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Standard Operating Procedure

GEN-Q111

Radiochemical Identification & Purity of 3'-Deoxy-3'-[^{18}F]Fluorothymidine ([^{18}F]FLT) Injection by Radio-Thin Layer Chromatography Analysis

Approval Signatures:

Printed Name	Date	Signature	Date
Author:			
Supervisor:			
Regulatory Affairs/QA:			

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Radiochemical Identification & Purity of 3'-Deoxy-3'-[^{18}F]Fluorothymidine ([^{18}F]FLT) Injection by Radio-Thin Layer Chromatography Analysis

1. **Purpose:** To describe the quality control procedure for determining the radiochemical identity and purity of 3'-deoxy-3'-[^{18}F]Fluorothymidine ([^{18}F]FLT) Injection by Radio-Thin Layer Chromatography Analysis.

2. **Scope:** The Radio-Thin Layer Chromatography Analysis procedure is one of the methods used for determining the radiochemical identity and purity of the final product at the *manufacturing sites* synthesizing [^{18}F]FLT under the *Sponsor's* IND. This quality control procedure is one of the QC release tests performed on the final product and it must be completed prior to releasing the batch of product for human administration.

3. **Responsibilities:**
   3.1. *Manufacturing Sites*: To follow this procedure as written and to ensure that all results, control information, and raw data are documented as specified in dedicated Quality Control Laboratory Notebooks and recorded on the appropriate testing forms.

4. **References**
   4.1. USP 26 ‹621› Chromatography
   4.2. USP 26 ‹821› Radioactivity
   4.3. USP 26 ‹823› Radiopharmaceuticals For Positron Emission Tomography—Compounding
   4.4. V. Shen, K.R. Hetzel, R.D. Ice: Procedure Manual, "Radiochemical Purity of Radiopharmaceuticals Using Gelman Seprachrom™ (ITLC™) Chromatography, Gelman Instrument Company, Technical Bulletin 32 (1974).
   4.5. K. Bauer, L. Gros, W. Sauer. Thin Layer Chromatography: An Introduction. EM Science, Heidelberg, Germany.
   4.6. **GEN-Q015**, "Investigating Out of Specification QC Test Results"
   4.7. FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—June 2002 (CDER)
   4.8. General Quality Control Procedures for Nuclear Pharmacy, http://nuclearpharmacy.uams.edu/general.htm

5. **Forms**
   - **Form FQC-001**, "[^{18}F]FLT Final Product QC Test Results" Form I
   - **Form IOS-001**, Investigating Out of Specification (OOS) QC Test Results Form

6. **Policies**
   6.1. TLC plates should be stored with a desiccant in well-closed containers.

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6.2. Date opened stock bottles of solvents upon receipt and when they are first opened for use. As a general rule, any unused solvents should be discarded after the manufacturer's expiration date on the bottle or 6 months. Keep a sealed bottle in inventory as a back-up. The purity of solvents that are used as mobile phase is important to ensure reliability of test results. Contaminants, such as water or other chemicals, can alter the system and give inaccurate results. Solvents can evaporate, absorb moisture from the air, or become contaminated with other solvents.

6.3. Always observe radiation safety rules. Check forceps, tweezers, tongs, tubes, and vials for radioactive contamination. Assure background counts in the well counter are not significantly different from background.

6.4. Use a counting rate between 10,000 and 200,000 counts per minute for the peak of highest activity if possible. If the counts are too high in the FLT peak, do the test and record a greater than for the purity value and recount the sample later when the count rate is below the dead time of the detector.

6.5. Always label tubes and vials to distinguish between: solvent types, samples, standards, and strip sections.

6.6. Both the [^{18}F]FLT sample and [^{19}F]FLT standard spots should be applied far enough apart to ensure that the spots do not overlap during development.

