Consultation documents

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The International Pharmacopoeia

Misoprostol
(Misoprostolum)

This is a revised draft proposal for The International Pharmacopoeia (Working document QAS/15.602/Rev.1, November 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

[Note from the Secretariat. The following monograph is proposed for inclusion in The International Pharmacopoeia.]

Molecular formula. C_{22}H_{38}O_{5}

Relative molecular mass. 382.5

Graphic formula

its epimer at C* and their enantiomers

Chemical name

Description. Clear, colourless or yellowish, oily liquid.

Solubility. Practically insoluble in water R, soluble in dehydrated ethanol R, sparingly soluble in acetonitrile R.
Category. Prostaglandin (PGE1) analogue.

Storage. Misoprostol neat oil should be kept in a tightly sealed container and stored at a temperature between -25 and -10°C.

Additional information. Misoprostol is hygroscopic. It is gradually degraded at room temperature, the degradation being faster at higher temperatures.

Requirements

Definition. Misoprostol contains not less than 96.5% and not more than 102.0% of C_{22}H_{38}O_{5} with reference to the anhydrous substance.

Identity tests

Either test A 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 misoprostol RS or with the reference spectrum of misoprostol.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R3 as the coating substance and a mixture of 8 volumes of toluene R, 2 volumes of ethyl acetate R, 1 volume of dehydrated ethanol R and 0.1 volume of glacial acetic acid R as the mobile phase, prepared immediately before use. Apply separately to the plate 100 μL of each of the following two solutions in dehydrated ethanol R. For solution (1) use 0.1 mg of the test substance per mL. For solution (2) use 0.1 mg of misoprostol RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air, expose it to the vapour of iodine R and examine the chromatogram in daylight.

   The principal spot obtained with solution (1) corresponds in position, appearance and intensity to that obtained with solution (2).

C. 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 from solution (1) corresponds to the retention time of the peak due to misoprostol in the chromatogram obtained from solution (2).

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using 1.0 mL of a 10 mg per mL solution of the test substance in dehydrated methanol R; the water content is not more than 10 mg/g.

Related substances

Prepare fresh solutions and perform the tests without delay.

Carry out the test as described under 1.14.4 High performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilyl silica gel (5 μm).\(^1\)

Use the following conditions for gradient elution:

   Mobile phase A: mix 28 volumes of acetonitrile for chromatography R with 69 volumes of water R and 3 volumes of methanol R;

   Mobile phase B: mix 47 volumes of acetonitrile for chromatography R with 50 volumes of water R and 3 volumes of methanol R.

\(^1\) An Ascentis Express C18 column was found suitable.
Prepare the following solutions using a mixture of 31 volumes of acetonitrile R and 69 volumes of water R as solvent. For solution (1) dissolve about 50 mg of the test substance in 10.0 mL and sonicate for about 10 minutes. Ensure that the temperature of the sonication bath is below room temperature to avoid degradation of misoprostol. For solution (2) dilute 1 volume of solution (1) to 500 volumes. For solution (3) heat 5 mL of solution (1) in a water bath at 75°C for 1 hour.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 200 nm. Store the samples at 4°C during analysis using a cooled autosampler.

Inject 20 μL of solution (3). The test is not valid unless the peak-to-valley ratio (Hp/Hv) is at least 5.0, where Hp is the height above the extrapolated baseline of the peak due to impurity A (with a relative retention of about 0.95 with reference to misoprostol (retention time about 21 minutes)) and Hv is the height above the extrapolated baseline at the lowest point of the curve separating the peak due to impurity A from the peak due to misoprostol.

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

The chromatogram obtained with solution (1) may show the following impurities at the following relative retentions with reference to misoprostol (retention time about 21 minutes): impurity E (1st peak): about 0.84; impurity E (2nd peak): about 0.86; impurity B (1st peak): about 0.90; impurity B (2nd peak): about 0.92; impurity A: about 0.95; impurity D: about 1.27; impurity C: about 1.37. Use also the chromatogram obtained with solution (3) to identify impurity A and C.

In the chromatogram obtained with solution (1):

- the sum of the areas of any peak corresponding to impurity A, B and E is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 0.76, is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);
- the area of any other impurity peak is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);
- the sum of the corrected area of any peak corresponding to impurity C and the areas of all other peaks, other than the principal peak, is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

<table>
<thead>
<tr>
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<tr>
<td>32–40</td>
<td>100</td>
<td>0</td>
<td>re-equilibration</td>
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</tbody>
</table>

Maintain the column temperature at 35°C.
Diastereoisomers

Carry out the test as described under 1.14.4 High performance liquid chromatography using a stainless steel column (15 cm × 2.1 mm) packed with silica gel for chromatography R (3.5 μm). As the mobile phase use a mixture of 4 volumes of 2-propanol R, 96 volumes of heptane R and 0.1 volume of trifluoroacetic acid R.

As the test solution use 1.0 mg of the test substance per mL of a mixture of 4 volumes of 2-propanol R and 96 volumes of heptane R.

Maintain the column temperature at 25°C.

Operate with a flow rate of 0.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 205 nm. Store the samples at 4°C during analysis using a cooled autosampler.

Inject 10 μL of the test solution.

The chromatogram shows two principal peaks due to misoprostol at retention times of about 14 and 16 minutes. The test is not valid unless the resolution between these two peaks is at least 2.0.

Measure the areas of the two peaks corresponding to misoprostol. The first peak of misoprostol is 45%–55% of the sum of the areas of the two peaks due to misoprostol.

Assay

Carry out the test as described under 1.14.4 High performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilyl silica gel (5 μm). As the mobile phase use a mixture of 45 volumes of acetonitrile R and 55 volumes of water.

Prepare the following solutions in the mobile phase. For solution (1) use 0.1 mg of misoprostol per mL. For solution (2) use 0.1 mg of misoprostol RS per mL.

Maintain the column temperature at 35°C.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 200 nm. Store the samples at 4°C during analysis using a cooled autosampler.

Inject alternately 20 μL each of solutions (1) and (2). The test is not valid unless the symmetry factor of the peak due to misoprostol is between 0.8 and 1.5.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of misoprostol (C_{22}H_{38}O_{5}), using the declared content of C_{22}H_{38}O_{5} in misoprostol RS.

