILI FUMARAS)

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”.


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.
TENOFOVIR DISOPROXIL FUMARATE

(TENOFOVIRI DISOPROXILI FUMARAS)

Graphic formula

Molecular formula. \( C_{19}H_{30}N_5O_{10}P.C_4H_4O_4 \)

Relative molecular mass. 635.5.

Chemical names. bis(1-methylethyl) 5-\(((1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy)methyl\)-5-oxo-2,4,6,8-tetraoxa-5-\(\lambda_5\)-phosphanonanedioate (ester) hydrogen \((2E)\)-but-2-enedioate (salt); bis\(((1-methylethoxy)carbonyl)oxy)methyl\) \(((1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy)methyl\)phosphonate (ester) hydrogen \((2E)\)-but-2-enedioate (salt); 5-\(((1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy)methyl\)-2,4,6,8-tetraoxa-5-phosphanonanedioic acid 1,9-bis(1-methylethyl) ester 5-oxide, \((2E)\)-2-butenedioate (1:1); CAS Reg. No. 202138 50 9

Description. White to almost-white, crystalline powder.

Solubility. Slightly soluble in water R, soluble in methanol R, very slightly soluble in dichloromethane R.

Category. Antiretroviral (Nucleotide Reverse Transcriptase Inhibitor).

Storage. Tenofovir disoproxil fumarate should be kept in a tightly closed container, protected from light and stored at a temperature between 2–8 °C.

Additional information. Tenofovir disoproxil fumarate may exhibit polymorphism.
Requirements

Definition. Tenofovir disoproxil fumarate contains not less than 98.5% and not more than 101.0% of tenofovir disoproxil fumarate ($\text{C}_{19}\text{H}_{30}\text{N}_{5}\text{O}_{10}\text{P}.\text{C}_{4}\text{H}_{4}\text{O}_{4}$), calculated with reference to the anhydrous substance.

Manufacture. The production method is validated to ensure that the substance, if tested, would comply with a limit of not more than 5 ppm for the mutagenic impurity 9-propenyladenine (impurity K), which may be a synthesis-related substance, using a suitable method.

- a limit of not more than 1.0% for the tenofovir disoproxil (S)-enantiomer (impurity G) using a suitable chiral chromatographic method.

Identity tests

Either tests A, B and C or test D 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 5 μL of each of two solutions in methanol containing (A) 10 mg of the test substance per mL and (B) 10 mg of tenofovir disoproxil fumarate 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 with 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. Stain the plate with iodine vapour and 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. 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 50 volumes of heptane R, 30 volumes of glacial acetic acid R and 20 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 μL of each of the following two solutions in ethanol R. For solution (A), use 10 mg of the test substance per mL and for solution (B), use 2 mg of fumaric acid R per mL. Develop the plate in an unsaturated tank over a path of 10 cm. 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).

One of the principal spots obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under test B.1 but using silica gel R5 as the coating substance. Spray lightly with a 16 g/L solution of potassium permanganate R and examine the chromatogram in daylight.

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

C. The absorption spectrum \((L_0)\) of a 25 µg/mL solution, when observed between 220 nm and 320 nm, exhibits a maximum at about 261 nm; the specific absorbance \(A_{\%}\) is 230 to 250.

D. 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 tenofovir disoproxil fumarate RS or with the reference spectrum of tenofovir disoproxil fumarate.

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

Specific optical rotation (1.4). Prepare a fresh solution and perform the test without delay. Use a 10.0 mg/mL solution in hydrochloric acid (0.1 mol/l) VS and calculate with reference to the anhydrous substance; \([\alpha]_D^{20} = -15 \text{ to } -20\).
**Water.** Determine as described under **2.8 Determination of water by the Karl Fischer method**, Method A. Use about 1.0 g of the substance; the water content is not more than 10 mg/g.

**Heavy metals.** Use 1.0 g in 30 mL of methanol R for the preparation of the test solution as described under **2.2.3 Limit test for heavy metals**, Procedure 2; determine the heavy metals content according to Method A; not more than 20 μg/g.

**Sulfated ash (2.3).** Not more than 2.0 mg/g.

**Impurity G.** Carry out test as described under **1.14.4 High-performance liquid chromatography** using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with amylose tris (3,5-dimethylphenyl carbamate) (5 μm). 15 As the mobile phase, use a mixture of 949 volumes of methanol R, 50 volumes of acetonitrile R and 1 volume of triethylamine R. Operate at a flow rate of 0.8 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm. Maintain the column temperature at 20 °C. Prepare the following solutions using mobile phase as the diluent. For solution (1), dissolve 40.0 mg of the test substance in 100.0 mL. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3), use a solution containing 0.4 mg of tenofovir disoproxil fumarate for epimer identification RS (containing tenofovir disoproxil and the impurities G) per mL. Inject 5 μL of solution (3). Record the chromatogram for about 15 minutes.

Impurity G is eluted at the relative retention of 1.6 with reference to tenofovir disoproxil (retention time about 5 minutes).

The test is not valid unless the resolution factor between the peaks due to impurity G and due to tenofovir disoproxil is at least 3.

Inject alternately 5 μL each of solutions (1) and (2).

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15 A Nucleocel Alpha-RP S or a Chiralpak AD-H column were found suitable.
In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity G is not greater than the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) (1.0%).

**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 base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecysilyl groups (5 μm).

The mobile phases for the gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions:

- Mobile phase A: 2 volumes of acetonitrile R, 20 volumes of phosphate buffer pH 6.0 and 78 volumes of water R; and
- Mobile phase B: 65 volumes of acetonitrile R, 20 volumes of phosphate buffer pH 6.0 and 15 volumes of water R.

Prepare the phosphate buffer pH 6.0 by dissolving 3.50 g of potassium dihydrogen phosphate R and 1.70 g of tetrabutyl ammonium hydrogen sulfate R in 800 mL of water R, adjust the pH to 6.0 by adding sodium hydroxide (1 mol/L) VS 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>0–5</td>
<td>81</td>
<td>19</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5–40</td>
<td>81–49</td>
<td>19–51</td>
<td>Linear gradient</td>
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<tr>
<td>40–60</td>
<td>49–0</td>
<td>51–100</td>
<td>Linear gradient</td>
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<tr>
<td>60–65</td>
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<td>Isocratic</td>
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<tr>
<td>65–70</td>
<td>0–81</td>
<td>100–19</td>
<td>Return to initial composition</td>
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<tr>
<td>70–80</td>
<td>81</td>
<td>19</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

After preparation, keep the solutions at about 6 °C or use an injector with cooling.

