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Cancer Imaging Program

National Cancer Institute

Standard Operating Procedure

GEN-Q219

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

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]Fluoromisonidazole ([^{18}F]FMISO) product solution.

2. **Scope:** The HPLC method described here will be used for determining the chemical and radiochemical purity of the final product, at the sites manufacturing [^{18}F]FMISO. 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**
   - **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   - **4.5** FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)
   - **4.6** **W5ETH-L**, Master Compounding Record for "Preparation of HPLC Buffer Solution for Purification of [^{18}F]FMISO"
   - **4.7** **HFM-L**, Master Compounding Record for the "Preparation of 95% 1 mM Formate in water"
   - **4.8** **GEN-M120**, "Documentation of Manufacturing Variances"

SOP**: GEN-Q219** Date:_____

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

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5. **Forms**
   - **Form FQC-010**, "[^{18}F]FMISO 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 Q219**, "Preparation of FMISO Standard Solution and Dilutions Form"

6. **Policies**

   N/A

7. **Materials and Equipment**

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

   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 FMISO Standard, (**SS-FMI**)

   7.4. **W5ETH-L**, Master Compounding Record for the "Preparation of HPLC Mobile Phase for Purification of [^{18}F]FMISO"

   7.5. **HFM-L**, Master Compounding Record for the "Preparation of 95% 1 mM Formate in Water" (Analytical Mobile Phase Consisting of 95% 1 mM HCOOH in HPLC grade water: 5% 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 FMISO standard solution consisting of fluoromisonidazole (FMISO) at 1 mg/mL concentration should be made as directed in **Form Q219**.

   8.1.2. Dilutions of the stock FMISO standard solution: The stock standard solution should be diluted through several 2-fold to 10-fold dilutions to obtain standard concentrations appropriate for the analysis. All dilutions made of the stock standard solution should be made using the preparative mobile phase (**HFM-L**). Use **Form Q219**, "Preparation of FMISO 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 standards 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, 15 µg for the total injected dose corrected for the volume injected. For example, if

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the injected dose is 10 mL then the minimum acceptable limit, for a 10 mL injection is 15 µg/10 mL = 1.5 µg/mL. The lowest concentration standard should be < 1.5 µg/mL and the concentration (UV absorbance peak area) of the highest standard must be greater than that for the product sample injection.

8.2.2. The retention time for FMISO is approximately 5.7 minutes. The FMISO precursor does not elute from the column with this analysis method. Sample chromatograms are shown in Figures 1-4. All standards must be baseline resolved (resolution > 1.5) for a valid analysis.

Parameters
UV Detector: **Set to 327, 254 or 280 nm (327 is preferred)**
Flow Rate: **0.3 mL/min**
Eluent: **5% Methanol : 95% 1 mM formate (v:v) (HFM-L)**
Temperature: **Room Temperature**
Column: **Aquasil 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]FMISO product peak or inject a sample of the [^{18}F]FMISO product spiked with sufficient FMISO standard to observe the UV absorbance from the standard. Either the mass spectrum must show the mass (M/Z) to be 190 M/Z(M+H) and / or M/Z 212 (M+Na) (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 FMISO chemical analysis obtained from the first injection was correct.

8.4. Data Analysis

8.4.1. Integrate all of the UV responses to obtain the peak areas for the FMISO standards, and the UV responses to obtain the peak areas for the FMISO.

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

8.4.3. Determine the correlation coefficient for the linear relationship.

NOTE: If the correlation coefficient is < 0.8, the standards must be rerun and an OOS investigation initiated.

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

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8.4.5. Typically the final concentration of the [^{19}F]FMISO in the [^{18}F]FMISO product to be tested will be < 1 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 1 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 327, 254 or 280 nm (whichever wavelength that is used for detection of FMISO) UV absorbing chromatographic peaks that elute after more than 3.0 minutes post injection, ie. post injection and mobile phase disturbances, must be integrated separately and the total of these impurities must be less than "35 µg" in the final product. Two specific impurities also have individual limits for release of the product (see 8.4.7). Assume that the molar absorption coefficients for the unknown contaminants are the same as for FMISO and that the FMISO standard curve is applicable to the unknown contaminants.