Impurities

A. Mixture of methyl rac-7-[(1R,2S,3S)-3-hydroxy-2-[(1E,4R)-4-hydroxy-4-methyloct-1-en-1-yl]-5-oxocyclopentyl]heptanoate and methyl rac-7-[(1R,2S,3S)-3-hydroxy-2-[(1E,4S)-4-hydroxy-4-methyloct-1-en-1-yl]-5-oxocyclopentyl]heptanoate (8-epimisoprostol)

2 An Xbridge HILIC column was found suitable.
3 An Ascentis Express C18 column was found suitable.
B. Mixture of methyl 7-[(1RS,2SR,3RS)-3-hydroxy-2-[(1E,4RS)-4-hydroxy-4-methyl-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1RS,2SR,3RS)-3-hydroxy-2-[(1E,4SR)-4-hydroxy-4-methyl-1-enyl]-5-oxocyclopentyl]heptanoate (12-epimisoprostol) (synthesis impurity)

C. Mixture of methyl rac-7-[(1R,2S)-2-[(1E,4R)-4-hydroxy-4-methyl-1-en-1-yl]-5-oxocyclopent-3-en-1-yl]heptanoate and methyl rac-7-[(1R,2S)-2-[(1E,4S)-4-hydroxy-4-methyl-1-en-1-yl]-5-oxocyclopentyl]heptanoate. (misoprostol A)

D. Methyl rac-7-2-[(1E,4R)-4-hydroxy-4-methyl-1-en-1-yl]-5-oxocyclopent-1-en-1-yl]heptanoate (misoprostol B)

E. (+) - methyl (1R,2R,3R)-3-hydroxy-2-[(1E,4RS)-(1,5-heptadien-1-yl)]-4-hydroxy-4,6-dimethyl-5-oxocyclopentaneheptanoate

*[Note from the Secretariat. Chemical name to be confirmed.]*
Misoprostol dispersion
(Misoprostoli dispersio)

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.642, November 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

[Note from the Secretariat. The following monograph is proposed for inclusion in The International Pharmacopoeia. Comments are in particular sought on the suitability of the proposed content limits for misoprostol (95.0% to 105.0% of the amount of Misoprostol stated on the label).]

Description. A white or almost white powder.

Category. Prostaglandin (PGE1) analogue.

Storage. Misoprostol is hygroscopic, it should be stored below 8°C under nitrogen in sealed containers and should not be exposed to moisture.

Requirements

Definition. Misoprostol dispersion is a mixture of Misoprostol and Hypromellose. It contains not less than 95.0% and not more than 105.0% of the amount of C_{22}H_{38}O_{5} stated on the label.

Identity tests

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

A. 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 from solution (1) corresponds to the retention time of the peak due to misoprostol in the chromatogram obtained from solution (2).

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R3 as the coating substance and a mixture of 8 volumes of toluene R, 2 volumes of ethyl acetate R, 1 volume of dehydrated ethanol R and 0.1 volume of glacial acetic acid R as the mobile phase, prepared immediately before use. Apply separately to the plate 100 μL of each of the following two solutions in dehydrated ethanol R. For solution (1) shake mechanically a quantity of the dispersion equivalent to 1 mg of misoprostol with 10.0 mL of dehydrated ethanol R for 10 minutes, filter and use the clear filtrate. For solution (2) use 0.1 mg of misoprostol RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air, expose it to the vapour of iodine R and examine the chromatogram in daylight.

The principal spot obtained with solution (1) corresponds in position, appearance and intensity to that obtained with solution (2).

C. Carry out test C1, C2 and C3.
C1. Add gently 1 g of the dispersion to 100 mL of water in a beaker. Allow the substance to disperse over the surface, tapping the top of the container to ensure an even dispersion of the substance. Allow the beaker to stand for about 5 hours until the substance becomes transparent and mucilaginous. Swirl the beaker to wet the remaining substance. Add a stirring bar and stir until the test substance is completely dissolved. To two 50 mL aliquots of this solution add an equal volume of either sodium hydroxide (~40 g/L) TS or hydrochloric acid (~36.5 g/L) TS. Both mixtures remain stable and clear.

C2. Add 1 g of the dispersion to 100 mL of boiling water and stir the mixture; a slurry is formed but the dispersion does not dissolve. Cool the slurry to 20°C and stir. The resulting liquid is clear or an opalescent, mucilaginous colloidal mixture.

C3. Pour a few mL of the mixture prepared for identity test C2 onto a glass plate and allow the water to evaporate. A thin, self-sustaining film is formed.

**Loss on drying.** Dry at 105°C for 2 hours; it loses not more than 50 mg/g.

**Related substances**

Prepare fresh solutions and perform the tests without delay.

Carry out the test as described under 1.14.4 High performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilyle silica gel (5 μm).³

Use the following conditions for gradient elution:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
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</tr>
<tr>
<td>32–40</td>
<td>100</td>
<td>0</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

Maintain the column temperature at 35°C.

Prepare the following solutions using a mixture of 31 volumes of acetonitrile R and 69 volumes of water as solvent. For solution (1) mix a quantity of the dispersion equivalent to about 8 mg of misoprostol, accurately weighed, with 20.0 mL of acetonitrile R and sonicate for 10 minutes. Ensure that the temperature of the sonication bath is below room temperature to avoid degradation of misoprostol. Centrifuge and filter the supernatant. Evaporate 8.0 mL of the filtrate to dryness with nitrogen, dissolve the residue in 4.0 mL of solvent, using a vortex mixer. For solution (2) dilute 1 volume of solution (1) to 500 volumes. For solution (3) heat 2 mL of solution (1) in a water bath at 75°C for 1 hour.

³ An Ascentis Express C18 column was found suitable.
Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 200 nm. Store the samples at 4°C during analysis, using a cooled autosampler.

Inject 100 µL of solution (3). The test is not valid unless the peak-to-valley ratio (Hp/Hv) is at least 5.0, where Hp is the height above the extrapolated baseline of the peak due to impurity A (with a relative retention of about 0.95 with reference to misoprostol (retention time about 21 minutes) and Hv is the height above the extrapolated baseline at the lowest point of the curve separating the peak due to impurity A from the peak due to misoprostol.