Prepare the following solutions using water R as diluent. For solution (1), use 1.0 mg of the test substance per mL. For solution (2), dilute a suitable volume of solution (1) to obtain a concentration of 5 µg of tenofovir disoproxil fumarate per mL. For solution (3), use 0.2 mg of fumaric acid R per mL.
For the system suitability test, prepare solution (4) by heating solution (1) carefully in a boiling water-bath for 20 minutes.

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 30 °C.

Inject 20 μL of solution (4). The test is not valid unless the resolution between the principal peak (retention time about 40 minutes) and the peak due to tenofovir monosoproxil (impurity A) (with a relative retention of about 0.5) is not less than 25.

Inject alternatively 20 μL each of solutions (1) and (2) and (3). In the chromatogram obtained with solution (1), the following peak is eluted at the following relative retention, with reference to tenofovir (retention time about 40 minutes): fumarate about 0.15.

In the chromatogram obtained with solution (1):
- the area of any peak due to tenofovir monosoproxil (impurity A) is not greater than twice the area of the principal peak obtained with solution (2) (1.0%);
- the area of any other impurity peak is not greater than the area of the principal peak obtained with solution (2) (0.5%), and
- the areas of not more than two such peaks are greater than 0.4 times the area of the principal peak obtained with solution (2) (0.2%).
- The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (2.5%). Disregard any peak corresponding to the peak obtained in the chromatogram with solution (3) and 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 0.40 g, accurately weighed, in 30 mL of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/l) VS is equivalent to 63.55 mg of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P$, $C_4H_4O_4$).
Impurities


B. (1-methyllethyl) (5RS,8R)-9-(6-amino-9H-purin-9-yl)-5-methoxy-8-methyl-5-oxo-2,4,7-trioxa-5-λ₅-phosphanonanoate.

C. Methyl (1-methylethyl) (5RS)-5-[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy)methyl]-5-oxo-2,4,6,8-tetraoxa-5-λ₅-phosphanonanedioate, (synthesis-related impurity).

E. (1-methylethyl) \((8R)\)-5-hydroxy-8-methyl-9-(6-\{[(1-methylethoxy)carbonyl]amino\}-9\text{H}-purin-9-yl)-5-oxo-2,4,7-trioxa-5-\(\lambda_5\)-phosphananoate, (synthesis-related impurity, degradation product).

F. Bis(1-methylethyl) 9,9'-[methylenebis(imino-9\text{H}-purine-6,9-diyl)]bis[(8R)-5-hydroxy-8-methyl-5-oxo-2,4,7-trioxa-5-\(\lambda_5\)-phosphananoate] (tenofovir monosoproxil dimer), (degradation product).

G. Bis(1-methylethyl) 5-\{[(1,5)-2-(6-amino-9\text{H}-purin-9-yl)-1-methylethoxy]methyl\}-5-oxo-2,4,6,8-tetraoxa-5-\(\lambda_5\)-phosphananedioate (tenofovir disoproxil (S)-enantiomer) [see under Manufacture].
H. 1-methylethyl propyl (5RS)-5-[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-5-oxo-2,4,6,8-tetraoxa-5-λ5-phosphanonanedioate, (synthesis-related impurity).

I. Bis(1-methylethyl) 5-[(1R)-2-(6-[[[(9-[(2R)-5-hydroxy-2,11-dimethyl-5,9-dioxo-3,6,8,10-tetraoxa-5-λ5-phosphadodecyl]-9H-purin-6-yl]amino)methyl]amino]-9H-purin-9-yl]-1-methylethoxy]methyl]-5-oxo-2,4,6,8-tetraoxa-5-λ5-phosphanonanedioate (tenofovir di- and monosoproxil heterodimer), (synthesis-related impurity, degradation product).
J. Tetrakis(1-methylethyl) 5,5'-((methylenebis(imino-9H-purine-6,9-diyl)(2R)-propane-1,2-diyloxy)methylene)bis[5-oxo-2,4,6,8-tetraoxa-5-\lambda^5-phosphanonanedioate] (tenofovir disoproxil dimer), (synthesis-related impurity, degradation product).

K. 9-(prop-1-enyl)-9H-purin-6-amine, [see under Manufacture],

and epimer at P

K\text{.} (1-methylethyl) (5RS)-5-\{(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy\}methyl\}-10-methyl-5,9-dioxo-2,4,6,8-tetraoxa-10-aza-5-\lambda^5-phosphaundecanoate, (synthesis-related impurity).
**LM.** Ethyl 1-methylethyl (5RS)-5-\{[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl\}-5-oxo-2,4,6,8-tetraoxa-5-\(\lambda^5\)-phosphanonanedioate (synthesis-related impurity, degradation product).

**MN.** 9-\{(R)-2-(Phosphonomethxy)propyl\}adenine (synthesis-related impurity, degradation product).

*[Note from the Secretariat. The structure of impurity M will be added at a later stage.]*

**Reference substances invoked**

**Tenofovir disoproxil fumarate RS.**

Established International Chemical Reference Substance (ICRS).

**Tenofovir disoproxil fumarate for epimer identification RS** (containing tenofovir disoproxil fumarate and impurities G)

ICRS to be established.
PROPOSAL TO DISCONTINUE THE TEST FOR UNDUE TOXICITY

(CHAPTER 3.7) 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 10 October 2019.

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

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PROPOSAL TO DISCONTINUE THE TEST FOR UNDUE TOXICITY
(CHapter 3.7) IN THE INTERNATIONAL PHARMACOPOEIA

At its Sixty-ninth meeting, held from 29 October to 02 November 2018, the World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) recommended to discontinue the inclusion of the innocuity test in future WHO documents on vaccines and other biologicals to be published in the Technical Report Series (TRS) (including WHO recommendations, guidelines and manuals). In addition, the inclusion of this test in previously published WHO TRS documents should be disregarded:

The scientific rationale and evidence for performing the innocuity test (also called the “abnormal toxicity test” or “general safety test”) as a measure of the safety of vaccines and other biological products, for the purpose of marketing authorization and lot release, were discussed by the Expert Committee. Current manufacturing processes, which include the implementation of Good Manufacturing Practices (GMP) and comprehensive quality control measures (including in-process controls), were considered to be more appropriate than the innocuity test in assuring the quality and safety of vaccines and other biological products. The Expert Committee reviewed the historical inclusion of the innocuity test in the documents published in the WHO TRS and concluded that its complete omission would not compromise the quality and safety of vaccines and other biological products. Therefore, the Expert Committee recommends the discontinuation of the inclusion of the innocuity test in all future WHO recommendations, guidelines and manuals for biological products published in the TRS, and that a clear indication be made in its report that the inclusion of this test in previously published WHO TRS documents be disregarded.16

The principle of the test consists of injecting the product under investigation into guinea pigs and/or mice. The sample passes the test if no animal shows any signs of illness, relevant body weight changes or dies within a certain period. The exact test design and name varies between the different pharmacopoeias and requirements.

