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Cancer Imaging Program
National Cancer Institute

Standard Operating Procedure

GEN-Q319

Chemical and Radiochemical Purity by HPLC for [^{18}F]FES

1. **Purpose:** To describe the quality control procedure for using HPLC to determine the mass concentration and chemical and radiochemical purity of the final [^{18}F]Fluoroestradiol ([^{18}F]FES) product solution.

2. **Scope:** The HPLC method described here will be used for determining the chemical and radiochemical purity of the final product, at all contract sites manufacturing [^{18}F]FES. This quality control procedure is one of the quality control release tests performed on the final product and it must be completed prior to releasing the batch of final 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**

   USP 28 ‹621› Chromatography
   USP 28 ‹821› Radioactivity
   USP 28 ‹823› Radiopharmaceuticals For Positron Emission Tomography—Compounding
   GEN-Q015, "Investigating Out of Specification QC Test Results"
   FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)
   ETH50-L, Master Compounding Record for "Preparation of HPLC Mobile phase for Purification of [^{18}F]FES"
   H60FES-L, Master Compounding Record for "Preparation of the [^{18}F]FES HPLC Analytical Mobile Phase"
   GEN-M120, "Documentation of Manufacturing Variances"

SOP: **GEN-Q319** 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
- **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"
- **Form MVR-001**, "Manufacturing Variance Report Form"
- **Form Q319**, "Preparation of FES Standard Solution and Dilutions Form"

6. Policies

N/A

7. Materials and Equipment

7.1. Analytical HPLC Column (Betabasic C_{18} 2.1 mm ID X 150 mm long, 5 µm particles, (Thermo-Hypersil) or equivalent) (**SS-BBA**)

7.2. High Performance Liquid Chromatography (HPLC) System consisting of a high-pressure pump, either an auto-sampler/injector, or manual injector; column and flow-through radioactivity detector; & either a variable or multi-wavelength UV detector, with data acquisition system. A mass spectrometer may also be used for the identity test in section 8.3.2.

7.3. Non-Radioactive FES Standard, (**SS-FES**)

7.4. **ETH50-L**, HPLC Mobile Phase for Purification of [^{18}F]FES

7.5. **H60FES-L**, Analytical Mobile Phase Consisting of 40% HPLC grade water: 60% HPLC Grade Methanol (v:v))

7.6. Automatic pipettors with disposable tips and glass graduated cylinders of appropriate sizes

7.7. Glass volumetric flasks and glass or plastic vials for dilutions

8. Procedures

8.1. Preparation of Standards

8.1.1. **A Stock FES standard solution** consisting of fluoroestradiol (FES) at 1 mg/mL concentration should be made as directed in **Form Q319**.

8.1.2. **Dilutions of the stock FES standard solution:** Several 10-fold to 2-fold dilutions of the stock standard solution are needed to obtain standard concentrations appropriate for the analysis. All stock standard solution dilutions should be made using the preparative mobile phase (**ETH50-L**). Use **Form Q319**, "Preparation of FES Standard Solution and Dilutions Form," to prepare the standard concentration appropriate for analysis.

8.2. Calibration Curves

8.2.1. Inject a minimum of either five dilutions or five volumes of the standard so that the concentrations measured in the standard curve either bracket the solution to be tested or are all greater than the sample to be tested. The lowest standard to be tested must be less than the maximum allowed, 5 µg for the total injected dose corrected for the volume injected. For example, if the injected dose is 20 mL then the minimum acceptable limit, for a 20 mL injection is 5 µg/10 mL = 0.25 µg/mL. The lowest concentration standard should be < 0.25 µg/mL and the concentration (UV absorbance or Mass peak

area) of the highest standard must be greater than that for the product sample injection.

8.2.2. The retention time for FES is approximately 6 minutes. The FES precursor does not elute from the column with this analysis method. Sample chromatograms are shown in Figures 1-3. All standards must be baseline resolved (resolution > 1.5) for a valid analysis.