Inject alternately 100 µL each of solutions (1) and (2).

The chromatogram obtained with solution (1) may show the following impurities at the following relative retentions with reference to misoprostol (retention time about 21 minutes): impurity E (1st peak): about 0.84; impurity E (2nd peak): about 0.86; impurity B (1st peak): about 0.90; impurity B (2nd peak): about 0.92; impurity A: about 0.95; impurity D: about 1.27; impurity C: about 1.37. Use also the chromatogram obtained with solution (3) to identify impurity A and C.

In the chromatogram obtained with solution (1):

- the sum of the areas of any peak corresponding to impurity A, B and E is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 0.76, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);
- the area of any other impurity peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
- the sum of the corrected area of any peak corresponding to impurity C and the areas of all other peaks, other than the principal peak, is not greater than 12.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Diastereoisomers

Carry out the test as described under 1.14.4 High performance liquid chromatography using a stainless steel column (15 cm × 2.1 mm) packed with silica gel for chromatography R (3.5 µm). ² As the mobile phase use a mixture of 4 volumes of 2-propanol R, 96 volumes of heptane R and 0.1 volume of trifluoroacetic acid R.

Prepare the following test solution using as a solvent a mixture of 4 volumes of 2-propanol R and 96 volumes of heptane R. Mix a quantity of the dispersion equivalent to about 2 mg of misoprostol with 5.0 mL of acetonitrile R and sonicate for 10 minutes ensuring that the temperature of the sonication bath is below room temperature to avoid degradation of misoprostol. Centrifuge and filter the supernatant. Evaporate 2.0 mL of the filtrate to dryness with nitrogen, dissolve the residue in 1.0 mL of solvent and vortex for 1 min.

Maintain the column temperature at 25°C.

Operate with a flow rate of 0.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 205 nm. Store the samples at 4°C during analysis, using a cooled autosampler.

² An Xbridge HILIC column was found suitable.
Inject 10 μL of the test solution.

The chromatogram shows two principal peaks due to misoprostol at retention times of about 14 and 16 minutes. The test is not valid unless the resolution between these two peaks is at least 2.0.

Measure the areas of the two peaks corresponding to misoprostol. The first peak of misoprostol is 45% to 55% of the sum of the areas of the two peaks due to misoprostol.

**Assay**

Carry out the test as described under [1.14.4 High performance liquid chromatography](#) using a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilyl silica gel (5 μm).³ As the mobile phase use a mixture of 45 volumes of acetonitrile R and 55 volumes of water.

Prepare the following solutions in the mobile phase. For solution (1) mix a quantity of the dispersion equivalent to about 4 mg of misoprostol, accurately weighed, with 200.0 mL of mobile phase and sonicate for 10 minutes. Ensure that the temperature of the sonication bath is below room temperature to avoid degradation of misoprostol. Filter a portion of this solution, discarding the first few mL of the filtrate. For solution (B) use 20 μg of misoprostol RS per mL.

Maintain the column temperature at 35°C.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 200 nm. Store the samples at 4°C during analysis, using a cooled autosampler.

Inject alternately 100 μL each of solutions (1) and (2). The test is not valid unless the symmetry factor of the peak due to misoprostol is between 0.8 and 1.5.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of misoprostol (C₂₂H₃₈O₅) in the dispersion, using the declared content of C₂₂H₃₈O₅ in misoprostol RS.

**Impurities**

The impurities limited by the requirements of this monograph are those listed in the monograph for Misoprostol.

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³ An Ascentis Express C18 column was found suitable.
Misoprostol tablets
*(Misoprostoli compressi)*

This is a draft proposal for *The International Pharmacopoeia* (Working document QAS/15.643, November 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

**Note from the Secretariat.** The following monograph is proposed for inclusion in *The International Pharmacopoeia.*

**Category.** Prostaglandin (PGE<sub>1</sub>), analogue.

**Storage.** Misoprostol tablets should be kept in tightly closed containers, protected from humidity.

**Additional information.** Strength in the current WHO Model list of essential medicines: 100 μg, 200 μg.

**Requirements**

Comply with the monograph for *Tablets.*

Misoprostol tablets contain not less than 90.0% and not more than 110.0% of the amount of C<sub>22</sub>H<sub>38</sub>O<sub>5</sub> stated on the label.

**Identity tests**

Either test A or B may be applied.

A. 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 from solution (1) corresponds to the retention time of the peak due to misoprostol in the chromatogram obtained from solution (2).

B. Carry out the test as described under 1.14.1 *Thin-layer chromatography* using silica gel R3 as the coating substance and a mixture of 8 volumes of toluene R, 2 volumes of ethyl acetate R, 1 volume of dehydrated ethanol R and 0.1 volume of glacial acetic acid R as the mobile phase, prepared immediately before use. Apply separately to the plate 100 μL of each of the following two solutions in dehydrated ethanol R. For solution (1) shake mechanically a quantity of the powdered tablets equivalent to 1 mg of misoprostol with 10 mL of dehydrated ethanol R for 10 minutes, filter and use the clear filtrate. For solution (2) use 0.1 mg of misoprostol RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air, expose it to the vapour of iodine R and examine the chromatogram in daylight.

The principal spot obtained with solution (1) corresponds in position, appearance and intensity to that obtained with solution (2).
Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms using as the dissolution medium 500 mL of purified water and rotating the paddle at 50 revolutions per minute. At 30 minutes withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature (solution (A)). For solution (B) dilute a suitable volume of solution (2) described under “Assay” with water R to obtain a concentration of 0.2 µg of misoprostol per mL for 100 µg tablets and 0.4 µg of misoprostol per mL for 200 µg tablets.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using the method described under “Assay”. Inject 250 µL each of solutions (A) and (B) and measure the areas of the peak responses corresponding to misoprostol obtained in the chromatograms. For each of the tablets tested calculate the total amount of misoprostol (C<sub>22</sub>H<sub>38</sub>O<sub>5</sub>) in the medium from the peak areas obtained using the declared content of C<sub>22</sub>H<sub>38</sub>O<sub>5</sub> in misoprostol RS. Use the requirements as described under 5.5 Dissolution test for solid oral dosage forms. Acceptance criteria to evaluate the results: the amount in solution is not less than 80% (Q) of the amount declared on the label.