7. Materials and Equipment

7.1. SS-TLA, Aluminum-backed Silica gel plates, Merck 0.2 mm, F_{254} (size 5 cm x 20 cm or 5 cm x 7.5 cm)

7.2. SS-ACN, Acetonitrile (mobile phase, HPLC Grade)

7.3. HBS-L, (92/8) Sodium Dihydrogen Phosphate 10 mM/ Ethanol) Buffer

7.4. SS-FLT, Non-radioactive 3'-Deoxy-3'-fluorothymidine [^{19}F]FLT (to use for standard)

7.5. UV Lamp with 254 nm wavelength

7.6. Radio-chromatogram scanner, or beta or gamma Radioactivity counter, or Phosphor imager

7.7. Cellophane Tape

7.8. Developing Chamber

7.9. Ruler with metric measurements

7.10. Soft-Lead Pencil

7.11. Syringe, pipette, or capillary tube (1-2 µL of sample needed)

7.12. Counting Tubes (only needed if you are cutting and counting the strips)

7.13. Dedicated QC Laboratory Notebooks

7.14. Photocopier (permanent record)

7.15. Glass container with Teflon® Lined Cap

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7.16. Calibrated Balance

8. Procedures

8.1. Preparation of the TLC Standard (HPLC Standard 1 mg/mL from GEN-Q119 can be used in place of this standard)

8.1.1. Weigh out 10 mg of non-radioactive [^{19}F]FLT into a glass container with a Teflon® lined cap.

8.1.2. Label the glass container non-radioactive [^{19}F]FLT TLC Stock Solution.

8.1.3. Transfer 1 mL of 50% ethanol into the glass container to make up a 10 mg/mL stock solution. Cap the container and mix thoroughly.

8.1.4. Store this stock solution at 2-8°C until ready for use.

8.1.5. Make up the TLC standard using 10-20 µL of the 10 mg/mL TLC stock solution prepared in 8.1.3 above and add 200 µL of **HBS-L,** (92/8) Sodium Dihydrogen Phosphate 10 mM/ Ethanol buffer into another glass container with a Teflon® lined cap to make up an approximate 1 mg/mL standard TLC solution.

8.2. Preparation of the TLC Chamber & Plate

8.2.1. Pour a sufficient volume of mobile-phase, (acetonitrile), into the TLC developing chamber such that the solvent just covers the bottom of the chamber (less than 1 cm high). Cover the chamber and allow the solvent to equilibrate.

8.2.2. Using a soft lead pencil and a ruler, lightly draw tick marks at the edges of the plate and 1 cm up from the bottom of the plate. Do not draw any lines across the plate, as that may disturb the solid phase and interfere with progression of the mobile phase, refer to diagram 1. This is the origin where the sample and standards will be spotted.

8.2.3. Draw a second line with the pencil 6.5 cm from the bottom of the TLC plate. This is the solvent front, *i.e.*, the maximum distance that the solvent should be allowed to travel on the plate.

8.2.4. Using a soft lead pencil, label the top of the TLC plate to indicate the channels where the freshly prepared [^{18}F]FLT sample (X) and [^{19}F]FLT standard (S) will be located. Write the date at the top of the TLC plate for reference purposes (example 4-9-04 in figures). Refer to Diagram 1 on page 5.

8.3. [^{18}F]FLT Sample & [^{19}F]FLT Standard Application

8.3.1. Spot approximately 1 µL of the sample onto the TLC plate at the origin below the (X).

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8.3.2. Spot approximately 1 µL of the [^{19}F]FLT standard onto the TLC plate at the origin below (S).