Parameters
UV Detector: **Set to 280 nm**
Flow Rate: **0.3 mL/min**
Eluent: **60% Methanol: 40% water (v:v) (H60FES-L)**
Temperature: **Room Temperature**
Column: **Betabasic C_{18}:** 2.1 mm ID X 150 mm long (5µm particles)

8.3. Preparation and Analysis of Sample

8.3.1. Inject the sample. (This must be done in triplicate but step 8.3.2 may be done after the first of three sample injections).

8.3.2. As an identity test, either use a mass spectrometer to determine the mass associated with the [^{18}F]FES product peak or inject a sample of the [^{18}F]FES product spiked with sufficient FES standard to observe the UV absorbance from the standard. Either the mass spectrum must show the mass (M/Z) to be 289 M/Z (M-H) (ES^-) in the mass peak associated with the UV absorbance and radioactivity peaks or the UV absorbance peak from the standard spiked sample must co-elute with the radioactive peak and not show two UV absorbance peaks at or near the radioactivity peak.

8.3.3. The test for release will be the completion of the standard curve and the first product injection meeting criteria for release and the identity test. (A reference standard curve can be used if the investigational site has documented consistency of the standard response curve and a single standard run on the day of the test falls correctly on the standard curve). Two more product injections will follow to assure the product FES chemical analysis obtained from the first injection was correct.

8.4. Data Analysis

8.4.1. Integrate all of the UV and, if applicable, MS responses to obtain the peak areas for the FES standards, and the UV or MS responses to obtain the peak areas for the FES.

8.4.2. Determine the equation for the line of regression between the UV or MS responses (area under the curve for the eluted peak) versus concentration.

8.4.3. Determine the correlation coefficient for the linear relationship.

<u>NOTE</u>: If the correlation coefficient is < 0.8, the standards must be re-run and an OOS investigation initiated.

8.4.4. Perform the reverse correlation to determine the concentration of FES in the final solution.

8.4.5. Typically the final concentration of the [^{19}F]FES in the [^{18}F]FES product to be tested will be < 0.25 µg/mL. It is possible that the product sample will give a UV absorbance (peak area) that is below or near the limit of

quantitation for the UV detector and is much less than the 0.25 µg per mL equivalent signal. If the peak area is less than 10 standard deviations of the baseline peak to peak noise of the UV absorbance signal (this is the limit of quantification), then the product will be reported as not quantifiable with a concentration that is less than the value for the limit of quantitation.

8.4.6. Any other 280 nm UV absorbing chromatographic peaks that elute after more than 3.0 minutes up to 80 minutes post injection, e.g. post injection and mobile phase disturbances, should be integrated individually and shown to sum to less than "5 µg" in the final product. Assume that the molar UV absorption coefficients for the unknown contaminants are the same as for FES and that the FES standard curve is applicable to the unknown contaminants.

8.4.7. Integrate all of the radioactive peaks in the chromatogram. Determine the percent of the total radioactivity that is in the [^{18}F]FES peak.

ACCEPTANCE CRITERIA

Radiochemical Purity: **≥ 95%**

Identity of radioactive peak:
Confirmed by co-injection of [^{19}F]FES or 289 M/Z(ES$^-$)

Sum of UV (280 nm) absorbing contaminants:
< 5 µg in the final product dose

Nonradioactive FES (UV 280 nm or 289 M/Z (ES$^-$):
< 5 µg in the final product dose

Sample chromatograms are presented in Figures 1-3. Figure 1 shows an UV absorbance from a chromatogram of [^{19}F]FES standard. Figure 2 shows chromatograms from an [^{18}F]FES product analysis. Figure 3 shows a chromatogram for the FES precursor. The precursor is not eluted with the analytical mobile phase (60% methanol), but is with 80% methanol. The chromatograms in figures 1-2 used the methods including mobile phase, 40% water: 60% methanol and Betabasic C_{18} column described in this procedure.