Related substances
Prepare fresh solutions and perform the tests without delay.

Carry out the test as described under 1.14.4 High performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilyl silica gel (5 µm).<sup>1</sup>

Use the following conditions for gradient elution:
- Mobile phase A: mix 28 volumes of acetonitrile for chromatography R with 69 volumes of water R and 3 volumes of methanol R.
- Mobile phase B: mix 47 volumes of acetonitrile for chromatography R with 50 volumes of water R and 3 volumes of methanol R.

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<td>100</td>
<td>0</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

Maintain the column temperature at 35°C.

Prepare the following solutions using a mixture of 31 volumes of acetonitrile R and 69 volumes of water as solvent. For solution (1) weigh and powder 20 tablets, mix a quantity of the powder equivalent to about 2000 µg of misoprostol, accurately weighed, with 10.0 mL of acetonitrile R and sonicate for about 10 minutes. Ensure that the temperature of the sonication bath is below room temperature to avoid degradation of misoprostol. Centrifuge and filter the supernatant. Evaporate 6.0 mL of the filtrate to dryness with nitrogen and dissolve the residue in 3.0 mL of solvent, using a vortex mixer. For solution (2) dilute 1 volume of solution (1) to 200 volumes. For solution (3) heat 1 mL of solution (1) in a water bath at 75°C for 1 hour.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 200 nm. Store the samples at 4°C during analysis, using a cooled autosampler.

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<sup>1</sup> An Ascentis Express C18 column was found suitable.
Inject 200 µL of solution (3). The test is not valid unless the peak-to-valley ratio (Hp/Hv) is at least 5.0, where Hp is the height above the extrapolated baseline of the peak due to impurity A (with a relative retention of about 0.95 with reference to misoprostol (retention time about 21 minutes) and Hv is the height above the extrapolated baseline at the lowest point of the curve separating the peak due to impurity A from the peak due to misoprostol.

Inject alternately 200 µL each of solutions (1) and (2).

The chromatogram obtained with solution (1) may show the following impurities at the following relative retentions with reference to misoprostol (retention time about 21 minutes): impurity E (1st peak): about 0.84; impurity E (2nd peak): about 0.86; impurity B (1st peak): about 0.90; impurity B (2nd peak): about 0.92; impurity A: about 0.95; impurity D: about 1.27; impurity C: about 1.37. Use also the chromatogram obtained with solution (3) to identify impurity A and C.

In the chromatogram obtained with solution (1):
- the sum of the areas of any peak corresponding to impurity A, B and E is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 0.76, is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);
- the area of any peak corresponding to impurity D is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

### Assay

Carry out the test as described under \[1.14.4\] **High performance liquid chromatography** using a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilyl silica gel (5 µm). As the mobile phase use a mixture of 45 volumes of acetonitrile R and 55 volumes of water R.

Prepare the following solutions in the mobile phase. For solution (1) weigh and powder 20 tablets, weigh accurately a quantity of the powder equivalent to about 400 µg of misoprostol in a 20.0 mL volumetric flask. Add about 10 mL and sonicate for 10 minutes. Ensure that the temperature of the sonication bath is below room temperature to avoid degradation of misoprostol and make up to volume. Filter a portion of this solution, discarding the first few mL of the filtrate. For solution (2) use 20 µg of misoprostol RS per mL.

Maintain the column temperature at 35°C.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 200 nm. Store the samples at 4°C during analysis using a cooled autosampler.

Inject alternately 100 µL each of solutions (1) and (2).

The test is not valid unless the symmetry factor of the peak due to misoprostol is between 0.8 and 1.5.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of misoprostol \(C_{22}H_{38}O_5\) in the tablets, using the declared content of \(C_{22}H_{38}O_5\) in misoprostol RS.

### Impurities

The impurities limited by the requirements of this monograph are those listed in the monograph for Misoprostol.

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2 An Ascentis Express C18 column was found suitable.
Carbamazepine  
*(Carbamazepinum)*

This is a revised draft proposal for *The International Pharmacopoeia* (Working document QAS/15.608/Rev.1, December 2015).

The working document with line numbers and tracked changes is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/). Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

[Note from the Secretariat. It is proposed to revise the monograph on Carbamazepine in The International Pharmacopoeia. Comments are in particular sought as to whether the impurities listed under the section Impurities are degradation products or synthesis impurities.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

Molecular formula. \( \text{C}_{15}\text{H}_{12}\text{N}_{2}\text{O} \)

Relative molecular mass. 236.3

Graphic formula.

![Chemical Structure](image)

Chemical name. 5H-Dibenz[b,f]azepine-5-carboxamide; CAS Reg. No. 298-46-4.

Description. A white to almost white, crystalline powder.

Solubility. Very slightly soluble in water; sparingly soluble in acetone; soluble in ethanol (~750 g/L) TS; freely soluble in dichloromethane.

Category. Antiepileptic.

Additional information. Carbamazepine exhibits polymorphism. The acceptable crystalline form is anhydrous polymorph form III\(^1\). It corresponds to carbamazepine RS.

Storage. Carbamazepine should be kept in a tightly closed container.

Requirements

Definition. Carbamazepine contains not less than 98.0% and not more than 102.0% of \( \text{C}_{15}\text{H}_{12}\text{N}_{2}\text{O} \), calculated with reference to the dried substance.

Identity tests

- Either test A or any two of tests B, C and D may be applied.
  
  A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum obtained from the test substance without pretreatment is concordant with the spectrum obtained from carbamazepine RS or with the reference spectrum of carbamazepine.

  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 78 volumes of toluene R and 22 volumes of methanol R as the mobile phase. Apply separately to the plate 2 μL of each of the following three solutions, prepared using a mixture of equal volumes of ethanol (~750 g/L) TS and dichloromethane R. For solution (A) use 5 mg of the test substance per mL. For solution (B) use 5 mg of carbamazepine RS per mL. For solution (C) use 5 mg of carbamazepine RS and 5 mg of diazepam R per mL. After removing the plate from the chromatographic chamber allow it to dry in air and 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). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

  B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described under test B.1 but using a plate containing silica gel R5 as the coating substance.