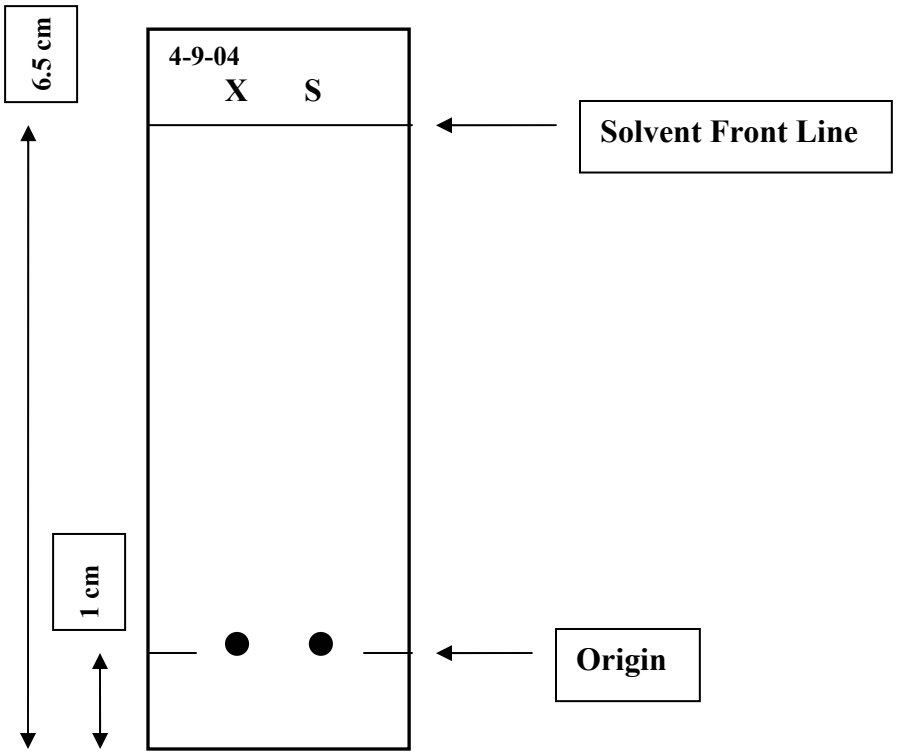

Diagram 1: Preparation

8.3.3. Allow the spots to dry completely prior to placing them in the developing chamber (approximately 5-10 minutes).

8.4. Migration

8.4.1. Develop the TLC plate, by carefully positioning it within the developing chamber and replacing the lid.

8.4.2. Allow the mobile phase to migrate up the TLC plate until it just reaches the Solvent Front line (refer to Diagram 1).

8.4.3. Immediately remove the TLC plate from the developing chamber and allow it to dry at room temperature.

8.5. Determine the R_f of the Non-Radioactive [^{19}F]FLT

8.5.1. Visualize the TLC plate under a UV light of 254 nm to determine the exact location of the non-radioactive [^{19}F]FLT standard. The

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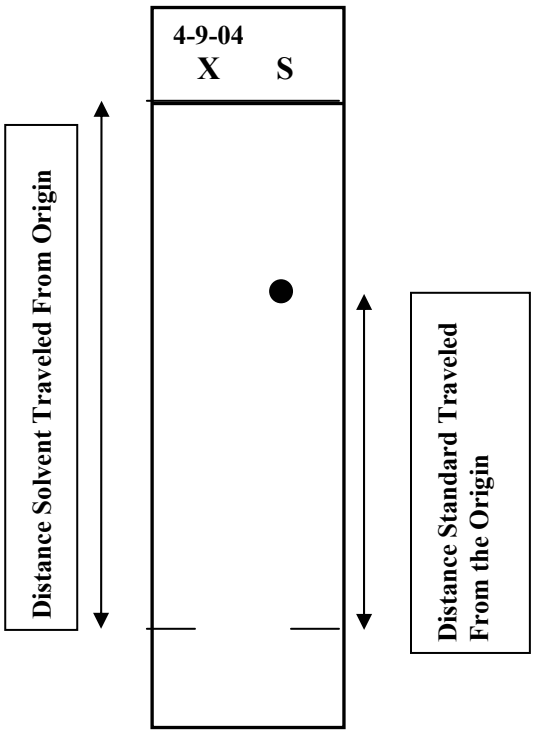
standard should have migrated approximately halfway up the plate, as the range for the R_f values is 0.4 – 0.7. Using a soft lead pencil, circle the spot on the plate corresponding to [^{19}F]FLT.

NOTE: The radioactive [^{18}F]FLT will not typically appear under UV light.