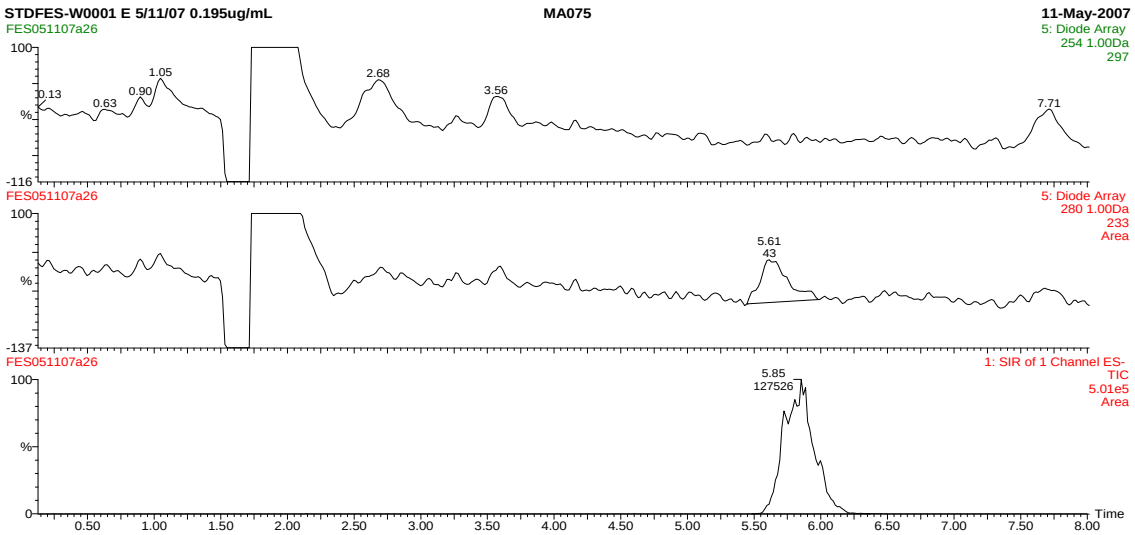

Figure 1. HPLC chromatograms for a 10 µL injection of a 0.195 µg/mL FES standard. Top trace is an expanded scale of the UV absorbance at 254 nm showing poorer FES sensitivity and mobile phase impurities at this wavelength. The middle trace is an expanded scale of the UV absorbance at 280 nm (near the lambda max for FES). The bottom trace is of the mass (ES^-) of FES (M/Z 289) confirming the identity of the UV peak. The slight elution time differences are due to the tubing volume between the detectors. The retention time of the FES is 5.6 minutes for UV and 5.8 for mass (k' = 3.7 (void at 1.2 min see fig 3)). This chromatogram shows that, although small, the minimum standard concentration needed <0.25 µg/mL can be detected with sufficient signal to noise for quantitation of the UV absorbance, although mass spectrometry is preferred because the signal is greater. If a photodiode array detector is used the combined wavelengths of 275 to 285 may be used to increase signal. The mass spectral peak shape is asymmetric due to the sampling times of several channels and data sampling rates of the MS not due to the chromatographic separation. Standard curves (not shown) indicate that despite the "noisiness" of the mass spectral peak, the integrated mass spectral peak is quantitative.

