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Cancer Imaging Program

National Cancer Institute

Standard Operating Procedure

GEN-Q311

Radiochemical Identification & Purity of [^{18}F]Fluoroestradiol ([^{18}F]FES) Injection by Radio-Thin Layer Chromatography Analysis

1. **Purpose:** To describe the quality control procedure for determining the radiochemical identity and purity of [^{18}F]Fluoroestradiol ([^{18}F]FES) Injection by Radio-Thin Layer Chromatography Analysis. The purpose of this assay is meant to measure any unbound fluorine-18 that might be retained on an HPLC column and is a complement to the HPLC 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 all of the sites manufacturing [^{18}F]FES. 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:** 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 28 ‹621› Chromatography
   - 4.2. USP 28 ‹821› Radioactivity
   - 4.3. USP 28 ‹823› Radiopharmaceuticals For Positron Emission Tomography-Compounding
   - 4.4. **GEN-Q015**, "Investigating Out of Specification QC Test Results"
   - 4.5. FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)

GEN-SOP: NCI-Q311 Date:_____

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

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5. Forms

Form FQC-005, "[^{18}F]FES Final Product QC Test Results"
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.
6.2. Date stock bottles of solvents upon receipt and when they are first opened for use. Methanol should be good for over a year if kept sealed when not in use. Keep a sealed bottle as a back-up. The purity of methanol that is used for the mobile phase is important, contaminants, such as water, can alter the R_f and give inaccurate results.
6.3. Always observe radiation safety rules. Check forceps, tweezers, tongs, tubes, and vials for radioactive contamination. Make certain the gamma counter is not contaminated.
6.4. If possible, use a counting rate between 10,000 and 200,000 counts per minute for the highest activity sample (peak).
6.5. Always label tubes, vials and strip sections to prevent errors.
6.6. Both the [^{18}F]FES sample and [^{19}F]FES standard spots should be applied far enough apart to ensure that the spots do not overlap during development.

7. Materials and Equipment

7.1. Aluminum-backed Silica gel plates, Merck 0.2 mm, F_{254} either as supplied or cut to size of > 1 cm wide by $\geq$ 7 cm in height (**SS-TLA)**
7.2. TLC mobile phase, methanol (**MTH-L)**
7.3. Non-radioactive fluoroestradiol ([^{19}F]FES) (use as standard), (1-10 mg/mL) (**SS-FES**)
7.4. Acidic ammonium molybdate indicating reagent with spray bottle. The indicating reagent consists of 25 g ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7)_{24}$, **SS-AMB**) added to 50 mL of concentrated sulfuric acid (**SS-SUL**) then made up to a volume of 500 mL with deionized water.
7.5. Beta or gamma radioactivity chromatogram scanner, or counter, or Phosphor imager
7.6. Cellophane Tape
7.7. Developing chamber
7.8. Ruler with metric measurements and a soft-lead pencil
7.9. Syringe, pipette, or capillary tube (~1 µL of sample needed)
7.10. Counting Tubes (only needed if you are cutting and counting the strips)
7.11. Dedicated QC Laboratory Notebooks for the Contract Sites
7.12. Photocopier (permanent record)
7.13. FES standard stock solution ~1 mg/mL (**FESSTD-___**)

8. Procedures

8.1. Preparation of the TLC Chamber & Plate

8.1.1. Pour a sufficient volume of methanol, **MTH-L**, 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.
8.1.2. 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.

8.1.3. 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.

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

8.2. [^{18}F]FES Sample & [^{19}F]FES Standard Application

8.2.1. Spot ~1 µL of the sample onto the TLC plate at the origin below the (**X**).

8.2.2. Spot approximately 1 µL of the [^{19}F]FES standard (**FESSTD- _____)** onto the TLC plate at the origin below **(S**), let dry between applications.

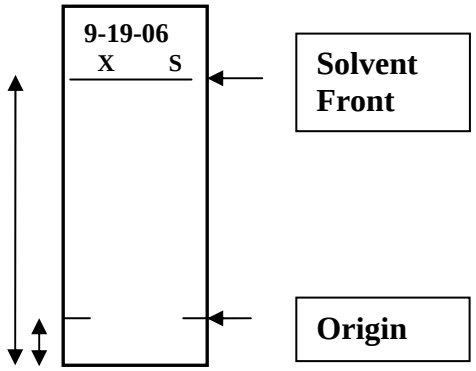
8.2.3. Allow the spots to dry completely prior to placing them in the developing chamber (approximately 5-10 minutes).