8.5.2. Once the TLC has been measured for UV, cover the entire silica surface of the plate with cellophane tape to prevent contamination later in the procedure.

8.5.3. Calculate the R_f value of the non-radioactive standard, [^{19}F]FLT using the equation, as illustrated in Diagram 2.

Diagram 2: Calculation of R_f Values



$$R_f \text{ (Standard)} = \frac{\text{Distance Standard Traveled from the Origin}}{\text{Distance Solvent Traveled from the Origin}}$$

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8.5.4. Verify that the R_f for the [^{19}F]FLT standard is within the range of 0.4 to 0.7. Record this data in the QC Laboratory notebook.

8.5.5. Make a photocopy copy of the TLC plate prior to cutting it up for radiochemical analysis. Retain this photocopy of the TLC plate in the QC Laboratory notebook as a permanent record of observations.

8.6. Methods of Assay for Radiochemical Purity and Identity

Radiochemical purity and identity can be determined by using either a radiochromatogram scanner or by the cutting and counting technique.

8.6.1. Scan or image the developed TLC plate with an appropriate radiation detecting instrument. Identify all peaks for calculation of radiochemical purity.

8.6.2. Cut the TLC plate into three sections (Refer to Diagram 3). The sections are transferred into separate labeled counting tubes, and counted for radioactivity with an appropriate radiation counter.

8.6.3. Verify for either method used that the major radioactive spot on the TLC plate is within the same R_f as that of the non-radioactive [^{19}F]FLT standard. It should be in the section labeled TLC B for 8.6.2, the cutting and counting method.

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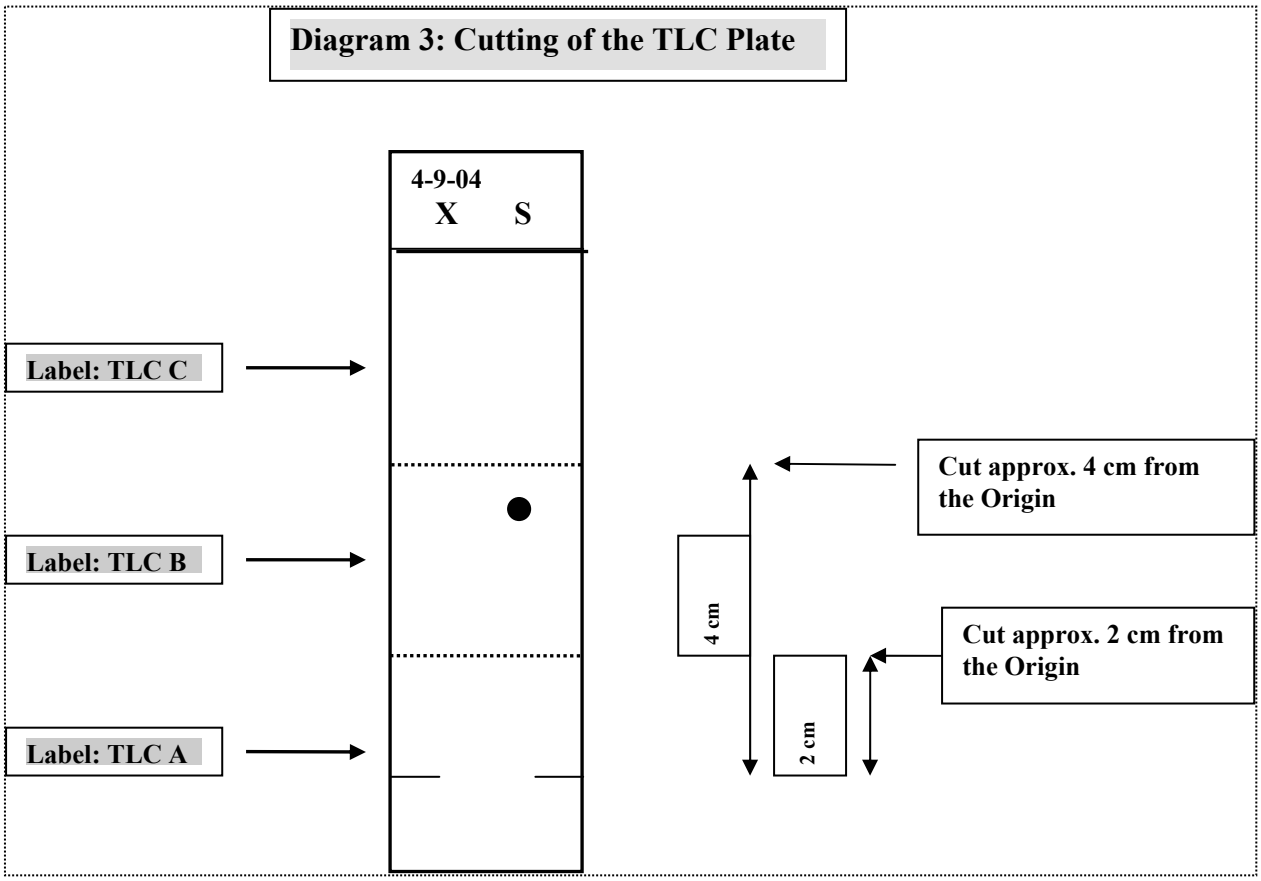