In The International Pharmacopoeia, the test is referred to as the test for undue toxicity (chapter 3.7) and stipulated in the monographs on Kanamycin acid sulfate and Kanamycin monosulfate.

16 Text reproduced from main outcomes of the meeting of the WHO Expert Committee on Biological Standardization held from 29 October to 2 November 2018 (https://www.who.int/biologicals/expert_committee/ECBS_Executive_Summary_final_20 NOV_2018.IK.pdf?ua=1).
Following the decision of the WHO Expert Committee on Biological Standardization (ECBS), it is proposed to omit chapter 3.7, “Undue Toxicity” in the *International Pharmacopoeia* and its reference in the monographs on Kanamycin acid sulfate and Kanamycin monosulfate (see below: changes from the current text/monographs are indicated by insert or delete.). Users of *The International Pharmacopoeia* are invited to provide their comments on this proposal.

### 3.7 Undue toxicity

The test is used to determine the absence of undue toxicity of antibiotics intended for parenteral administration.

**Recommended procedure**

Use healthy mice of a single strain that have not previously been used for any test. Select 5 mice, each weighing between 18 g and 22 g. Prepare the solution of the test substance as specified in the monograph. Inject a test dose of 0.5 mL intravenously into a tail vein at a uniform rate, the injection occupying 5 seconds. Keep the mice under observation for 48 hours after the injection. The product meets the requirements for freedom from undue toxicity if no animal dies within 48 hours.

If 1 or 2 mice die within the observation period, repeat the procedure once, using respectively 5 or 15 mice, healthy and not previously used for any test, each weighing between 19.5 g and 20.5 g. The product under test meets the requirements for freedom from undue toxicity if no animal dies in the repeat test within the observation period (48 hours).

**Monograph on Kanamycin acid sulfate**

**Manufacture**

Kanamycin acid sulfate is produced by methods of manufacture designed to eliminate or minimize substances lowering blood pressure. The method of manufacture is validated to demonstrate that the product, if tested, would comply with the test as described under 3.7 Undue toxicity, using 0.5 mL of a solution containing 2 mg per mL.
Monograph on Kanamycin monosulfate

Manufacture

Kanamycin monosulfate is produced by methods of manufacture designed to eliminate or minimize substances lowering blood pressure. The method of manufacture is validated to demonstrate that the product, if tested, would comply with the test as described under 3.7 Undue toxicity, using 0.5 mL of a solution containing 2 mg per mL.
GUIDELINE ON DATA INTEGRITY

(October 2019)

DRAFT FOR COMMENTS

Please send any comments you may have to Dr Sabine Kopp, Group Lead, Medicines Quality Assurance, Technologies Standards and Norms (email: kopps@who.int) with a copy to Ms Claire Vogel (vogelc@who.int) by 15 January 2020.

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.

The new text on data integrity is intended to replace the following:

Guidance on good data and record management practices
GUIDELINE ON DATA INTEGRITY

1. INTRODUCTION AND BACKGROUND

1.1. Data governance and data integrity (DI) are important elements in ensuring the reliability of data and information obtained in production and control of pharmaceutical products. The data and information should be complete as well as being attributable, legible, contemporaneous, original and accurate, commonly referred to as meeting “ALCOA” principles.

1.2. In recent years, the number of observations made regarding the integrity of data, documentation and record management practices during inspections of good manufacturing practice (GMP), good clinical practice (GCP) and good laboratory practice (GLP) has been increasing. Possible causes for this may include (i) too much reliance on human practices; (ii) the use of computerized systems that are not appropriately managed and validated; and (iii) failure to adequately review and manage original data and records.

1.3. Quality risk management (QRM), control strategies and sound scientific principles are required to mitigate such risks. Examples of controls may include, but are not limited to:

- the establishment and implementation of a DI policy;
- the establishment and implementation of procedures that will facilitate compliance with DI requirements and expectations;
- adoption of a quality culture within the company that encourages personnel to be transparent about failures which includes a reporting mechanism;
- application of QRM with identification of all areas of risk to DI through data integrity risk assessment (DIRA) and implementation of appropriate controls to eliminate or reduce risks to an acceptable level throughout the life cycle of the data;
- ensuring sufficient resources to monitor compliance with DI policies and procedures and processes, and facilitate continuous improvement;
- provision of necessary training for personnel in, for example, good practices (GXP), computerized systems and DI;
- implementation and validation of computerized systems appropriate for their intended use;
- definition and management of appropriate roles and responsibilities for quality agreements and contracts entered into by contract givers and contract acceptors.
2. SCOPE

2.1. This guideline provides information, guidance and recommendations to facilitate compliance with DI, GXP in documentation and record keeping requirements.

2.2. The scope of this guideline is designated as ‘GXP’. It does not, however, cover medical devices.

2.3. Where possible, this guideline has been harmonised with other published documents. The guideline should be read with other WHO GXP guidelines and publications.

2.4. In line with the current approach in GMP, it recommends a risk-based approach over the life cycle of data. DIRA should be carried out in order to identify and assess areas of risk.

2.5. The principles of this guideline apply to contract givers and contract acceptors. Contract givers are ultimately responsible for the integrity of data provided to them by contract acceptors. Contract givers should therefore ensure that contract acceptors comply with the principles contained in this guideline.

2.6. Efficient risk-based controls and review of data and documents should be identified and implemented. The effectiveness of the controls should be verified.

3. GLOSSARY

(Note: This section will be updated)

The definitions given below apply to the terms used in these guidelines. They may have different meanings in other contexts.

**ALCOA.**
A commonly used acronym for “attributable, legible, contemporaneous, original and accurate”.

**ALCOA+.**
A commonly used acronym for “attributable, legible, contemporaneous, original and accurate” which puts additional emphasis on the attributes of being complete, consistent, enduring and available – implicit basic ALCOA principles.
**Archiving, archival.**
Archiving is the process of storage and protecting records from the possibility of being accessed, further altered or deleted, and storing these records under the control of independent data management personnel throughout the required retention period. Archived records should include, for example, associated metadata and electronic signatures.

**Archivist.**
An independent individual designated in GLP who has been authorized by management to be responsible for the management of the archive, i.e. for the operations and procedures for archiving.

