Consultation documents

_The International Pharmacopoeia_

**Flucytosinum**

**Flucytosine**

This is a draft proposal for _The International Pharmacopoeia_ (Working document QAS/14.599, December 2014).

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. It is proposed to revise the monograph on Flucytosine in The International Pharmacopoeia.]

[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.** C₄H₄FN₃O

**Relative molecular mass.** 129.1

**Graphic formula.**

![Graphic formula of Flucytosine](image)

**Chemical name.** 5-Fluorocytosine; 4-amino-5-fluoro-2(1H)-pyrimidinone; CAS Reg. No. 2022-85-7.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Sparingly soluble in water; slightly soluble in ethanol (~750 g/L) TS; practically insoluble in ether R.

**Category.** Antifungal.

**Storage.** Flucytosine should be kept in a tightly closed container, protected from light.

**Additional information.** Flucytosine melts at about 295°C.
Requirements

Definition. Flucytosine contains not less than 99.0% and not more than 101.0% of \( \text{C}_4\text{H}_8\text{FN}_3\text{O} \), calculated with reference to the dried substance.

Identity tests

• Either tests 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 flucytosine RS or with the reference spectrum of flucytosine.

B. The absorption spectrum of a 5.0 µg/mL solution in hydrochloric acid (0.1 mol/L) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 286 nm; the absorbance of a 1 cm layer at this wavelength is about 0.36.

C. See the test described below under Related Substances, Test A. The principal spot obtained with solution (1) corresponds in position, appearance and intensity with that obtained with solution (2).

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 using a platinum crucible; not more than 20 µg/g.

Clarity and colour of solution. Dissolve 0.5 g in carbon dioxide-free water R and dilute to 50 mL with the same solvent. This solution is clear and not more intensely coloured than standard colour solution Yw0 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Determine the sulfated ash content as described under (2.3) using a platinum crucible; not more than 1 mg/g.

Loss on drying. Dry to constant weight at 105°C; it loses not more than 10 mg/g.

Fluorides. Prepare and store all solutions in plastic containers.

Prepare the following buffer solution. Dissolve 58 g of sodium chloride R in 500 mL of water R. Add 57 mL of glacial acetic R and 200 mL of a 100 g/L solution of cyclohexylenedinitrilotetra-acetic acid R in sodium hydroxide (~40 g/L) TS. Adjust the pH to 5.0–5.5 with sodium hydroxide (~200 g/L) TS and dilute to 1000 mL with water R.

Prepare the following solutions. For solution (1) dissolve 1.00 g of the test substance in water R and dilute to 100.0 mL with the same solvent. For solution (2) dissolve 4.42 g of sodium fluoride R, previously dried at 120°C for 2 hours in water R to obtain a solution containing 1.9 mg fluoride ion per mL. Dilute solution (2) further to obtain standard solutions with the following concentrations: solution (3) 19 µg/mL; solution (4) 1.9 µg/mL; and solution (5) 0.19 µg/mL.

Add to 20.0 mL each of solution (1), (3), (4) and (5) 10.0 mL of the buffer solution and stir the solution using a magnetic stirrer and a plastic-coated stirring bar. Use a fluoride-ion-selective electrode and a silver/silver chloride reference electrode system, connected to a potentiometer capable of indicating reproducibly a minimum of ±0.2 mV. Insert the previously rinsed and dried electrodes into the solutions, stir for 5 minutes and read the potential in mV. Plot the logarithms of the fluoride ion concentration in solution (3), (4) and (5) versus the measured potential.

Determine the concentration of fluoride ion in solution (1), reading off from the standard curve the value of µg of fluoride ion per mL correlating with the measured potential and divide by the sample mass taken to obtain the content in the sample; not more than 200 µg/g.
Related Substances.

Either test A or test B may be applied.

