Identification—
A: Ultraviolet Absorption (197U)—
Solution: 8 µg per mL.
Medium: dilute hydrochloric acid (1 in 100). Absorptivities at 285 nm, calculated on the dried basis, do not differ by more than 2.0%.
B: The Rf value of the principal spot in the specimen chromatogram in the test for Fluorouracil corresponds to that obtained with the solution of USP Fluorotinsy RS.

Loss on drying (731)—Dry it at 105° for 4 hours: it loses not more than 1.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Heavy metals, Method II (231): 0.002%.

Fluoride ions—Note—All glassware and/or plasticware used in this test should be scrupulously clean and even free from trace amounts of fluoride. The use of plasticware to contain the solutions while the potential is measured is recommended.

Buffer solution—To 110 g of sodium chloride in a 2-L volumetric flask add 1 g of sodium citrate and 700 mL of water, and dissolve with shaking. Carefully add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and while stirring, cautiously add 450 mL of glacial acetic acid. Cool to room temperature, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix. The pH of this solution is between 5.0 and 5.5.

Standard stock solution—Accurately weigh 2.211 g of sodium fluoride, previously dried at 150° for 4 hours, into a 1-L volumetric flask, and dissolve in about 200 mL of water. Add 1.0 mL of sodium hydroxide solution (1 in 250), dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ion. Store the solution in a closed plastic container.

Standard preparations— Dilute a portion of Standard stock solution quantitatively and stepwise with Buffer solution to obtain a Standard preparation having a fluoride concentration of 1 µg per mL. Prepare the final dilution in a 100-mL volumetric flask. In the same manner, prepare additional Standard preparations having fluoride concentrations of 3 µg per mL, 5 µg per mL, and 10 µg per mL, respectively.

Test preparation—Place 1 g of Flucytosine, accurately weighed, in a 100-mL volumetric flask, and dissolve in and dilute with Buffer solution to volume.

Procedure—Concomitantly measure the potential (see Titrimetry (541)), in mV, of the Standard preparations and the Test preparation, with a suitable pH meter equipped with a fluoride-specific ion electrode and a glass-sleeved calomel reference electrode that has been modified in the following manner. Mix 70 mL of freshly prepared saturated potassium chloride solution with 30 mL of isopropyl alcohol, fill the electrode with the clear supernatant, and allow the electrode to remain in the mixture for not less than 2 hours prior to use, or preferably overnight.

When taking the measurements, transfer the solution to a 150-mL beaker, and immerse the electrodes. Insert a polyethylene-coated stirring bar into the beaker, place the beaker on a magnetic stirrer having an insulated top, and allow to stir until equilibrium is attained (about 1 to 2 minutes). Rinse and dry the electrodes between measurements, taking care not to scratch the crystal in the specific ion electrode.

Measure the potential of each Standard preparation, and plot the fluoride concentration, in mg per 100 mL, versus the potential, in mV, on semilogarithmic paper. Measure the potential of the Test preparation, and determine from the standard curve the fluoride concentration, in mg per 100 mL. Calculate the percentage of fluoride in the portion of Flucytosine taken by the formula:

\[ C / 10 \]

in which C is the fluoride concentration, in mg per 100 mL, from the standard curve: not more than 0.05% of fluoride is found.

Fluorouracil—Dissolve 250 mg in 10 mL of a mixture of glacial acetic acid and water (4:1). Apply 20.0 µL of this solution to a thin-layer chromatographic plate (see Chromatography (621)) coated with a 0.5-mm layer of chromatographic silica gel mixture. To the same plate apply 20 µL, in 10-µL increments, of a 0.025 mg per mL solution of USP Fluorouracil RS in a mixture of glacial acetic acid and water (4:1). Develop the chromatogram in a mixture of chloroform and glacial acetic acid (13:7) until the solvent front has moved not less than 14 cm from the origin. Remove the plate from the developing chamber, and allow the solvent to evaporate. Locate the spots on the plate by observing under short-wavelength UV radiation: any spot from the solution under test is not greater in size and intensity than the spot at the respective Rf produced by the Standard solution, corresponding to not more than 0.1% of fluorouracil.

Assay—Place about 400 mg of Flucytosine, accurately weighed, in a 250-mL beaker, add 150 mL of a mixture of 2 volumes of glacial acetic acid and 1 volume of acetic anhydride, and dissolve, warming gently if necessary. Titrate potentiometrically with 0.1 N per chloric acid VS, using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N per chloric acid is equivalent to 12.91 mg of C4H4FN3O.

Flucytosine Capsules

Flucytosine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flucytosine (C4H4FN3O).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—
USP Fluorotinsy RS

Identification—
A: The UV absorption spectrum of the solution from the Capsule contents obtained in the Assay exhibits maximum absorption at the same wavelength as that of the Standard solution, and the two spectra are similar between 260 nm and 350 nm.