**Audit trail.**
The audit trail is a form of metadata containing information associated with actions that relate to the creation, modification or deletion of GXP records. An audit trail provides for secure recording of life cycle details such as creation, additions, deletions or alterations of information in a record, either paper or electronic, without obscuring or overwriting the original record. An audit trail facilitates the reconstruction of the history of such events relating to the record regardless of its medium, including the “who, what, when and why” of the action.

**Data governance.**
The arrangements to ensure that data, irrespective of the format in which they are generated, are recorded, processed, retained and used to ensure the record throughout the data life cycle.

**Data life cycle.**
All phases of the process by which data are created, recorded, processed, modified, transmitted, reviewed, reported, used, approved, archived and restored until destruction.

**Electronic signatures.**
A signature in digital form (bio-metric or non-biometric) that represents the signatory. This should be equivalent in legal terms to the handwritten signature of the signatory.

**Good practices (GXP).**
Acronym for the group of good practice guides governing the preclinical, clinical, manufacturing, testing, storage, distribution and post-market activities for regulated pharmaceuticals, biologicals and medical devices, such as GLP, GCP, GMP, good pharmacovigilance practices (GPP) and good distribution practices (GDP).
**metadata.**
Metadata are data that describe the attributes of other data and provide context and meaning and form an integral part of original records. An audit trail record is an example of metadata.

**raw data (source data).**
The original record (data) which can be described as the first-capture of information, whether recorded on paper or electronically.

**routine data review.**
Routine data review is a process where the raw data and metadata are reviewed for their integrity in an individual data set.

**periodic data review.**
Periodic data review is a process where an audit of the data generated is done, on a periodic basis (e.g. monthly), where data are selected on a random basis to verify the effectiveness of existing control measures and identification of the possibility of unauthorised activity at all interfaces.

### 4. PRINCIPLES OF DATA INTEGRITY AND GOOD DOCUMENTATION PRACTICES

4.1. There should be a written DI policy.

4.2. Senior management is responsible for the establishment and implementation of an effective quality system and a data governance system. This applies to paper and electronic generated data.

4.3. Data should be Attributable, Legible, Contemporaneous, Original, and Accurate (ALCOA) and be Complete, Consistent, Enduring, and Available (+). This is generally referred to as ALCOA+. (There is no difference in expectations regardless of which acronym is used).

4.4. The quality system, including documentation such as procedures and formats for recording data, should be appropriately designed and implemented to provide assurance that records and data meet the principles contained in this guideline.

4.5. Data governance should address data ownership and accountability throughout the life cycle and consider the design, operation and monitoring of processes/systems to comply with the principles of DI, including control over intentional and unintentional changes to data.
4.6. Data governance systems should include:

- training in the importance of DI principles;
- the creation of an appropriate working environment; and
- active encouragement of the reporting of errors, omissions and undesirable results.

4.7. Senior management should be accountable for the implementation of systems and procedures in order to minimise the potential risk to DI, and to identify the residual risk using risk management techniques such as the principles of the International Conference on Harmonisation (ICH) Q9.

4.8. The data governance programme should include policies and procedures addressing data management. Elements of effective management governance should include:

- management oversight and commitment;
- application of QRM;
- good data management principles;
- quality metrics and performance indicators;
- validation;
- change management;
- security and access control;
- configuration control;
- prevention of commercial, political, financial and other organizational pressures;
- prevention of incentives that may adversely affect the quality and integrity of work;
- adequate resources, systems;
- workload and facilities to facilitate the right environment that supports DI and effective controls;
- monitoring;
- record keeping;
- training; and
- awareness of the importance of DI, product quality and patient safety.

4.9. There should be a system for the regular review of documents and data to identify any DI failures. This includes paper records and electronic records in day-to-day work, system and facility audits and self-inspections.
4.10. The effort and resources applied to assure the integrity of the data should be commensurate with the risk and impact of a DI failure.

4.11. Where DI weaknesses are identified, appropriate corrective and preventive actions (CAPA) should be implemented across all relevant activities and systems and not in isolation.

4.12. Significant DI lapses identified should be reported to the national medicine regulatory authority.

4.13. Changing from automated or computerised systems to paper-based manual systems or vice-versa will not in itself remove the need for appropriate DI controls.

4.14. Good documentation practices should be followed to ensure that all records are complete.

4.15. Records (paper and electronic) should be kept in a manner that ensures compliance with the principles of this guideline. These include, but are not limited to:

- restricting the ability to change dates and times for recording events;
- using controlled documents and forms for recording GXP data;
- controlling the issuance of blank paper templates for data recording of GXP activities, with reconciliation;
- defining access and privilege rights to automated systems;
- enabling audit trails;
- having automated data capture systems and printers connected to equipment and instruments in production and quality control where possible;
- ensuring proximity of printers to sites of relevant activities; and
- ensuring access to original electronic data for personnel responsible for reviewing and checking data.

4.16. Data and recorded media should be durable. Ink should be indelible. Temperature-sensitive or photosensitive inks and other erasable inks should not be used, or other means should be identified to ensure traceability of the data over their life cycle.

4.17. Paper should not be temperature-sensitive, photosensitive or easily oxidizable. If this is not feasible or limited, then true or certified copies should be available.

4.18. Systems, procedures and methodology used to record and store data should be periodically reviewed for effectiveness and updated, as necessary, in relation to new technology.
5. **QUALITY RISK MANAGEMENT**

5.1. The DIRA should be documented. This should cover systems and processes that produce data or, where data are obtained, data criticality and inherent risks.

5.2. The risk assessment should include, for example, computerised systems, supporting personnel, training and quality systems.

5.3. Record and DI risks should be assessed, mitigated, communicated and reviewed throughout the document and data life cycle.

5.4. Where the DIRA has highlighted areas for remediation, prioritisation of actions (including acceptance of an appropriate level of residual risk) and controls should be documented and communicated. Where long-term remediation actions are identified, risk-reducing short-term measures should be implemented to provide acceptable data governance in the interim.

5.5. Controls identified may include organizational and functional controls such as procedures, processes, equipment, instruments and other systems to both prevent and detect situations that may impact on DI. (Examples include appropriate content and design of procedures, formats for recording, access control, the use of computerized systems and other means).

5.6. Controls should cover risks to data. Risks include deletion of, changes to, and excluding data and results from data sets without written authorisation and detection of those activities and events.

6. **MANAGEMENT REVIEW**

6.1. Compliance with DI policy and procedures should be reported in the periodic management review meetings.

6.2. The effectiveness of the controls implemented should be measured against the quality metrics and performance indicators. These should include for example:

- The tracking and trending of data;
- lapse in DI rates;
- review of audit trails in, for example, production, quality control, GLP, case report forms and data processing;
- routine audits and/or self-inspections including DI and computerized systems; and
- DI lapses at outsourced facilities (contract acceptors).
7. **OUTSOURCING**

7.1. Outsourcing of activities and responsibilities of each party (contract giver and contract accepter) should be clearly described in written agreements. Specific attention should be given to ensuring compliance with DI requirements.