A. Impurity A (fluorouracil) and impurity B. 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 60 volumes of nitromethane R, 20 volumes of methanol R, 10 volumes of ethyl acetate R and 10 volumes of water R as the mobile phase. Apply separately to the plate 1 μL of each of the following two solutions. Use a mixture composed of 60 volumes of methanol R, 35 volumes of water R and 5 volumes of glacial acetic acid R as the solvent. For solution (1) use 10 mg of the test substance per mL. For solution (2) use 10 mg of flucytosine RS per mL. Apply also 20 μL of each of the following two solutions. Use the same solvent as described above. For solution (3) use 20 mg of the test substance per mL. For solution (4) use 30 μg of fluorouracil RS per mL. After application allow the spots to dry in a current of cool air. Develop over a path of 9 cm in an unsaturated chromatographic chamber. After removing the plate from the chromatographic chamber allow it to dry exhaustively in a current of air. Examine the chromatogram in ultraviolet light (254 nm). Flucytosine, impurity A (fluorouracil) and impurity B are eluted with the following Rf values: flucytosine about 0.26, impurity A (fluorouracil) about 0.54 and impurity B about 0.74.

In the chromatogram obtained with solution (3) any spot corresponding to impurity A (fluorouracil) or impurity B is not more intense than the principal spot in the chromatogram obtained with solution (4) (0.15%).

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

As the mobile phase use a solution prepared as follows. Dissolve 13.6 g of potassium dihydrogen phosphate R in 950 mL of water R, adjust to pH 2.0 by adding phosphoric acid R and add 50 mL of methanol R.

Prepare the following solutions in a dissolution solvent prepared by dissolving 13.6 g of potassium dihydrogen phosphate R in 950 mL of water R and adding 50 mL of methanol R. For solution (1) use 0.3 mg of the test substance per mL. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 0.3 μg of flucytosine per mL. For solution (3) use 0.3 μg of fluorouracil RS per mL. For solution (4) mix 1.0 mL of solution (2) and 1.0 mL of solution (3).

Operate with a flow rate of 1.1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 260 nm.

Inject separately 20 μL each of solution (1), (2), (3) and (4) and record the chromatograms for 15 times the retention time of flucytosine.

Use the chromatogram obtained with solution (3) to identify the peak due to impurity A (fluorouracil). Impurity B is eluted at a relative retention of about 12 with reference to flucytosine (retention time about 2.2 minutes).

The test is not valid unless the resolution between the peaks due to flucytosine and impurity A (fluorouracil) in the chromatogram obtained with solution (4) is not less than 5.0 and the symmetry factor for the peak due to flucytosine in the chromatogram obtained with solution (2) is not more than 2.0.
In the chromatogram obtained with solution (1):

- the area of any peak due to impurity A (fluorouracil) is not greater than 1.5 times the area of the corresponding peak obtained with solution (3) (0.15%);
- the area of any peak due to the impurity B, when multiplied by a correction factor of 0.6, is not greater than 1.5 times the area of the principal peak obtained with solution (2) (0.15%);
- the area of any other peak, other than the principal peak, is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%);
- the sum of the area of any peak corresponding to impurity A (fluorouracil), the corrected area of any peak corresponding to impurity B and the areas of all other peaks, other than the principal peak, is not greater than 3 times the area of the principal peak obtained with solution (2) (0.3%). Disregard any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.03%).

**Assay**

Dissolve about 0.1 g, accurately weighed, in a mixture of 40 mL of acetic anhydride R and 100 mL of glacial acetic acid R1, and 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 12.91 mg of C\(_4\)H\(_4\)FN\(_3\)O.

**Impurities**

**A. 5-fluoropyrimidine-2,4(1H,3H)-dione (fluorouracil)**

![Image of fluorouracil structure]

**B. 2-ethoxy-5-fluoropyrimidin-4(3H)-one**

**Reagent to be established**

**Cyclohexylenedinitrilotetra-acetic acid R**

Trans-Cyclohexylene-1,2-dinitrilo-N,N,N',N'-tetra-acetic acid, C\(_{14}\)H\(_{22}\)N\(_2\)O\(_8\)H\(_2\)O.

*Description.* A white or almost white, crystalline powder.

*Melting point.* About 204°C.
Flucytosini infusio intraveno
Flucytosine intravenous infusion

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Description. Flucytosine intravenous infusion is a clear, colourless or almost colourless solution.