B: Shake a portion of the contents of Capsules, equivalent to about 500 mg of flucytosine, with 10 mL of water. Filter, and to 2 mL of the filtrate add 1 mL of sodium pentacyanoaminoferrocene reagent [prepared by dissolving 100 mg of sodium (tripentacyanoaminoferrocene in 20 mL of sodium carbonate solution (1 in 100)] and 1 mL of 3 per cent hydrogen peroxide: on standing, a darker green is produced than that produced by a blank.

Dissolution (711)—
Medium: water; 900 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.

Procedure—Determine the amount of C4H4FN3O dissolved by employing UV absorption at the wavelength of maximum absorbance at about 276 nm on filtered portions of the solution under test, suitably diluted with Dissolution Medium, if necessary, in comparison with a Standard solution having a known concentration of USP Flucytosine RS in the same Medium.

Tolerances—Not less than 80% (Q) of the labeled amount of C4H4FN3O is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—Weigh the contents of not fewer than 20 Capsules, and determine the average weight per Capsule. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 250 mg of flucytosine, to a 250-mL volumetric flask. Add about 50 mL of 0.1 N hydrochloric acid, shake by mechanical means for 30 minutes, then add 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute 10 mL of the clear filtrate with 0.1 N hydrochloric acid to 250 mL. Dilute 10.0 mL of this solu-

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tion with 0.1 N hydrochloric acid to 50 mL. Dissolve an accurately weighed quantity of USP Flucytosine RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 8 μg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 285 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of flucytosine (C₄H₄FN₃O) in the portion of Capsule contents taken by the formula:

\[ 31.25(C_0 / A_0) \]

in which \( C \) is the concentration, in μg per mL, of USP Flucytosine RS in the Standard solution; and \( A_0 \) and \( A_\lambda \) are the absorbances of the solution from the Capsule contents and the Standard solution, respectively.

### Flucytosine Oral Suspension

Flucytosine Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flucytosine (C₄H₄FN₃O). Prepare Flucytosine Oral Suspension 10 mg per mL as follows (see Pharmaceutical Compounding—Nonsterile Preparations (795)):

Flucytosine .................. 1.0 g
Vehicle: a mixture of V vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF (1:1), a sufficient quantity to make .... 100 mL

If using Flucytosine Capsules, empty the contents of the capsules into a suitable mortar, or add Flucytosine powder to the mortar. Add about 10 mL of the Vehicle, and mix to a uniform paste. Add the Vehicle in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough Vehicle to bring to final volume, and mix well.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store in a cold place.

**Labeling**—Label it to state that it is to be well shaken, and to state the beyond-use date.

**USP Reference standards** (11)—
USP Flucytosine RS

**pH** (791): between 4.0 and 5.0.

**Beyond-use date:** 60 days after the day on which it was compounded.

**Assay**—

**Buffer**—Dissolve 1 g of ammonium acetate and 1 mL of di-isopropylamine in 1 L of water, and adjust with glacial acetic acid to a pH of 7.5.

**Mobile phase**—Prepare a filtered and degassed solution of methanol and Buffer (1:1). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Flucytosine RS in Mobile phase, and dilute quantitatively with Mobile phase to obtain a solution having a known concentration of about 50 μg per mL.

**Assay preparation**—Agitate the container of Oral Suspension for 30 minutes on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70 °C until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 seconds. Pipet 0.5 mL of the sample into a 100-mL volumetric flask, and dilute with Mobile phase to volume.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-× 200-mm analytical column that contains 5-μm packing L3. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the retention time is about 3 minutes, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of Flucytosine (C₄H₄FN₃O) in the volume of Oral Suspension taken by the formula:

\[ 200(C/V)(r_U / r_S) \]

in which \( C \) is the concentration, in μg per mL, of USP Flucytosine RS in the Standard preparation; \( V \) is the volume, in mL, of Oral Suspension taken; and \( r_U \) and \( r_S \) are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

### Fludarabine Phosphate

![Fludarabine Phosphate](https://example.com/fludarabine-phosphate.png)

C₉H₁₁F₁₄N₇O₇P 365.21
9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-β-D-arabinofuranosyl)-
9-β-D-Arabinofuranosyl-2-fluoroadenine 5′-(dihydrogen phosphate) [75607-67-9].

Fludarabine Phosphate contains not less than 98.0 percent and not more than 102.0 percent of C₉H₁₁F₁₄N₇O₇P, calculated on the anhydrous, solvent-free basis.

**Caution**—Fludarabine Phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store in a refrigerator.

**USP Reference standards** (11)—
USP Fludarabine Phosphate RS

**Identification, Infrared Absorption (197K).**

**Specific rotation** (7815): between +10° and +14°.

**Test solution:** 5 mg per mL, in water.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total aerobic microbial count does not exceed 1000 cfu per g.

**Water, Method I (921):** not more than 3.0%.

**Chloride**—

**Standard chloride solution**—Transfer 82.4 mg of sodium chloride to a 100-mL volumetric flask, and dissolve in and dilute