7.2. Compliance with the principles and responsibilities should be verified during periodic site audits. This should include the review of procedures and data (including raw data and metadata, paper records, electronic data, audit trails and other related data) held by the contracted organization that are relevant to the contract giver’s product or services.

7.3. Where data and document retention are contracted to a third party, particular attention should be paid to understanding the ownership and retrieval of data held under that agreement, as well as controls to ensure the integrity of data over their life cycle.

7.4. No activity, including outsourcing databases, should be sub-contracted to a third party without the prior approval of the contract giver.

7.5. All contracted parties should be aware of the requirements relating to data governance, DI and data management.

8. **TRAINING**

8.1. Personnel should be trained in DI policies and procedures.

8.2. Personnel should agree to abide by DI principles and should be made aware of the potential consequences in cases of non-compliance.

8.3. Personnel should be trained in good documentation practices and measures to prevent and detect DI issues. This may require specific training in evaluating the configuration settings and reviewing electronic data and metadata, such as audit trails, for individual computerized systems used in the generation, processing and reporting of data.
9. DATA

9.1. Data may be presented by manually recording an observation, result or other data and information on paper, or electronically recording thereof, by using equipment and instruments including those linked to computerised systems. A combination of manual and electronic systems may also be used.

9.2. The same considerations for DI apply for other data sets (such as photographs, videos, DVD, imagery and chromatography plates) as for the other data sets, together with any additional controls required for that format such as copying, photography or digitisation. There should be a documented rationale for the selection of such a method.

9.3. Where possible, risk-reducing supervisory measures should be implemented.

9.4. Results and data sets require independent verification if deemed necessary from the DIRA or by another requirement.

10. DATA INTEGRITY

10.1. Data integrity (DI) is the degree to which data are complete, consistent, accurate, trustworthy and reliable.

10.2. Risk-based system design and controls should enable the detection of errors, lapses and omissions of results and data during the data life cycle. Controls may include procedural controls, organizational controls and functional controls.

10.3. The DI policy should clearly define what constitutes raw data, source data, metadata and a “complete data set”.

10.4. Data should be contemporaneously recorded, collected and maintained in a secure manner. Controls should ensure that they are attributable, legible, original (or a true copy) and accurate. Assuring DI requires appropriate QRM systems, including adherence to sound scientific principles and good documentation practices.
10.5. Systems should be established and implemented to ensure that all data acquired, processed and reported are in accordance with the principles in this guideline. Data should be:

- A = attributable to the person generating the data
- L = legible and permanent
- C = contemporaneous
- O = original record (or certified true copy)
- A = accurate

10.6. Data governance measures should also ensure that data are complete, consistent, enduring and available throughout the life cycle, where:

- Complete = the data must be whole; a complete set.
- Consistent = the data must be self-consistent.
- Enduring = durable; lasting throughout the data life cycle.
- Available = readily available for review or inspection purposes.

10.7. Original data should be reviewed, retained, complete, enduring and readily retrievable and readable throughout the records retention period.

11. GOOD DOCUMENTATION PRACTICES

11.1. The principles contained in this guideline are applicable to paper and electronic data.

11.2. Specific controls should be identified through DIRA, to ensure the integrity of data and results recorded on paper records. These may include, but are not limited to:

- the use of permanent, indelible ink;
- no use of pencil or erasers;
- the use of single-line cross-outs to record changes with name, date and reason recorded (i.e. the paper equivalent to the audit trail);
- no use of correction fluid or otherwise obscuring the record;
- controlled issuance of bound, paginated notebooks;
- controlled issuance of sequentially numbered copies of blank forms; and
- archival of paper records by independent, designated personnel in secure and controlled archives.
12. COMPUTERIZED SYSTEMS

(Note. This section highlights some specific aspects relating to the use of computerized systems. It is not intended to repeat the information presented in the other WHO Guidelines here, such as the WHO Guideline on Computerized systems, WHO Guideline on Validation, and WHO Guideline on Good Chromatography Practices. See references.)

12.1. The computerized system selected should be suitable for its intended use.

12.2. Where GXP systems are used to acquire, record, store or process data, management should have appropriate knowledge of the risks that the system and users may have on the data.

12.3. Suitably configured and validated software should be used where instruments and equipment with computerised systems are used. The potential for manipulation of data should be eliminated during the data life cycle.

12.4. Where electronic systems with no configurable software and no electronic data retention (e.g. pH meters, balances and thermometers) are used, controls should be put in place to prevent manipulation of data and repeat testing to achieve the desired result.

12.5. Appropriate means of detection for lapses in DI principles should be in place. Additional means should be implemented where stand-alone systems with a user-configurable output is used, for example, Fourier-transform infrared spectroscopy (FTIR) and UV spectrophotometers.

12.6. All records that are defined by the data set should be reviewed and retained. Reduced effort and/or frequency may be justifiable.

Access and privileges

12.7. There should be a documented system in place that defines the access and privileges of users of computerized systems. The paper and electronic records should be in line with the electronic information including the creation and deletion of users.

12.8. Access and privileges should be in accordance with the responsibility and functionality of the individual with appropriate controls to ensure DI (e.g. no modification, deletion or creation of data outside the application is possible).
12.9. A limited number of personnel, with no conflict of interest in data, should be appointed as system administrators. Certain privileges such as data deletion, database amendment or system configuration changes should not be assigned to administrators without justification - and such activities should only be done with documented evidence of authorization by another responsible person. Records should be maintained.

12.10. Unique usernames and passwords should be used for systems as appropriate.

12.11. Programmes and methods (such as acquisition and processing methods) should ensure that data meet ALCOA principles. Where results or data are processed using a different method/parameters than the acquisition method should be recorded. Audit trails and details should allow reconstruction of all data processing activities.

12.12. Data transfer should not result in any changes to the content or meaning of the data. The transfer should be tracked in the audit trail.

12.13. Data transfer should be validated.

**Audit Trail**

12.14. GXP systems should provide for the retention of audit trails. Audit trails should reflect, for example, users, dates, times, original data and results, changes and reasons for changes.

12.15. Audit trails should be enabled when software is installed, and remain enabled all times. Proof of enabling and verification during the life cycle of data should be maintained.

12.16. Where add-on software or legacy systems are used (with no audit trail), mitigation measures may be taken for defined temporary periods. This should be addressed within defined timelines.