  After removing the plate from the chromatographic chamber allow it to dry in air. Spray the plate with potassium dichromate TS3, then heat it at 105°C for 15 minutes. Examine the chromatogram in daylight.

  The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

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

  D. Heat 0.1 g with 2 mL of nitric acid (~1000 g/L) TS in a water-bath for 3 minutes; an orange-red colour is produced.

**Chlorides.** For the preparation of the test solution boil 3.57 g in 50 mL of water for 10 minutes, cool, again adjust the volume, filter. To 25 mL of the filtrate add 10 mL of nitric acid (~130 g/L) TS and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.14 mg/g.

**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 10 μg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant weight at 105°C; it loses not more than 5.0 mg/g.
**Acidity or alkalinity.** To 1.0 g add 20 mL of carbon-dioxide-free water R, shake for 15 minutes and filter. To 10 mL of the filtrate add 0.05 mL of phenolphthalein/ethanol TS and 0.5 mL of carbonate-free sodium hydroxide (0.01 mol/L) VS; the solution is red. Add 1.0 mL of hydrochloric acid (0.01 mol/L) VS; the solution is colourless. Add 0.15 mL of methyl red/ethanol TS; the solution is red.

**Related substances.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the chromatographic conditions given under “Assay, method B”.

Prepare the following solutions. For solution (1) dissolve about 75 mg of the test substance in 25 mL of methanol R, sonicate and dilute to 50 mL with water R. For solution (2) dilute 1 volume of solution (1) to 1000 volumes with a mixture of equal volumes of methanol R and water R. For solution (3) use a solution containing 10 µg of carbamazepine RS and 10 µg of carbamazepine impurity A RS per mL of a mixture of equal volumes of methanol R and water R. For solution (4) use a solution containing 10 µg of iminodibenzyl R (impurity E) per mL.

Inject 20 µL of solution (3). The test is not valid unless the resolution between carbamazepine and carbamazepine impurity A is not less than 1.7.

Inject alternately 20 µL each of solutions (1), (2) and (4). Record the chromatograms for eight times the retention time of carbamazepine. In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to carbamazepine (retention time about 9 minutes): impurity A about 0.9; impurity D about 2.1; and impurity E about 3.5. Use the chromatogram obtained with solution (3) to identify the peak due to impurity A and the chromatogram obtained with solution (4) to identify the peak due to impurity E.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 2.8, is not greater than 1.5 times the area of the principal peak 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 0.4, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
- the area of any peak corresponding to impurity E, when multiplied by a correction factor of 2.7, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);
- the area of any other impurity peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.10%);
- the sum of the corrected areas of the peaks corresponding to impurity A, impurity D and impurity E and the areas of all other peaks, other than the principal peak, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (2) (0.05%).

**Assay**

- Either method A or B may be applied.

**A.** Dissolve about 0.1 g, accurately weighed, in sufficient ethanol (~750 g/L) TS to produce 100.0 mL. Dilute 10.0 mL of this solution to 100.0 mL with the same solvent, and again dilute 10.0 mL of this dilution to 100.0 mL with ethanol (~750 g/L) TS. Measure the
absorbance (1.6) of a 1 cm layer of the resulting solution at the maximum at about 285 nm. Calculate the percentage content of $C_{15}H_{12}N_2O$ in the substance being tested, using the absorptivity value of 49.0 ($A_{10}^{1%} = 490$).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded cyanopropyl groups (10 μm). As the mobile phase use a mixture of 30 volumes of tetrahydrofuran R, 120 volumes of methanol R, 850 volumes of water R, 0.2 volume of anhydrous formic acid R and 0.5 volume of triethylamine R.

Operate with a flow rate of 2.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 230 nm.

Prepare the following solutions. For solution (1) dissolve about 10 mg of the test substance, accurately weighed, in 25 mL of methanol R, sonicate and dilute to 50.0 mL with water R. For solution (2) use carbamazepine RS to obtain a solution containing 0.2 mg per mL of equal volumes of methanol R and water R.

Inject alternately 20 μL each of solution (1) and (2). The assay is not valid unless the column efficiency (N) is at least 5000, determined for the peak due to carbamazepine in the chromatogram obtained with solution (2).

Measure the areas of the peaks corresponding to carbamazepine obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of carbamazepine ($C_{15}H_{12}N_2O$) in the samples using the declared content of $C_{15}H_{12}N_2O$ in carbamazepine RS.

Impurities

A. 10,11-dihydro-dibenzo[b,f]azepine-5H-carboxamide (10,11-dihydrocarbamazepine) (synthesis impurity)

B. 9-methylacridine

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2 A Nucleosil 100-10 CN column was found suitable.
C. (5\textit{H}-dibenzo[\textit{b,f}]azepin-5-ylcarbonyl)urea (\textit{N-carbamoylcarbamazepine})

D. 5\textit{H}-dibenzo[\textit{b,f}]azepine (iminostilbene)

E. 10,11-dihydro-5\textit{H}-dibenzo[\textit{b,f}]azepine (iminodibenzyl)

F. 5\textit{H}-dibenzo[\textit{b,f}]azepine-5-carbonyl chloride (5-chlorocarbonyliminostilbene)

G. 10-bromo-5\textit{H}-dibenzo[\textit{b,f}]azepine-5-carboxamide (10-bromocarbamazepine)

\textbf{Reagent to be established}

Diazepam R

Diazepam of a suitable quality should be used.
Carbamazepine tablets
*(Carbamazepini compressi)*

This is a revised draft proposal for *The International Pharmacopoeia* (Working document QAS/15.632/ Rev.1, December 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, CH-1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

*Note from the Secretariat. It is proposed to revise the monograph on Carbamazepine tablets in The International Pharmacopoeia.*

**Category.** Antiepileptic.

**Storage.** Carbamazepine tablets should be kept in a tightly closed container.

**Additional information.** Strength in the current WHO Model list of essential medicines (EML): 100 mg, 200 mg. Strength in the current WHO EML for children: 100 mg, 200 mg.

