DACARBAZINE
(DACARBAZINUM)

Draft proposal for The International Pharmacopoeia
(August 2017)
DRAFT FOR COMMENT

Should you have any comments on this draft, please send these to Dr Herbert Schmidt,
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by 30 September 2017.
In order to speed up the process for receiving draft monographs and for sending
comments, please let us have your email address (to bonnyw@who.int) and we will add it
to our electronic mailing list. Please specify if you wish to receive monographs.
### SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/17.711: DACARBAZINE

<table>
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<tr>
<td>Revision drafted following requests from the custodian centre for ICRS</td>
<td>February 2017</td>
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<tr>
<td>Discussion at the consultation on new medicines, quality control and laboratory standards</td>
<td>May 2017</td>
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<tr>
<td>Draft revision of the monograph sent out for public consultation</td>
<td>August–September 2017</td>
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<td>Presentation to the WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
<td>October 2017</td>
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[Note from the Secretariat. It is proposed to revise the monograph on Dacarbazine based on information found in the United States Pharmacopoeia, in the European Pharmacopoeia and in the scientific literature.]

Changes from the current monograph are indicated in the text by insert or delete.
DACARBAZINE
(DACARBAZINUM)

Molecular formula. \( \text{C}_6\text{H}_{10}\text{N}_6\text{O} \)

Relative molecular mass. 182.2

Graphic formula

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{N} \quad \text{N} \\
& \quad \text{H} \\
\text{H}_3\text{C} & \quad \text{N} \quad \text{N} \\
& \quad \text{O}
\end{align*}
\]

Chemical name. \( 5-\text{(3,3-dimethyltriaz-1-en-1-yl)}-1H\text{-imidazole-4-carboxamide} \); CAS Reg. No. 4342-03-4.

Description. A colourless or pale yellow, crystalline powder.

Solubility. Slightly soluble in water and ethanol (~750 g/L) TS, practically insoluble in Dichloromethane R.

Category. Cytotoxic drug.

Storage. Dacarbazine should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 8 °C.

Additional information. CAUTION: Dacarbazine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Dacarbazine contains not less than 98.5–99.0% and not more than 101.0–102.0% of \( \text{C}_6\text{H}_{10}\text{N}_6\text{O} \), calculated with reference to the anhydrous dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied. 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 dacarbazine RS or with the reference spectrum of dacarbazine.

B. The absorption spectrum of a 6 µg/mL solution in hydrochloric acid (0.1 mol/L) VS hydrochloric acid (~4 g/L) TS, when observed between 200 230 nm and 400 350 nm, exhibits a maximum at about 323 nm and a pronounced shoulder at 275 nm. The absorbance of a 1 cm layer at the maximum wavelength of 323 nm is about 0.64.

C. Dissolve 25 mg in 5 mL of water, add 1 drop of cobalt(II) chloride (30 g/l) TS and 1 drop of ammonia (~100 g/l) TS; a violet-red solution is produced.

D. Dissolve 25 mg in 5 mL of hydrochloric acid (~70 g/l) TS, add about 0.2 g of zinc R powder and allow to stand for 5 minutes. Filter, and to the filtrate add 3 drops of sodium nitrite (10 g/l) TS and 0.5 mL of ammonium sulfamate (5 g/l) TS. After the reaction has subsided add 5 drops of N-(1 naphthyl)ethylenediamine hydrochloride/ethanol TS; a deep red solution is produced.

C. 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 glacial acetic acid R, water R and butanol R (1:2:5 V/V/V) as the mobile phase. Apply separately to the plate 10 µL of each of the following 2 solutions in methanol R: containing (A) 0.4 mg of the test substance per mL and (B) 0.4 mg of dacarbazine RS per mL. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber allow it to dry in air or in a current of air. Examine the chromatogram under ultraviolet light (254 nm). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to dacarbazine in the chromatogram obtained with solution (B).

Clarity and colour of solution. A solution of 0.20 g in 10 mL of citric acid (20 g/l) TS is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11.1 Colour of liquids. Dissolve 0.25 g of the test substance in a 210 g/L solution of citric acid R and dilute to 25.0 mL with the same solution. The solution is clear and not more intensely coloured than reference solution BY6, when analysed as described under 1.11.2 Degree of coloration of liquids, Method II.

[Note from the Secretariat. Chapter 1.11 Colour of liquids is currently under revision. Reference is already made to a new test procedure to be added under the section 1.11.2 Degree of coloration of liquids.]

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 60° C to constant mass under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer
method, method A. Use 1.00 g of the test substance. The water content is not more than 5 mg/g.

**Impurity D**

Carry out the test as described under 1.14.5 *Gas chromatography* with the apparatus equipped with an injection system for the performance of static head-space chromatography. Use a fused-silica capillary column 30 m long and 0.53 mm in internal diameter coated with base-deactivated polyethyleneglycol R (film thickness: 1.0 µm).

As a detector use a flame ionization detector.

Use helium for chromatography R as the carrier gas with a flow rate of 13 mL/min.

Use a split ratio of 1:1.

The following head-space injection conditions may be used:

<table>
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<th>Equilibration temperature (°C)</th>
<th>60</th>
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<tbody>
<tr>
<td>Equilibration time (min)</td>
<td>10</td>
</tr>
<tr>
<td>Transfer line temperature (°C)</td>
<td>90</td>
</tr>
<tr>
<td>Pressurization time (s)</td>
<td>30</td>
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<tr>
<td>Injection volume (mL)</td>
<td>1</td>
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</table>

Maintain the temperature of the column at 35 °C for 3 minutes, then raise the temperature within 8 minutes to 165 °C, maintaining the temperature of the injection port at 180 °C and that of the flame ionization detector at 220 °C.