12.17. Routine data review should include a review of audit trails. Evidence should be maintained.
Electronic signatures

12.18. Each electronic signature should be appropriately controlled. An electronic signature should be:

- validated;
- attributable to an individual;
- free from alteration and manipulation; and
- compliant with the requirements of international standards.

12.19. An inserted image of a signature or a footnote indicating that the document has been electronically signed is not adequate.

Data review and approval

12.20. There should be a documented procedure for the routine and periodic review, as well as approval of data.

12.21. CAPAs should be recorded where errors, discrepancies or omissions are identified.

12.22. A conclusion following the review of original data, metadata and audit trail records should be documented, signed and dated.

Data backup, retention, and restoration

12.23. Data should be backed up and archived according to written procedures. The validated procedures and controls should ensure the protection of data and records.

12.24. Data and records should be kept in a secure area which provides appropriate protection. Access should be controlled.

12.25. Retention periods should be defined in authorized procedures.

12.26. Records reflecting documented reasons for the destruction of data should be maintained.

12.27. Backup and restoration processes should be validated and periodically tested, including verification of data size, completeness and accuracy of data and metadata.
13. CORRECTIVE AND PREVENTIVE ACTIONS

13.1. Where organizations use computerized systems (e.g. for GXP data acquisition, processing, interpretation, reporting) which do not meet current GMP requirements, a workplan towards upgrading such systems should be documented and implemented to ensure compliance with current GMP.

13.2. When GMP lapses in DI are identified, root cause analysis, impact and risk assessment should be carried out. Appropriate CAPAs should be established and implemented. Health authorities and other relevant organizations should be notified if the investigation identifies significant impact or risk to materials, products, patients, reported information or data in application dossiers, clinical trial reports, and so on.

References and further reading

(Note: This section will be updated)

1. WHO Basic Principles in Good Manufacturing Practices
2. WHO Guideline on Validation
3. WHO Guideline on Computerized Systems
4. WHO Guideline on Good Chromatography Practices
5. Medicines and Healthcare Products Guideline
6. U.S. Food and Drug Administration Guideline
7. Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S) Guideline
8. International Society for Pharmaceutical Engineering (ISPE) Baseline
ANNEX 1
EXAMPLES IN DATA INTEGRITY MANAGEMENT

This Annex reflects on some examples in data integrity (DI) management, to support the main text on DI. It should be noted that these are examples and are intended for the purpose of clarification only.

Example 1: Quality risk management and data integrity risk assessment

Risk management is an important part of good manufacturing practices (GMP). Risks should be identified and assessed, control identified and implemented to assist manufacturers in preventing possible DI lapses.

As an example, a Failure Mode and Effects Analysis (FMEA) model (or any other tool) can be used to identify and assess the risks relating to any system where data are, for example, acquired, processed, recorded, saved and archived. Based on severity, occurrence and detection classification and an overall risk priority number or risk factor, corrective and preventive action (CAPA) should be identified, implemented and assessed for its effectiveness.

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<table>
<thead>
<tr>
<th>Detection</th>
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For example, if during the weighing of a sample, the entry of the date was not contemporaneously recorded on the worksheet but the date is available on the print-out from a weighing balance and log book for the balance for that particular activity, this is still considered DI. The risk is however different when there is no other means of traceability for the activity. When assessing the risk relating to the lapse in DI, the severity could be classified as “low” (the data is available on the print-out); it does not happen on a regular basis (occurrence is “low”), and it could easily be detected by the reviewer (detection is “high”) – therefore the overall risk factor may be considered low. The root cause as to why the record was not made in the analytical report at the time of weighing should still be identified and the appropriate action taken to prevent this from happening.

Example 2: Good documentation practices in data integrity

Documentation should be managed with care. These should be appropriately designed to assist in eliminating erroneous entries, manipulation and human error.

**Paper systems**

*Formats*

Formats should be designed and prepared to enable personnel to record the correct information at the right time. Provision should be made for entries such as dates, time (start, finish), signatures, initials, results, batch numbers, equipment identification numbers and so on. The system should prompt the personnel to make the entries at the appropriate step.

*Blank forms*

The use of blank forms is not encouraged. Where blank forms are used (e.g. to supplement worksheets, laboratory notebooks and master production and control records), appropriate controls have to be in place and may include, for example, a numbered set of blank forms issued which are reconciled upon completion. Similarly, bound paginated notebooks, stamped or formally issued by a document control group, allow the detection of unofficial notebooks and any gaps in notebook pages. Authorization may include two or three signatures with dates, for example, “prepared by” or “entered by”, “reviewed by” and “approved by”.

*Error in recording data*

Entries of data and results (electronic and paper records) should be free from mistakes. Entries should be made with care. Where incorrect information had been recorded, this may be corrected provided that the reason for the error is documented, the original entry remains readable, and the correction is signed and dated.
Example 3: Data entry

Data entry includes examples such as sample receiving registration, sample analysis result recording, logbook entries, registers, batch manufacturing record entries, and information in case report forms. The recording of source data on paper records should be in indelible ink and free from errors. Direct entry into electronic records should be done by responsible, appropriately trained individuals. Entries should be traceable to an individual (in electronic records thus having a unique username and password) and traceable to the date (and time, where possible). Where appropriate, the entry should be verified by a second person or entered through technical means such as bar-coding, where possible, for the intended use of these data. Additional controls may include locking critical data entries after the data are verified and review of audit trails for critical data to detect if they have been altered.

Example 4: Dataset

All data should be included in the dataset unless there is a documented, justifiable, scientific explanation and procedure for the exclusion of any result or data. Whenever out of trend or atypical results are obtained, they should be investigated in accordance with written procedures. This includes investigating and determining CAPA for invalid runs, failures, repeats and other atypical data. The review of original electronic data should include checks of all locations where data may have been stored, including locations where voided, deleted, invalid or rejected data may have been stored. Data and metadata should not be found in other electronic folders or in other operating system logs. Electronic data should be archived in accordance with a standard operating procedure. It is important to ensure that associated metadata are archived with the relevant data set or securely traceable to the data set through relevant documentation. It should be possible to successfully retrieve data and datasets from the archives. This includes metadata. This should be done in accordance with a procedure and verified at defined intervals.

Example 5: Enduring

Data and metadata should be readable during the life cycle of the data. Risks include the fading of microfilm records, the decreasing readability of the coatings of optical media such as compact disks (CDs) and digital versatile/video disks (DVDs), and the fact that these media may become brittle. Similarly, historical data stored on magnetic media will also become unreadable over time as a result of deterioration. Data and records should be stored in an appropriate manner, under the appropriate conditions.
Example 6: Attributable

Data should be attributable, thus being traceable to an individual. In paper records, this could be done through the use of initials, full handwritten signature or personal seal. In electronic records, this could be done through the use of unique user logons that link the user to actions that create, modify or delete data; or unique electronic signatures which can be either biometric or non-biometric. An audit trail that captures user identification (ID), date and time stamps, and the electronic signature must be securely and permanently linked to the signed record.