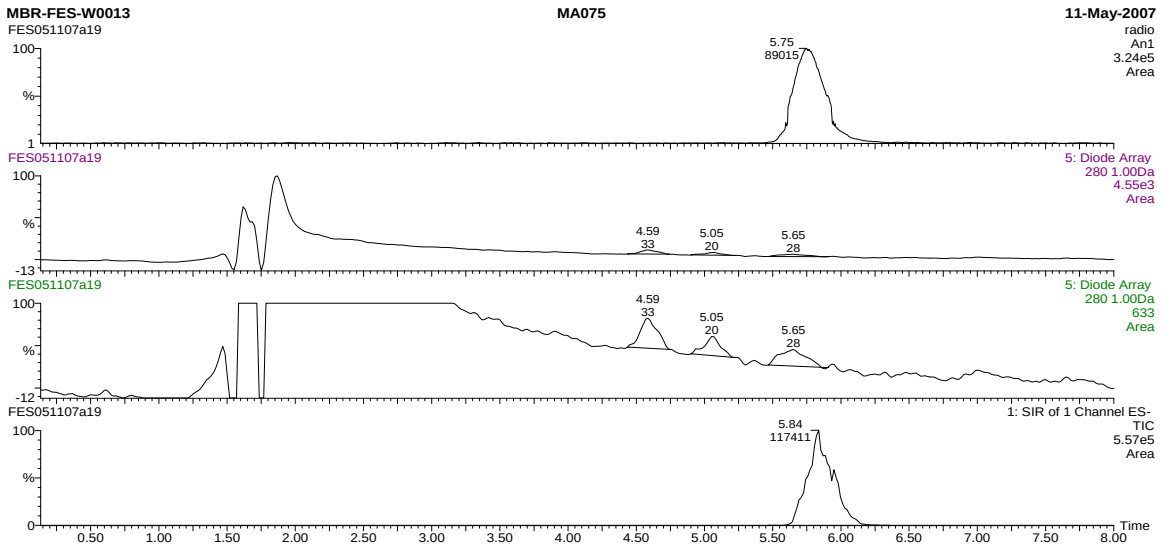

Figure 2. Typical HPLC chromatogram of [^{18}F]FES product. Bottom trace shows the mass of FES (M/Z 289) (ES^-) confirming identity. Top trace is the radioactivity. UV absorbance at 280 nm is shown in the two middle traces. The trace second from bottom is expanded so that the FES mass peak (0.1 µg/mL in this product by mass spectrometry) and impurities can be seen. There is tubing between the UV, radiation and MS detectors so that the FES is eluted at ~5.7 min for UV, ~5.8 min for radiation and 5.9 min for MS. Minor impurities in the USP solvents used for the mobile phase and formulation account for large first peak seen with the UV at ~ 1-3 min.

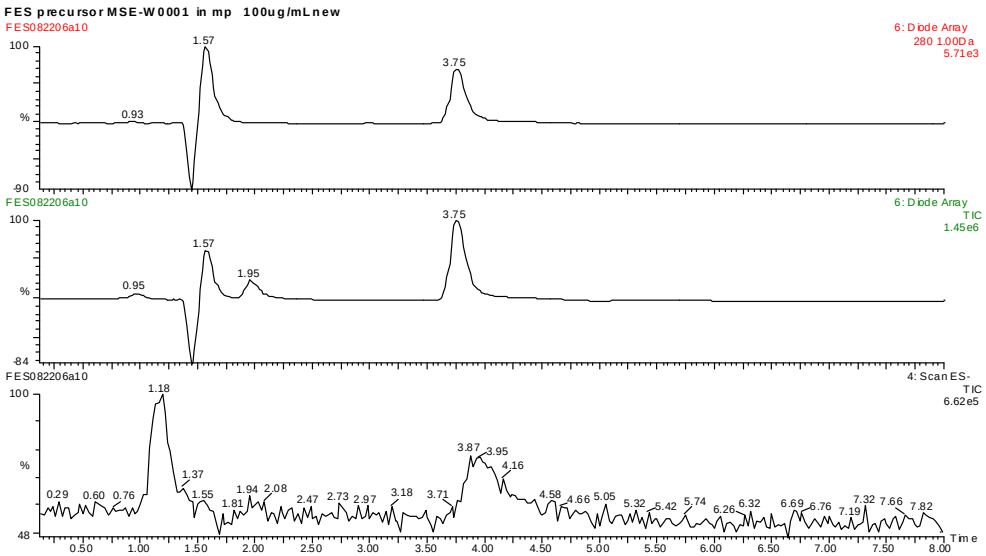

Figure 3 shows the elution of the FES precursor from ABX (**SS-MSE**) (10 µL injected 100 µg/mL solution), using 20% water: 80% methanol (v:v) and the Betabasic C_{18} column with a flow rate of 0.3 mL /min. The precursor is too lipophilic to elute with the 60% methanol used for analytical evaluation of the FES product. The traces from the bottom up, respectively, show; a scan of masses from 50 to 500 M/Z (ES-), a full UV spectrum from 210 to 300 nm, and UV absorbance at 280 nm. The UV absorbance associated with the peak eluted at 1.95 min showed no aromatic absorbance (ie no absorbance above 240 nm). The mass of the peak at 1.18 minutes was 61. The peak at 3.9 minutes is associated with mass 393 the M-H mass of the FES precursor.