**Requirements**

Complies with the monograph for *Tablets*.

**Definition.** Carbamazepine tablets contain not less than 90.0% and not more than 110.0% of the amount of carbamazepine \((C_{15}H_{12}N_2O)\) stated on the label.

**Identity tests**

- Either test A alone or any two of tests B, C and D may be applied

Transfer a quantity of the powdered tablets equivalent to about 0.25 g of carbamazepine to a 50 mL beaker, add 15 mL of acetone R and boil the solution. Filter while hot, evaporate the filtrate to dryness on a water-bath and dry at 80°C for 30 minutes. Dissolve in acetone R, allow to recrystallize and use the crystals for the following tests.

A. Carry out the examination with the crystals as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from carbamazepine RS or with the reference spectrum of carbamazepine.

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 78 volumes of toluene R and 22 volumes of methanol R as the mobile phase. Apply separately to the plate 2 µL of each of the following three solutions, prepared using as a solvent a mixture of equal volumes of ethanol (~750 g/L) TS and dichloromethane R. For solution (A) use 5 mg of the crystals per mL. For solution (B) use 5 mg of carbamazepine RS per mL. For solution (C) use 5 mg of carbamazepine RS and 5 mg of diazepam R per mL. After removing the plate from the chromatographic chamber allow it to dry in air and 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). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described under test B.1 but using a plate containing silica gel R5 as the coating substance.

After removing the plate from the chromatographic chamber allow it to dry in air. Spray the plate with potassium dichromate TS3 then heat the plate at 105°C for 15 minutes. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

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

D. Heat 0.1 g of the crystals with 2 mL of nitric acid (~1000 g/L) TS in a water-bath for 3 minutes; an orange-red colour is produced.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under Assay B.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 0.15 g of carbamazepine into a 100 mL volumetric flask, shake with 50 mL of methanol R for about 15 minutes, dilute to volume with water R and filter. For solution (2) dilute 1 volume of solution (1) to 500 volumes with equal volumes of methanol R and water R. For solution (3) use a solution containing 10 µg of carbamazepine RS and 10 µg of carbamazepine impurity A RS per mL of a mixture of equal volumes of methanol R and water R.

Inject 20 µL of solution (3). The test is not valid unless the resolution between carbamazepine and carbamazepine impurity A is not less than 1.7.

Inject alternately 20 µL each of solution (1) and solution (2). Record the chromatograms for four times the retention time of carbamazepine. In the chromatogram obtained with solution (1) the following impurity, if present, is eluted at the following relative retention with reference to carbamazepine (retention time about 9 minutes): impurity D about 2.1.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 0.4, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

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 1% solution of sodium dodecyl sulfate R in water and rotating the paddle at 75 revolutions per minute. At 60 minutes withdraw a sample of about 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. Measure the absorbance (1.6) of a 1 cm layer of the filtered sample, suitably diluted if necessary, at the maximum at about 288 nm.
For each of the tablets tested calculate the amount of carbamazepine ($C_{15}H_{12}N_2O$) in the medium using the absorptivity value of 49.0 ($A_{1%}^{1cm} = 490$). Evaluate the results as described under 5.5 Dissolution test for solid dosage forms, Acceptance criteria.

The amount of carbamazepine in solution for each tablet is not less than 75% (Q) of the amount declared on the label.

**Assay**

- Either method A or B may be applied.

  **A.** Weigh and powder 20 tablets. To an accurately weighed quantity of the powder, containing about 0.06 g of carbamazepine, add 25 mL of ethanol (~750 g/L) TS and boil for a few minutes. Stir the hot mixture in a closed flask for 10 minutes and filter. Wash the flask with ethanol (~750 g/L) TS, filter and dilute the cooled filtrate with sufficient ethanol (~750 g/L) TS to produce 100.0 mL. Dilute 5.0 mL to 250.0 mL with the same solvent.

  Measure the absorbance of a 1 cm layer of the solution at the maximum at about 285 nm against a solvent cell containing ethanol (~750 g/L) TS. Calculate the content of $C_{15}H_{12}N_2O$ using the absorptivity value of 49.0 ($A_{1%}^{1cm} = 490$).

  **B.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm), packed with particles of silica gel, the surface of which has been modified with chemically-bonded cyanopropyl groups (10 μm). As the mobile phase use a mixture of 30 volumes of tetrahydrofuran R, 120 volumes of methanol R, 850 volumes of water R, 0.2 volume of anhydrous formic acid R and 0.5 volume of triethylamine R.

  Operate with a flow rate of 2.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 230 nm.

  Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.1 g of carbamazepine to a 100 mL volumetric flask, add 50 mL of methanol R and sonicate for about 15 minutes. Allow to cool to room temperature, make up to volume with water R and filter the solution. Dilute 10.0 mL of the filtrate to 50.0 mL with a mixture of equal volumes of methanol R and water R. For solution (2) use carbamazepine RS to obtain a solution containing 0.2 mg per mL of equal volumes of methanol R and water R.

  Inject alternately 20 μL each of solution (1) and (2). The assay is not valid unless the column efficiency is at least 5000, determined for the peak due to carbamazepine in the chromatogram obtained with solution (2).

  Measure the areas of the peaks corresponding to carbamazepine and calculate the content of carbamazepine ($C_{15}H_{12}N_2O$) in the tablets using the declared content of $C_{15}H_{12}N_2O$ in carbamazepine RS.

**Impurities.** The impurity limited by the requirements of this monograph is impurity D listed in the monograph for carbamazepine.

**Reagent to be established**

Diazepam R
Diazepam of a suitable quality should be used.

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1 A Nucleosil 100-10 CN column was found suitable.
Carbamazepine chewable tablets
*Carbamazepini compressi manducabili*

This is a revised draft proposal for *The International Pharmacopoeia* (Working document QAS/15.609/Rev.1, December 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.

Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, CH-1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. The following draft monograph on Carbamazepine chewable tablets is proposed for inclusion in The International Pharmacopoeia.]

**Category.** Antiepileptic.

**Storage.** Carbamazepine chewable tablets should be kept in a tightly closed container.

**Additional information.** Strengths in the current WHO Model list of essential medicines (EML): 100 mg, 200 mg. Strengths in the current WHO EML for children: 100 mg, 200 mg.

