QUALITY CONTROL DEPARTMENT

PRODUCT STANDARD TEST PROCEDURE

PRODUCT NAME	**Fluorouracil Injection BP 50 mg/ml, 10ml**		
GENRIC NAME	Fluorouracil Injection BP 50 mg/ml, 10ml		
PRODUCT CODE	FU/002	**EFFECTIVE DATE**	14/04/2025
GRADE	BP	**REVIEW DATE**	13/04/2025
STP NO.	KPL/STP/IN/032-02	**VERSION NO.**	02
SPECIFICATION NO.	KPL/SPC/IN/032-02	**SUPERCEDES NO.**	01
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1. DESCRIPTION:

For Bulk: A clear colourless or almost colourless solution.

For Finish: A clear colourless or almost colourless solution filled in amber/clear colour glass vial USP type I.

(Note: Ensure the appearance of solution after reconstitution/preparation of intravenous administration as per respective patient information leaflet and record the observation).

2. IDENTIFICATION:

A. BY INFRARED ABSORPTION:

Sample Preparation:

Carefully acidify 2 ml of sample (equivalent of 0.1gm of Fluorouracil) with glacial acetic acid, stir, cool and filter. Wash the precipitate with 1ml of water and dry over phosphorus pentaoxide at 80°C at a pressure of 2kPa for 4 hours.

Procedure: Before start analysis performs the background and then start analysis. Place reference/working standard on ATR and record the chromatogram at 650 cm^{-1} to 4000 cm^{-1}. Similar procedure follows for sample.

Acceptance criteria: The infrared absorption spectrum obtained from the residue of sample should be concordant to the infrared absorption spectrum obtained from the working standard or reference standard.

B. BY UV:

Scan the sample prepared in assay in the range 230nm to 350nm.

Acceptance Criteria:

The UV spectrum of sample solution should exhibits a maxima only at about 266 nm.

3. pH: [8.5 to 9.1]

For Bulk: Take 5-10 ml sample in the container and measure the pH of the sample.

For finish: Take 5-10 ml sample in the container and measure the pH of the sample.

4. EXTRACTABLE VOLUME: [NLT 10.0 ml]

Select 3 containers and remove the flip of seal and transfer the content into a dry syringe of a capacity not exceeding three times the volume to be measured and fitted with a 21-gauge needle NLT 2.5 cm (1 inch) in length. Expel any air bubbles from the syringe and needle, and then discharge the contents of the syringe, without emptying the needle, into a standardized, dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its graduated volume. The volume is NLT the nominal volume of the container. Alternatively, the volume of the contents in milliliters may be calculated as the mass in grams divided by the density.

For containers with a nominal volume of 2 mL or less the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement, provided that a separate, dry syringe assembly is used for each container. The contents of containers holding 10 mL or more may be determined by opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the nominal volume in case of containers examined individually, or, in the case of containers with a nominal volume of 2 mL or less, is not less than the sum of the nominal volumes of the containers taken collectively.

5. UREA: (By Thin layer chromatography)

Mobile phase: A mixture of 70 volumes of ethyl acetate, 15 volumes of methanol and 15 volumes of water.

Plate: Merck silica gel 60 F_{254} plate.

Application: 20 µl; thoroughly dry the points of application

Development: Over a path of 15 cm.

Test solution: Dilute 1 ml of sample (equivalent of 50mg Fluorouracil) to 10ml with water.

Standard solution: Transfer accurately 2 mg of Urea standard into 10 ml volumetric flask, dissolve and dilute to 10 ml with water.

4-dimethylaminobenzaldehyde Solution: Transfer accurately 0.250 gm of 4-dimethylaminobenzaldehyde into 25 ml volumetric flask, dissolve and dilute to 25 ml with ethanol (96%).

Spraying Mixture: Prepare a mixture of 10 volumes of 4-dimethylaminobenzaldehyde solution and 1 volume of hydrochloric acid.

Procedure:

Apply separately to the plates 20 µl of standard solution and sample solution. After removal of the plate, allow it to dry in air. Spray with spraying mixture and heat at 100°C until maximum intensity of the spots is obtained.

Acceptance Criteria:

Any spot corresponding to urea in the chromatogram obtained with sample solution should be not more intense than the spot in the chromatogram obtained with Urea standard.

6. RELATED SUBSTANCES: (By Thin layer chromatography)

Mobile phase: A mixture of 70 volumes of ethyl acetate, 15 volumes of methanol and 15 volumes of water.

Plate: Merck silica gel 60 F_{254} plate.