8.7. Calculation of Radiochemical Purity

8.7.1. When using the method, as described in 8.6.1 above, the % radiochemical purity of the [^{18}F]FLT may be determined in two ways:

8.7.1.1. One way is to measure the areas under the peaks with a planimeter and using the following equations:

$$\textbf{\% Radiochemical Purity} = \frac{\textbf{Area of Peak at } R_{(0.4-0.7)}}{\textbf{(Sum of Areas of All Peaks)}} \times 100$$

Where **Area of Peak at $R_{(0.4-0.7)}$ = Area under the peak of the radiopharmaceutical**

Area of Peak at R_0 = Area under the peak of radiochemical impurity of F^-

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8.7.1.2. Or by using the count data from the digital output from the scanner unit and calculating the radiochemical purity with the following equation:

$$\textbf{\% Radiochemical Purity} = \frac{\textbf{Counts of Peak at } R_{(0.4-0.7)}}{\textbf{(Sum of Counts of All Peaks)}} \times 100$$

8.7.2. When using the method in 8.6.2, then the radiochemical purity of the [^{18}F]FLT can be determined by counting the individual sections in an appropriate radiation device and calculating the purity using the following equation:

$$\textbf{\% Radiochemical Purity} = \frac{\textbf{Counts Section B}}{\textbf{(Counts Section A + Counts Section B + Counts Section C)}} \times 100$$

Where **Section B = Portion of TLC Plate containing the Radiopharmaceutical**

Section A = Portion of TLC Plate containing the Radiochemical Impurity F^-

8.8. Interpretation and Reporting of the QC Results

8.8.1. The R_f of the [^{18}F]FLT sample must be in a range equivalent to that of the non-radioactive [^{19}F]FLT standard, whose R_f range is 0.4 – 0.7.

8.8.2. The Radiochemical purity of [^{18}F]FLT must be ≥ 95%.

8.8.3. Record the R_f of the [^{18}F]FLT sample, the R_f of the non-radioactive [^{19}F]FLT standard, and the calculated radiochemical purity results on both, the Form **FQC-001**, "[^{18}F]FLT Final Product QC Test Results" Form I, and in the QC Laboratory notebook.

NOTE: The final product must meet both release specifications outlined in 8.8.1 and 8.8.2 in order for the product to pass this quality control testing requirement.

8.8.4. If the product fails to meet the Radiochemical purity criteria, reject the product and conduct an investigation as per **GEN-Q015**, "Investigating Out of Specification QC Test Results," and record the observations on **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form."

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REVISION HISTORY

Version Number	Description	Reason for Revision	Date	Author
V0	Original Record	Revised NCI-Q111 Version January 28, 2005 to create this new SOP.	March 1, 2005	Teresa R. Zuber

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