DOLUTEGRAVIR SODIUM
DOLUTEGRAVIRUM 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 (schmidt@who.int) by 31 October 2019.

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

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SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/18.779:  

**DOLUTEGRAVIR SODIUM**  
**DOLUTEGRAVIRUM NATRICUM**

| Description                                                                 | Date                        |
|-----------------------------------------------------------------------------|                            |
| First draft received from collaborating laboratory                           | August 2018                 |
| Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations | October 2018                |
| Discussion at the consultation on screening technologies. Laboratory tools and pharmacopoeial specifications for medicines | 2-3 May 2019                |
| Draft monograph sent out for public consultation                            | September – October 2019    |
| Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations | October 2019                |
| Further follow-up action as required                                        |                            |

**Note from the Secretariat.** The monograph on Dolutegravir sodium is proposed for inclusion in The International Pharmacopoeia.

Being the first public standard, the monograph is expected to play an important role in ensuring access to quality assured Dolutegravir API worldwide. Manufacturers of this product, regulatory authorities, procurement agencies and other stakeholders are therefore invited to provide their feedback to the Secretariat of The International Pharmacopoeia. If not already done, manufacturers are also invited to submit information and samples of their products. With their support manufacturers will help to ensure that the proposed monograph adequately controls the quality of the products they manufacture.

The World Health Organization (WHO) develops, establishes, and promotes international standards following the mandate given by WHO’s currently 194 Member States. WHO’s norms and standards are developed for adoption as legally binding national regulations and to serve as a basis for national standards and technical regulations. Besides, they play a prominent role for WHO Prequalification of medicine as well as UN/WHO procurement activities.

For information regarding the submission of specifications and samples, please contact Dr Herbert Schmidt at schmidt@who.int.
**Dolutegravir Sodium**

**Dolutegravirum Natricum**

69 **Molecular formula.** C\(_{20}\)H\(_{18}\)F\(_2\)N\(_3\)NaO\(_5\)

70 **Relative molecular mass.** 441.37

71 **Graphic formula.**

73 **Chemical name.** (4\(_R\),12\(_a\)S)-N-[(2,4-Difluorphenyl)methyl]-3,4,6,8,12,12\(_a\)-hexahydro-7-hydroxy-4-methyl-6,8-dioxo-2\(_H\)-pyrido[1′,2′:4,5]pyrazino[2,1-\(b\)][1,3]oxazine-9-carboxamide sodium salt; CAS Reg. No. 1051375-19-9.

76 **Description.** A white to pale yellow powder.

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

78 **Category.** Antiretroviral (integrase inhibitor).

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

80 **Additional information.** Dolutegravir sodium may exhibit polymorphism.

81 **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.

84 **Identity tests**

85 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 und 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).\(^1\) 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

\(^1\) A Lux Cellulose-4 column was found suitable.
for peak identification RS (containing dolutegravir sodium and the impurities A, B and D) per
mL.

143 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).²

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.

² A Kinetex F5 column or an Ascentis Express F5 column were found suitable.
<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</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>composition</td>
</tr>
<tr>
<td>57–65</td>
<td>60</td>
<td>40</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

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).³

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.

³ A Kinetex F5 column or an Ascentis Express F5 column were found suitable
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 (C_{20}H_{18}F_{2}N_{3}NaO_{5}) using the declared content of C_{20}H_{18}F_{2}N_{3}NaO_{5} 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 C_{20}H_{18}F_{2}N_{3}NaO_{5}.

**Impurities**

![](image)

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\text{-difluorophenyl})methyl]\text{-}7\text{-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-}2H\text{-pyrido}[1',2':4,5]\text{pyrazino}[2,1\text{-b}][1,3]\text{oxazine-9-carboxamide (dolutegravir diastereomer)(synthesis-related impurity)}.

C. \((4R,12\alpha S)-N-[(\text{phenyl})methyl]\text{-}7\text{-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12\alpha-hexahydro-}2H\text{-pyrido}[1',2':4,5]\text{pyrazino}[2,1\text{-b}][1,3] \text{oxazine-9-carboxamide, Desfluoro dolutegravir (synthesis-related impurity)}.

D. \((4R,12\alpha S)-N-[(2\text{-fluorophenyl})methyl]\text{-}7\text{-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12\alpha-hexahydro-}2H\text{-pyrido}[1',2':4,5]\text{pyrazino}[2,1\text{-b}][1,3] \text{oxazine-9-carboxamide, 2-Fluoro dolutegravir (synthesis-related impurity)}.

E. \((4R,12\alpha S)-N-[(4\text{-fluorophenyl})methyl]\text{-}7\text{-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12\alpha-hexahydro-}2H\text{-pyrido}[1',2':4,5]\text{pyrazino}[2,1\text{-b}][1,3] \text{oxazine-9-carboxamide, 4-Fluoro dolutegravir (synthesis-related impurity)}.
(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.