8.3. Migration

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

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

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



NOTE: The radioactive [^{18}F]FES will not typically be visible after indicator is applied.

8.4. Determine the R_f of the Non-Radioactive [^{19}F]FES

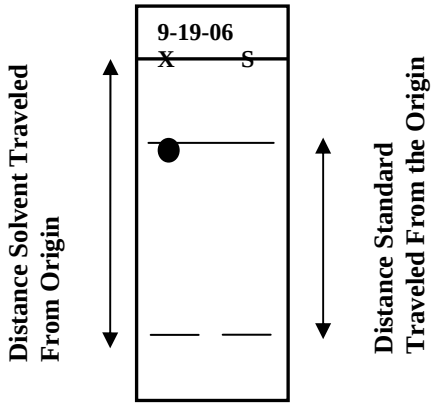
8.4.1. Spray the TLC plate with the acidic ammonium molybdate solution then heat the plate from the back with a heat gun until the standard spot is visible. Look at the TLC plate to determine the exact location of the non-radioactive [^{19}F]FES standard.

8.4.2. Cover the entire silica surface of the plate with cellophane tape to prevent contamination later in the procedure. The standard should have migrated approximately halfway up the plate, as the range for the R_f values is ≥ 0.5 (~0.8).

8.4.3. Make a photocopy copy of the TLC plate prior to cutting it up for radiochemical analysis.

8.4.4. Calculate the R_f value of the non-radioactive standard, [^{19}F]FES using the equation, as illustrated in Diagram 2. Verify that the R_f for the [^{19}F]FES standard is ≥0.5 (typically between 0.8 and 0.9). Retain the photocopy of the TLC plate, labeled with the batch number in the Batch record as a permanent record.

Diagram 2: Calculation of R_f Values



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

8.5. Methods of Assay for Radiochemical Purity and Identity

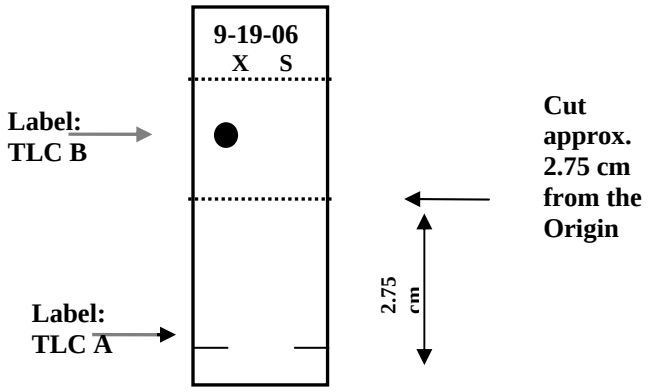
Radiochemical purity and identity can be determined by using either a radiochromatogram scanner or by the cutting and counting technique.

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

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

8.5.3. 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]FES standard. It should be in the section labeled TLC B for 8.5.2, the cutting and counting method.

Diagram 3:



8.6. Calculation of Radiochemical Purity

8.6.1. When using the method, as described in 8.5.1 above, the % radiochemical purity of the [^{18}F]FES may be determined in two ways:

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

$$\textbf{\% Radiochemical Purity} = \frac{\textbf{Area of Peak at } R_{(> 0.5)}}{\textbf{(Sum of Areas of All Peaks)}} \times 100$$

Where Area of Peak at $R_{(> 0.5)}$ = Area under the peak of the radiopharmaceutical
Area of Peak at R_0 = Area under the peak of radiochemical impurity of F^-

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

$$\textbf{\% Radiochemical Purity} = \frac{\textbf{Counts of Peak at } R_{(> 0.5)}}{\textbf{(Sum of Counts of All Peaks)}} \times 100$$

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

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

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

8.7. Interpretation and Reporting of the QC Results

8.7.1. The R_f of the [^{18}F]FES sample must be within 10% of that of the non-radioactive [^{19}F]FES standard, whose R_f is ≥ 0.5 and is typically between 0.8 and 0.9.

8.7.2. The Radiochemical purity of [^{18}F]FES must be $\geq$ 95% using this assay method.

8.7.3. Record the R_f of the [^{18}F]FES sample, the R_f of the non-radioactive [^{19}F]FES standard, and the calculated radiochemical purity results on both the Form **FQC-005**, "[^{18}F]FES Final Product QC Test Results", and in the QC Laboratory notebook.

NOTE: The final product must meet both release specifications outlined in 8.7.1 and 8.7.2 in order for the product to pass this quality control testing requirement.

8.7.4. 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."