**Requirements**

Complies with the monograph for Tablets.

**Definition.** Carbamazepine chewable tablets contain Carbamazepine in a suitable basis that may contain suitable flavouring agents. Carbamazepine chewable tablets contain not less than 90.0% and not more than 110.0% of the amount of carbamazepine (\(C_{15}H_{12}N_2O\)) stated on the label.

**Identity tests**

- Either test A alone or any two of tests B, C and D may be applied

Transfer a quantity of the powdered tablets equivalent to about 0.25 g of carbamazepine to a 50 mL beaker, add 15 mL of acetone R and boil the solution. Filter while hot, evaporate the filtrate to dryness on a water-bath and dry at 80°C for 30 minutes. Dissolve in acetone R, allow to recrystallize and use the crystals for the following tests.

**A.** Carry out the examination with the crystals as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from carbamazepine RS or with the reference spectrum of carbamazepine.

**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 78 volumes of toluene R and 22 volumes of methanol R as the mobile phase. Apply separately to the plate 2 μL of each of the following three solutions, prepared using as a solvent a mixture of equal volumes of ethanol (~750 g/L) TS and dichloromethane R. For solution (A) use 5 mg of the crystals per mL. For solution (B) use 5 mg of carbamazepine RS per mL. For solution (C) use 5 mg of carbamazepine RS and 5 mg of diazepam R per mL. After removing the plate from the chromatographic chamber allow it to dry in air and 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). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described under test B.1 but using a plate containing silica gel R5 as the coating substance.

After removing the plate from the chromatographic chamber allow it to dry in air. Spray the plate with potassium dichromate TS3 then heat the plate at 105°C for 15 minutes. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

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

D. Heat 0.1 g of the crystals with 2 mL of nitric acid (~1000 g/L) TS in a water-bath for 3 minutes; an orange-red colour is produced.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under Assay B.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 0.15 g of carbamazepine into a 100 mL volumetric flask, shake with 50 mL of methanol R for about 15 minutes, dilute to volume with water R and filter. For solution (2) dilute 1 volume of solution (1) to 500 volumes with equal volumes of methanol R and water R. For solution (3) use a solution containing 10 µg of carbamazepine RS and 10 µg of carbamazepine impurity A RS per mL of a mixture of equal volumes of methanol R and water R.

Inject 20 µL of solution (3). The test is not valid unless the resolution between carbamazepine and carbamazepine impurity A is not less than 1.7.

Inject alternately 20 µL each of solution (1) and solution (2). Record the chromatograms for four times the retention time of carbamazepine. In the chromatogram obtained with solution (1) the following impurity, if present, is eluted at the following relative retention with reference to carbamazepine (retention time about 9 minutes): impurity D about 2.1.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 0.4, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

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 1% solution of sodium dodecyl sulfate R in water and rotating the paddle at 75 revolutions per minute. At 60 minutes withdraw a sample of about 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. Measure the absorbance (1.6) of a 1 cm layer of the filtered sample, suitably diluted if necessary, at the maximum at about 288 nm.
For each of the tablets tested calculate the amount of carbamazepine \((C_{15}H_{12}N_2O)\) in the medium using the absorptivity value of 49.0 \((\lambda_{1\%} = 490)\). Evaluate the results as described under 5.5 Dissolution test for solid dosage forms, Acceptance criteria.

The amount of carbamazepine in solution for each tablet is not less than 75% \((Q)\) of the amount declared on the label.

**Assay**

- Either method A or B may be applied.

**A.** Weigh and powder 20 tablets. To an accurately weighed quantity of the powder, containing about 0.06 g of carbamazepine, add 25 mL of ethanol (~750 g/L) TS and boil for a few minutes. Stir the hot mixture in a closed flask for 10 minutes and filter. Wash the flask with ethanol (~750 g/L) TS, filter and dilute the cooled filtrate with sufficient ethanol (~750 g/L) TS to produce 100.0 mL. Dilute 5.0 mL to 250.0 mL with the same solvent.

Measure the absorbance of a 1 cm layer of the solution at the maximum at about 285 nm against a solvent cell containing ethanol (~750 g/L) TS. Calculate the content of \(C_{15}H_{12}N_2O\) using the absorptivity value of 49.0 \((\lambda_{1\%} = 490)\).

**B.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm), packed with particles of silica gel, the surface of which has been modified with chemically-bonded cyanopropyl groups (10 μm).\(^1\) As the mobile phase use a mixture of 30 volumes of tetrahydrofuran R, 120 volumes of methanol R, 850 volumes of water R, 0.2 volume of anhydrous formic acid R and 0.5 volume of triethylamine R.

Operate with a flow rate of 2.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 230 nm.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.1 g of carbamazepine to a 100 mL volumetric flask, add 50 mL of methanol R and sonicate for about 15 minutes. Allow to cool to room temperature, make up to volume with water R and filter the solution. Dilute 10.0 mL of the filtrate to 50.0 mL with a mixture of equal volumes of methanol R and water R. For solution (2) use carbamazepine RS to obtain a solution containing 0.2 mg per mL of equal volumes of methanol R and water R.

Inject alternately 20 µL each of solution (1) and (2). The assay is not valid unless the column efficiency is at least 5000, determined for the peak due to carbamazepine in the chromatogram obtained with solution (2).

Measure the areas of the peaks corresponding to carbamazepine and calculate the content of carbamazepine \((C_{15}H_{12}N_2O)\) in the tablets using the declared content of \(C_{15}H_{12}N_2O\) in carbamazepine RS.

**Impurities.** The impurity limited by the requirements of this monograph is impurity D listed in the monograph for carbamazepine.

**Reagent to be established**

Diazepam R
Diazepam of a suitable quality should be used.

\(^{1}\) A Nucleosil 100-10 CN column was found suitable.
Carbamazepine oral suspension
(Carbamazepini suspensio peroralis)

This is a revised draft proposal for The International Pharmacopoeia (Working document QAS/15.610/Rev.1, December 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.
Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, CH-1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. The following draft monograph on Carbamazepine oral solution is proposed for inclusion in The International Pharmacopoeia.]