Example 7: Contemporaneous

Personnel should record data and information at the time these are generated and acquired. For example, when a sample is weighed or prepared, the weight of the sample (date, time, name of the person, balance identification number) should be recorded at that time and not before or at a later stage. In the case of electronic data, these should be automatically date and time stamped. The use of hybrid systems is discouraged but where legacy systems are awaiting replacement, documented mitigating controls should be in place. (Replacement of hybrid systems should be a priority with a documented CAPA plan). The use of a scribe to record an activity on behalf of another operator should be considered only on an exceptional basis and should only take place where, for example, the act of recording places the product or activity at risk, such as, documenting line interventions by aseptic area operators.

Example 8: Changes

When changes are made to any result or data, the change should be traceable to the person who made the change, the date, time and reason for the change. In electronic systems, this traceability should be documented via computer generated audit trails or in other metadata fields or system features that meet these requirements. Where an existing computerized system lacks computer-generated audit trails, personnel may use alternative means such as procedurally controlled use of log-books, change control, record version control or other combinations of paper and electronic records to meet GXP regulatory expectations for traceability to document the what, who, when and why of an action.
Example 9: Original

Original data include the first or source capture of data or information and all subsequent data required to fully reconstruct the conduct of the GXP activity (see the definition of raw data). In some cases, the electronic data (electronic chromatogram acquired through high-performance liquid chromatography (HPLC)) may be the original data, and in other cases, the recording of the temperature on a log sheet in a room - by reading the value on a data logger – may be considered the original data. Original data should be reviewed. Proof of review should be presented (e.g. as a signature (reviewed by:) and date of the review). For electronic records, this is typically signified by electronically signing the electronic data set that has been reviewed and approved. Written procedures for data review should clarify the meaning of the review and approval signatures to ensure that the personnel concerned understand their responsibility as reviewers and approvers to assure the integrity, accuracy, consistency and compliance with established standards of the electronic data and metadata subject to review and approval. Written procedures for data review should define the frequency, roles and responsibilities and approach to review of meaningful metadata, such as audit trails. These procedures should also describe how aberrant data are to be handled if found during the review. Personnel who conduct such reviews should have adequate and appropriate training in the review process as well as in the software systems containing the data subject to review.

Example 10: Controls

Based on the outcome of the data integrity risk assessment (DIRA) (which should cover all areas of data governance and data management) – appropriate and effective controls should be identified and implemented to assure that all data, whether in paper records or electronic records, will meet ALCOA+ principles. Examples of controls may include, but are not limited to:

- qualification, calibration and maintenance of equipment, such as balances and pH meters, that generate printouts;
- validation of computerized systems that acquire, process, generate, maintain, distribute or archive electronic records;
- validation of systems to ensure that the integrity of data will remain while transmitting between/among computerized systems;
- validation of analytical procedures;
- validation of production processes;
- review of GXP records; and
- investigation of deviations, doubtful, out of trend and out of specifications results.
Points to consider for assuring accurate GXP records:

- The entry of critical data into a computer by an authorized person (e.g. entry of a master processing formula) requires an additional check on the accuracy of the data entered manually. This check may be done by independent verification and release for use by a second authorized person or by validated electronic means. For example, to detect and manage risks associated with critical data, procedures would require verification by a second person, such as a member of the quality unit staff;
- formulae for calculations entered into spreadsheets;
- master data entered into the laboratory information management system (LIMS) such as fields for specification ranges used to flag out of specification values on the certificate of analysis;
- other critical master data, as appropriate. Once verified, these critical data fields should normally be locked to prevent further modification and only be modified through a formal change control process;
- the process of data transfer between systems should be validated;
- the migration of data into and exported from systems requires specific planned testing and control; and
- when the activity is time-critical, printed records should display the date and time stamp.
ATC/DDD Classification (Temporary)

The following ATC codes and DDDs were agreed at the meeting of the WHO International Working Group for Drug Statistics Methodology in October 2019.

Comments or objections to the decisions from the meeting should be forwarded to the WHO Collaborating Centre for Drug Statistics Methodology before 1 February 2020. If no objections are received before this date, the new ATC codes and DDDs will be considered final and included in the January 2021 version of the ATC/DDD Index.

New ATC 5th level codes:

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1) Oral formulations only indicated in treatment of renal secondary hyperparathyroidism
2) Oral formulations only indicated in treatment of Cushing’s syndrome
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<td>Other antiparkinson drugs</td>
<td>N04CX</td>
</tr>
</tbody>
</table>

New DDDs:

<table>
<thead>
<tr>
<th>ATC level name/INN</th>
<th>DDD</th>
<th>unit</th>
<th>Adm.R</th>
<th>ATC code</th>
</tr>
</thead>
<tbody>
<tr>
<td>budesonide</td>
<td>2</td>
<td>mg</td>
<td>SL</td>
<td>A07EA06</td>
</tr>
<tr>
<td>buprenorphine</td>
<td>2.1</td>
<td>mg</td>
<td>P</td>
<td>N07BC01</td>
</tr>
<tr>
<td>caplacizumab</td>
<td>10</td>
<td>mg</td>
<td>P</td>
<td>B01AX07</td>
</tr>
<tr>
<td>lobeglitazone</td>
<td>0.5</td>
<td>mg</td>
<td>O</td>
<td>A10BG04</td>
</tr>
<tr>
<td>risankizumab</td>
<td>1.67</td>
<td>mg</td>
<td>P</td>
<td>L04AC18</td>
</tr>
<tr>
<td>tildrakizumab</td>
<td>1.11</td>
<td>mg</td>
<td>P</td>
<td>L04AC17</td>
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</tbody>
</table>
ATC/DDD Classification (Final)

The following ATC codes and DDDs were agreed at the meeting of the WHO International Working Group for Drug Statistics Methodology in March 2019. These are considered as final and will be included in the January 2020 version of the ATC/DDD Index.