Category. Antifungal.

Storage. Flucytosine intravenous infusion should be kept in a tightly-closed container, protected from light.

Additional information. Strengths in the current WHO Model List of Essential Medicines (EML): 2.5 g in 250 mL. Strengths in the current EML for Children: 2.5 g in 250 mL.

Requirements
Comply with the monograph for Parenteral preparations.

Definition. Flucytosine intravenous infusion is a sterile solution containing Flucytosine. It is supplied as a ready-to-use solution.

Flucytosine intravenous infusion contains not less than 90.0% and not more than 110.0% of the amount of Flucytosine (C₄H₄FN₃O) stated on the label.

Identity tests
• Either test A or tests B and C may be applied.

A. Evaporate 10 mL of the infusion to dryness on a water-bath and dry the residue at 105 °C for about 1 hour. 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 flucytosine RS or with the reference spectrum of flucytosine.

B. 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 60 volumes of nitromethane R, 20 volumes of methanol R, 10 volumes of ethyl acetate R and 10 volumes of water as the mobile phase. Apply separately to the plate 1 μL of each of the following two solutions. Use a mixture composed of 60 volumes of methanol R, 35 volumes of water R and 5 volumes of glacial acetic acid R as the solvent. For solution (A) use an aliquot of the infusion to be tested. For solution (B) use 10 mg of flucytosine RS per mL. After application allow the spots to dry in a current of cool air. Develop over a path of 9 cm in an unsaturated chromatographic chamber. After removing the plate from the chromatographic chamber allow it to dry exhaustively in a current of air. Examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).
C. The absorption spectrum (1.6) of the final solution prepared for Assay A, when observed between 230 nm and 350 nm, exhibits a maximum at about 286 nm and a minimum at about 245 nm.

**pH value (1.13).** pH of the infusion, 6.0–8.0.

**Pyrogens.** Carry out the test as described under 3.5 Test for pyrogens, per kg of the rabbit’s weight, 10 ml.

**Related substances**

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

As the mobile phase use a solution prepared as follows. Dissolve 13.6 g of potassium dihydrogen phosphate R in 950 mL of water R, adjust to pH 2.0 by adding phosphoric acid R and add 50 mL of methanol R.

Prepare the following solutions in a dissolution solvent prepared by dissolving 13.6 g of potassium dihydrogen phosphate R in 950 mL of water R and adding 50 mL of methanol R. For solution (1) dilute a quantity of the infusion to obtain a concentration of 0.3 mg of flucytosine per mL. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 0.3 μg of flucytosine per mL. For solution (3) use 0.3 μg of fluorouracil RS per mL. For solution (4) mix 1.0 mL of solution (2) add 1.0 mL solution (3).

Operate with a flow rate of 1.1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 260 nm.

Inject separately 20 μL each of solution (1), (2), (3) and (4) and record the chromatograms for 15 times the retention time of flucytosine.

Use the chromatogram obtained with solution (3) to identify the peak due to impurity A (fluorouracil). Flucytosine is eluted at a retention time about 2.2 minutes.

The test is not valid unless the resolution between the peaks due to flucytosine and impurity A (fluorouracil) in the chromatogram obtained with solution (4) is not less than 5.0 and the symmetry factor for the peak due to flucytosine in the chromatogram obtained with solution (2) is not more than 2.0.

In the chromatogram obtained with solution (1):

- the area of any peak due to the impurity A (fluorouracil) is not greater than 5 times the area of the corresponding peak obtained with solution (3) (0.5%);

**Assay**

Dilute an accurately measured volume of the infusion with hydrochloric acid (0.1 mol/L) VS to give a solution containing about 0.1 mg per mL of Flucytosine. Dilute 5.0 mL of the resulting solution to 100.0 mL with the same solvent. Measure the absorbance of the resulting solution in a 1 cm layer at the maximum at about 286 nm. Calculate the content of Flucytosine (C₄H₆F₃N₃O) using the absorptivity value of 70.9 (λ1% cm⁻¹ = 709).

**Impurities**

The impurity limited by the requirements of this monograph is listed in the monograph for Flucytosine.

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