Prepare the following solutions. For solution (1) transfer 0.200 g of the test substance into a 20 mL headspace vial and firmly attach the septum and cap. Using a 10 µL syringe, inject 5 µL of water R into the vial. For solution (2) dilute 1.00 g of dimethylamine R (impurity D) to 100.0 mL with water R. Firmly attach the septum and cap to a 20 mL vial. Using a 10 µL syringe, inject 10 µL of solution (2) into the vial. For solution (3) dilute 1.00 g of trimethylamine R to 100.0 mL with water R. Firmly attach the septum and cap to a 20 mL vial. Using a 10 µL syringe, inject 10 µL of solution (2) and 10 µL of solution (3) into the vial.

Analyse solution (3). The test is not valid unless the resolution between the peaks due to impurity D and trimethylamine is at least 2.5.

Analyse solution (1) and (2).
In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.05%).

**Related substances**

Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and 5 volumes of 1-butanol R, 2 volumes of water and 1 volume of acetic acid (~300 g/l) TS as the mobile phase. Apply separately to the plate 5 μl of each of the 3 following solutions in methanol R containing (A) 0.04 g of Dacarbazine per mL, (B) 0.4 mg of dacarbazine related compound impurity A RS per mL, and (C) 0.4 mg of dacarbazine related compound impurity B RS 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).

Any spot obtained with solution A, other than the principal spot, is not more intense or greater in size than that obtained with solution B (1%) and solution C (1%). Use freshly prepared solutions and protect them from light.

- Perform test A and B.

**A.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsil groups (5 μm).

Prepare the mobile phase by dissolving 15.63 g of docusate sodium R in a solution containing 2.33 g of glacial acetic acid R per L of water R and dilute to 1000 mL with the same solution. Prepare the mobile phase freshly every day and flush the column with a mixture of equal volumes of methanol R and water R after all tests have been completed or at the end of the day, for at least 2 hours.

Operate with a flow rate of 1.2 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Prepare the following solutions in distilled water R. For solution (1) dissolve 50.0 mg of the test substance and 75 mg of citric acid R and dilute to 5.0 mL. For solution (2) dissolve 5.0 mg of dacarbazine impurity A RS and dilute to 50.0 mL. Dilute 5.0 mL of this solution to 25.0 mL.

Inject alternately 25 μL each of solution (1) and (2). Record the chromatograms for about 3 times the retention time of impurity A (retention time about 3 minutes).
In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.2%);
- the area of any other impurity peak eluting after impurity A is not greater than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with solution (2) (0.10%).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under test A with the following modifications.

Prepare the mobile phase by mixing 45 volumes of the mobile phase described under test A with 55 volumes of methanol R.

Prepare the following additional solution in distilled water R. For solution (3) dissolve 5.0 mg of dacarbazine impurity B RS, add 0.5 mL of solution (1) and dilute to 10.0 mL. Dilute 1.0 mL of this solution to 50.0 mL.

Inject alternately 10 μL each of solution (1) and (3). Record the chromatograms for about twice the retention time of dacarbazine (retention time about 12 minutes). The test is not valid unless the resolution between the peaks due to impurity B (with a relative retention of about 0.7) and dacarbazine is at least 1.5.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);
- the area of any other impurity peak is not greater than the area of the peak due to dacarbazine in the chromatogram obtained with solution (3) (0.10%);
- the sum of the areas of all impurities peaks is not greater than 5 times the area of the peak due to dacarbazine in the chromatogram obtained with solution (3) (0.5%). Disregard any peak with an area less than 0.5 times the area of the peak due to dacarbazine in the chromatogram obtained with solution (3) (0.05%).

Assay

- Protect the solutions from light throughout the assay.

Dissolve about 30 mg, accurately weighed, in sufficient hydrochloric acid (0.1 mol/l) VS to produce 50 mL of stock solution. For solution S dilute 1.0 mL of the stock
solution to 100 mL with hydrochloric acid (0.1 mol/l) VS. For solution S₂-dilute a further 1.0 mL aliquot of the stock solution to 100 mL with phosphate buffer, pH 7.0, TS. Measure the absorbance of a 1-cm layer of solution S₂ at the maximum at about 323 nm against a solvent cell containing hydrochloric acid (0.1 mol/l) VS. Measure the absorbance of a 1-cm layer of solution S₁ at the maximum at about 329 nm against a solvent cell containing phosphate buffer, pH 7.0, TS. Calculate the percentage content of C₆H₁₀N₆O.

Dissolve about 0.150 g, accurately weighed, in 30 mL of anhydrous acetic acid R. Titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 18.22 mg of C₆H₁₀N₆O.

Impurities

A. 1,5-dihydro-4H-imidazo[4,5-P]-1,2,3-triazin-4-one (2-azahypoxanthine) (degradation product)

B. 5-amino-1H-imidazole-4-carboxamide (synthesis-related impurity)

[Note from the Secretariat. Chemical structure to be added.]

C. 5-diazenyl-1H-imidazole-4-carboxamide

[Note from the Secretariat. Chemical structure to be added.]

D. N-methylmethanamine

Reagents to be established

Water, distilled R

Water R prepared by distillation.

Polyethyleneglycol, base-deactivated R
Cross-linked, base-deactivated polyethyleneglycol, specially designed to be used as a stationary phase for gas chromatographic analysis of amine.