New ATC 5th level codes:

<table>
<thead>
<tr>
<th>ATC level name/INN</th>
<th>ATC code</th>
</tr>
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<tbody>
<tr>
<td>$^{13}$C-urea</td>
<td>V04CX05</td>
</tr>
<tr>
<td>alpelisib</td>
<td>L01XX65</td>
</tr>
<tr>
<td>belotecan</td>
<td>L01XX68</td>
</tr>
<tr>
<td>bempedoic acid</td>
<td>C10AX15</td>
</tr>
<tr>
<td>carbon monoxide</td>
<td>V04CX08</td>
</tr>
<tr>
<td>Centella asiatica herba</td>
<td>D03AX14</td>
</tr>
<tr>
<td>darunavir and ritonavir</td>
<td>J05AR26</td>
</tr>
<tr>
<td>darvadstrocel</td>
<td>L04AX08</td>
</tr>
<tr>
<td>econazole, combinations</td>
<td>G01AF55</td>
</tr>
<tr>
<td>edrophonium</td>
<td>V04CX07</td>
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<tr>
<td>elapegademase</td>
<td>L03AX21</td>
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<tr>
<td>entrectinib</td>
<td>L01XE56</td>
</tr>
<tr>
<td>fedratinib</td>
<td>L01XE57</td>
</tr>
<tr>
<td>folic acid</td>
<td>V04CX02</td>
</tr>
<tr>
<td>formoterol and tiotropium bromide</td>
<td>R03AL10</td>
</tr>
<tr>
<td>formoterol, glycopyrronium bromide and budesonide</td>
<td>R03AL11</td>
</tr>
<tr>
<td>fremanezumab</td>
<td>N02CD03$^1$</td>
</tr>
<tr>
<td>hexaminolevulinate</td>
<td>V04CX06</td>
</tr>
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</table>

1) New ATC 4th level, N02CD Calcitonin gene-related peptide (CGRP) antagonists
<table>
<thead>
<tr>
<th>ATC level name/INN</th>
<th>ATC code</th>
</tr>
</thead>
<tbody>
<tr>
<td>homatropine methylbromide</td>
<td>A03BB06</td>
</tr>
<tr>
<td>indacaterol and mometasone</td>
<td>R03AK14</td>
</tr>
<tr>
<td>indacaterol, glycopyrroinium bromide and mometasone</td>
<td>R03AL12</td>
</tr>
<tr>
<td>lamivudine, tenofovir disoproxil and dolutegravir</td>
<td>J05AR27</td>
</tr>
<tr>
<td>lefamulin</td>
<td>J01XX12</td>
</tr>
<tr>
<td>mannitol</td>
<td>V04CX04</td>
</tr>
<tr>
<td>methacholine</td>
<td>V04CX03</td>
</tr>
<tr>
<td>omadacycline</td>
<td>J01AA15</td>
</tr>
<tr>
<td>osilodrostat</td>
<td>H02CA02</td>
</tr>
<tr>
<td>patent blue</td>
<td>V04CX09</td>
</tr>
<tr>
<td>remimazolam</td>
<td>N05CD14</td>
</tr>
<tr>
<td>revefenacin</td>
<td>R03BB08</td>
</tr>
<tr>
<td>rifamycin</td>
<td>D06AX15</td>
</tr>
<tr>
<td>rosvastatin and fenofibrate</td>
<td>C10BA09</td>
</tr>
<tr>
<td>rosvastatin and ramipril</td>
<td>C10BX17</td>
</tr>
<tr>
<td>sarecycline</td>
<td>J01AA14</td>
</tr>
<tr>
<td>selinexor</td>
<td>L01XX66</td>
</tr>
<tr>
<td>tagraxofusp</td>
<td>L01XX67</td>
</tr>
<tr>
<td>upadacitinib</td>
<td>L04AA44</td>
</tr>
<tr>
<td>vonoprazan</td>
<td>A02BC08</td>
</tr>
<tr>
<td>voretigene neparvovec</td>
<td>S01XA27</td>
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</table>
### New ATC level codes (other than 5th levels):

<table>
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</thead>
<tbody>
<tr>
<td>Calcitonin gene-related peptide (CGRP) antagonists</td>
<td>N02CD</td>
</tr>
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### Change of ATC level codes:

<table>
<thead>
<tr>
<th>ATC-level names</th>
<th>Previous ATC-code</th>
<th>New ATC code</th>
</tr>
</thead>
<tbody>
<tr>
<td>erenumab</td>
<td>N02CX07</td>
<td>N02CD01</td>
</tr>
<tr>
<td>galcanezumab</td>
<td>N02CX08</td>
<td>N02CD02</td>
</tr>
<tr>
<td>miltefosine</td>
<td>L01XX09</td>
<td>P01CX04</td>
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</tbody>
</table>

### Change of ATC level names:

<table>
<thead>
<tr>
<th>ATC-code</th>
<th>Previous ATC-level name</th>
<th>New ATC-level name</th>
</tr>
</thead>
<tbody>
<tr>
<td>A03CB04</td>
<td>methylhomatropine and psycholeptics</td>
<td>homatropine methylbromide and psycholeptics</td>
</tr>
<tr>
<td>B06AB</td>
<td>Other hem products</td>
<td>Heme products</td>
</tr>
<tr>
<td>B06AB01</td>
<td>hematin</td>
<td>hemin</td>
</tr>
</tbody>
</table>
New DDDs:

<table>
<thead>
<tr>
<th>ATC level name/INN</th>
<th>DDD</th>
<th>unit</th>
<th>Adm.R</th>
<th>ATC code</th>
</tr>
</thead>
<tbody>
<tr>
<td>baloxavir marboxil</td>
<td>40</td>
<td>mg</td>
<td>O</td>
<td>J05AX25</td>
</tr>
<tr>
<td>doravirine</td>
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<td>g</td>
<td>O</td>
<td>J05AG06</td>
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<tr>
<td>fexinidazole</td>
<td>1.44</td>
<td>g</td>
<td>O</td>
<td>P01CA03</td>
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<tr>
<td>fremanezumab&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>7.5</td>
<td>mg</td>
<td>P</td>
<td>N02CD03</td>
</tr>
<tr>
<td>galcanezumab&lt;sup&gt;2)&lt;/sup&gt;</td>
<td>4</td>
<td>mg</td>
<td>P</td>
<td>N02CD02</td>
</tr>
<tr>
<td>lesinurad</td>
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<td>g</td>
<td>O</td>
<td>M04AB05</td>
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<tr>
<td>omadacycline</td>
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<td>g</td>
<td>O</td>
<td>J01AA15</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>g</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>prasterone</td>
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<td>mg</td>
<td>V</td>
<td>G03XX01</td>
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<tr>
<td>sarecycline</td>
<td>0.1</td>
<td>g</td>
<td>O</td>
<td>J01AA14</td>
</tr>
</tbody>
</table>

1) New ATC 4th level N02CD valid from January 2020.
2) ATC code altered from N02CX08. New ATC 4th level N02CD valid from January 2020.

WHO Collaborating Centre for Drug Statistics Methodology
Oslo, November 2019