Application: 10 µl; thoroughly dry the points of application

Development: Over a path of 15 cm.

Test solution: Dilute 2 ml of sample (equivalent of 100mg Fluorouracil) to 5ml with water.

Standard Solution (1): Dilute 1 ml of the test solution to 400 ml with methanol (50.0%).

Standard solution (2): Transfer accurately 2.50 mg of 5-hydroxyuracil standard into 50 ml volumetric flask, dissolve and dilute to 50 ml with methanol (50%).

Fast Blue B Salt solution: Transfer accurately 0.125 gm of Fast blue B salt into 25 ml volumetric flask, dissolve and dilute to 25 ml with water.

(Note: Use freshly prepared solution of Fast Blue B salt)

Sodium Hydroxide Solution, 0.1M: Transfer 0.200 gm of Sodium hydroxide into 50 ml volumetric flask, dissolve and dilute to 50 ml with water.

Procedure:

Apply separately to the plates 10 µl of standard solution (1), standard solution (2) and test solution. After removal of the plate, allow it to dry in current of air and examine the plate under ultraviolet light (254nm). **(**For Any Individual Impurity).

Then Spray the plate with Fast Blue B Salt solution spraying mixture and then spray with 0.1M sodium hydroxide solution. Dry the plate in the current of air and examine the plate. (For 5-hydroxyuracil)

Acceptance Criteria:

Any Individual Impurity: When examine the plate at 254nm, any secondary spot in the chromatogram obtained with test solution should be not more than the spot in the chromatogram obtained with standard solution (1).

5-hydroxyuracil: Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with sample solution should be not more intense than the spot in the chromatogram obtained with 5-hydroxyuracil standard.

Disregard: Any spots with RF value lower than 0.2.

7. **BACTERIAL ENDOTOXINS TEST:** Procedure refers to GTP No. KPL/GTP/009.
8. **STERILITY:** Procedure refers to GTP No. KPL/GTP/008.
9. **PARTICULATE MATTER:**

Procedure: Open 5 containers to get the 25ml of sample by slowly inverting the container. Clean the outer surface of the container with particle free water and remove the closure avoiding any contamination of the container.

The preparation complies with the test if the average number of particles present in the unit tested does not exceed 6000 per container equal to or greater than 10µm and does not exceed 600 per container equal to or greater than 25µm.

Limit: The particles above 10 µm should not exceed above 6000 and particles above 25 µm should not exceed 600.

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10. ASSAY:

Procedure: Mix the content of five vials and make pooled sample. Transfer accurately pooled sample equivalent to 75 mg of Fluorouracil into 200 ml volumetric flask, add 20 ml 1M hydrochloric acid, mix and dilute to 200 ml with water. Dilute 3 ml of this solution to 100 ml with 0.1M hydrochloric acid. Measure the absorbance of the resulting solution at 266nm.

Calculate the content of Fluorouracil, taking 552 as the value of A (1%, 1cm) at the maximum at 266nm.

Calculation:

$$\frac{\text{Sample Absorbance}}{552} \times \frac{200}{\text{Sample weight}} \times \frac{100}{3} \times \frac{1000}{100} \times \text{weight/ml} \times 10$$

10.1 Weight/ml:

Procedure: The weight per milliliter of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 20°, unless otherwise specified in the standard testing procedure of the material being analyzed. The weight per milliliter is determined by dividing the weight in air, expressed in g, of the quantity of liquid that fills a pycnometer at the specified temperature by the capacity, expressed in ml, of the pycnometer at the same temperature. The capacity of the pycnometer is ascertained from the weight in air, expressed in g, of the quantity of water required to fill the pycnometer at that temperature. The weight of a litre of water at specified temperatures when weighed against brass weights in air of density 0.0012g per ml is given in the following table. Ordinary deviations in the density of air from the above value, here taken as the mean, do not affect the result of a determination in the significant figures prescribed for Pharmacopoeia substances.

Temperature °C	Weight of Liter of water(gm)

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20	997.18
25	996.02
30	994.62

REVISION HISTORY:

Sr. No.	Change Control No.	Revision No.	Reason for Revision
01	CC/20/04/QC/037	00	New STP
02	NA	01	Schedule Revision
03	NA	02	Schedule Revision

End of Document

PREPARED BY(SIGN/DATE) (QC PERSONNEL)	CHECKED BY (SIGN/DATE) (HEAD – QC)	APPROVED BY (SIGN/DATE) (HEAD QA)	CONTROLLED COPY
14/04/25	14/04/2025	14/04/25	