Category. Antiepileptic.

Storage. Carbamazepine oral suspension should be kept in tightly closed, light-resistant containers, protected from freezing and from excessive heat.

Additional information. Strength in the current WHO Model list of essential medicines (EML): 100 mg per 5 mL. Strength in the current WHO EML for children: 100 mg per 5 mL.

Requirements

Complies with the monograph for Liquid preparations for oral use.

Definition. Carbamazepine oral suspension is a suspension of Carbamazepine in a suitable vehicle, which may be flavoured. It contains not less than 90.0% and not more than 110.0% of the amount of carbamazepine (C\textsubscript{15}H\textsubscript{12}N\textsubscript{2}O) stated on the label.

Identity tests

• Either test A alone or any two of tests B, C and D may be applied.

Transfer a quantity of the oral suspension equivalent to about 0.25 g of carbamazepine to a centrifuge tube, centrifuge and wash the precipitate with two quantities of 10 mL of water R. Dissolve the precipitate as completely as possible in 10 mL of dichloromethane R, filter and evaporate the filtrate to dryness in air, dry the residue at 80°C for 30 minutes and use it for the following tests.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from carbamazepine RS or with the reference spectrum of carbamazepine.

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 78 volumes of toluene R and 22 volumes of methanol R as the mobile phase. Apply separately to the plate 2 μL of each of the following three solutions, prepared using as a solvent in a mixture of equal volumes of ethanol (~750 g/L) TS and dichloromethane R.

For solution (A) use 5 mg of the residue per mL. For solution (B) use 5 mg of carbamazepine RS per mL. For solution (C) use 5 mg of carbamazepine RS and
5 mg of diazepam R per mL. After removing the plate from the chromatographic chamber allow it to dry in air and 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). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described under test B.1 but using a plate containing silica gel R5 as the coating substance.

After removing the plate from the chromatographic chamber allow it to dry in air. Spray the plate with potassium dichromate TS3 then heat the plate at 105°C for 15 minutes. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B). The test is not valid unless the chromatogram obtained with solution (C) shows 2 clearly separated spots.

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

D. Heat 0.1 g of the residue with 2 mL of nitric acid (~1000 g/L) TS in a water-bath for 3 minutes; an orange-red colour is produced.

**pH value** (1.13). pH of the oral suspension, 3.5–4.5.

**Related substances.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under Assay B.

Prepare the following solutions. For solution (1), if necessary, shake the container of oral suspension to resuspend any settled material. Transfer a quantity of it, containing about 0.2 g of Carbamazepine, into a 100 mL volumetric flask, add 50 mL of methanol R and sonicate for about 15 minutes. Allow the suspension to cool to room temperature and dilute to volume with water R. Centrifuge 10 mL of the suspension. Transfer 5.0 mL of the supernatant to a 10 mL volumetric flask and dilute to volume with equal volumes of methanol R and water R. For solution (2) dilute 1 volume of solution (1) to 500 volumes with equal volumes of methanol R and water R. For solution (3) use a solution containing 10 µg of carbamazepine RS and 10 µg of carbamazepine impurity A RS per mL of a mixture of equal volumes of methanol R and water R.

Inject 20 µL of solution (3). The test is not valid unless the resolution between carbamazepine and carbamazepine impurity A is not less than 1.7.

Inject alternately 20 µL each of solution (1) and solution (2). Record the chromatograms for four times the retention time of carbamazepine. In the chromatogram obtained with solution (1) the following impurity, if present, is eluted at the following relative retention with reference to carbamazepine (retention time about 9 minutes): impurity D about 2.1.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 0.4, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).
Assay

- Either method A or B may be applied.

A. If necessary, shake the container of oral suspension to resuspend any settled material. Transfer an accurately weighed quantity of it, containing about 0.1 g of Carbamazepine, to a 100 mL volumetric flask, add about 50 mL of ethanol (~750g/L) TS and sonicate for about 15 minutes. Allow the suspension to cool to room temperature, dilute with the same solvent to volume and filter the solution. Dilute 1.0 mL of the filtrate to 100.0 mL with ethanol (~750g/L) TS.

Measure the absorbance of a 1 cm layer of the solution at the maximum at about 285 nm against a solvent cell containing ethanol (~750 g/L) TS. Determine the weight per mL (1.3.1) of the oral suspension and calculate the content of C_{15}H_{12}N_{2}O, weight in volume, of the oral suspension using the absorptivity value of 49.0 (A_{1\%} = 490).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm), packed with particles of silica gel, the surface of which has been modified with chemically-bonded cyanopropyl groups (10 μm). As the mobile phase use a mixture of 30 volumes of tetrahydrofuran R, 120 volumes of methanol R, 850 volumes of water R, 0.2 volume of anhydrous formic acid R and 0.5 volume of triethylamine R.

Operate with a flow rate of 2.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 230 nm.

Prepare the following solutions. For solution (1), if necessary, shake the container of oral suspension to resuspend any settled material. Transfer an accurately weighed quantity of it, containing about 200 mg of Carbamazepine, to a 100 mL volumetric flask, add 50 mL of methanol R and sonicate for about 15 minutes. Allow the suspension to cool to room temperature, dilute to volume with water R and filter the solution. Dilute 5.0 mL of the filtrate to 50.0 mL with equal volumes of methanol R and water R. For solution (2) use carbamazepine RS to obtain a solution containing 0.2 mg per mL of equal volumes of methanol R and water R.

Inject alternately 20 μL each solution (1) and (2). The assay is not valid unless the column efficiency is at least 5000, determined for the peak due to carbamazepine in the chromatogram obtained with solution (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2). Determine the weight per mL (1.3.1) of the oral suspension and calculate the content of carbamazepine (C_{15}H_{12}N_{2}O), weight in volume, of the oral suspension using the declared content of C_{15}H_{12}N_{2}O in carbamazepine RS.

Impurities. The impurity limited by the requirements of this monograph is impurity D listed in the monograph for carbamazepine.

Reagent to be established

Diazepam R
Diazepam of a suitable quality should be used.

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1. A Nucleosil 100-10 CN column was found suitable.