8.4.7. Any peaks that elute after 3.0 minutes must be quantitated in the UV analysis. The primary chemical byproduct, 1-(2,3-dihydroxypropyl)-2-nitroimidazole, elutes from the column at ~ 3.8 min post injection (see figure 3) and must be ≤ 3 µg/mL for the product to be accepted for use. If a specific impurity is observed at ~ 6.0 min, it must be ≤ 4 µg/mL for the product to be accepted for use.

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

ACCEPTANCE CRITERIA

Radiochemical Purity	**≥ 95%**
Identity of radioactive peak confirmed by co-injection of FMISO	**or 190 and /or 212 M/Z**
Non Radioactive FMISO	**< 15 µg in the final product**
Other UV absorbing contaminants	**< 35 µg total in the final product**
	≤ 3 µg/mL for impurity at ~ 4 min
	≤ 4 µg/mL for any impurity at ~ 6 min

8.4.9. Sample chromatograms are presented in Figures 1-4. Figure 1 shows an UV chromatogram of a [^{19}F]FMISO standard. The retention time of the FMISO is ~5.8 minutes (k' = 2.5). Figure 2 shows chromatograms from an [^{18}F]FMISO product analysis. Figure 3 shows a chromatogram for a 1-(2,3-dihydroxypropyl)-2-nitroimidazole standard with a retention time of 3.8 minutes (k' = 1.3). The molecule, 1-(2,3-dihydroxypropyl)-2-nitroimidazole is the major synthetic byproduct of the [^{18}F]FMISO radiosynthesis. Figure 4 shows the retention of the NITTP FMISO precursor . The precursor is not eluted with the analytical mobile phase (5% methanol), but is with 50% methanol. The chromatograms in figures 1-3 used the mobile phase, 95% 1 mM formate: 5% methanol of this procedure.

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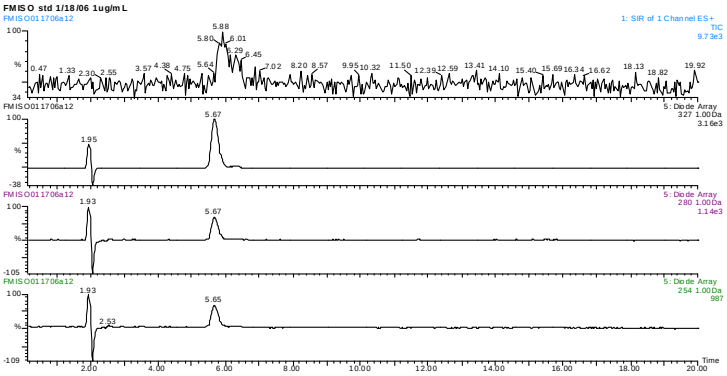

Figure 1: HPLC chromatograms for a 10 µL injection of a 1 µg/mL FMISO standard. Bottom trace is UV absorbance at 254 nm. Second trace from the bottom is the UV absorbance at 280 nm. The second trace from the top shows the UV absorbance at 327 nm. The 327 nm absorbance gave the maximum absorbance. Top trace is of the mass (ES+) of FMISO (M/Z 190) confirming the identity of the UV peak. The slight elution time differences are due to the tubing volume between the detectors.

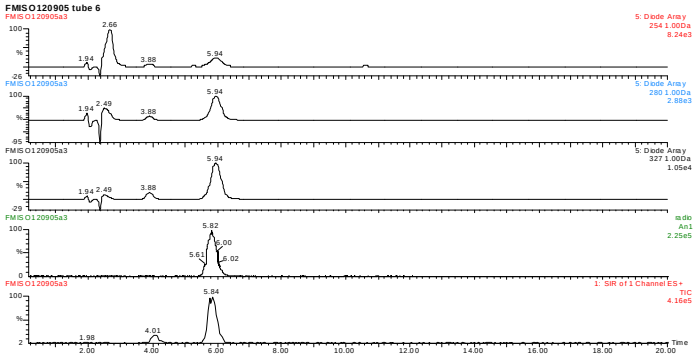

Figure 2: HPLC chromatogram of [^{18}F]FMISO product. Bottom trace shows the mass of FMISO (M/Z 190 (ES+) confirming identity. Trace second from bottom is the radioactivity. UV absorbance at 327 is shown in the middle trace. The trace second from top shows UV absorbance at 280 nm and the top trace shows the UV absorbance at 254nm.

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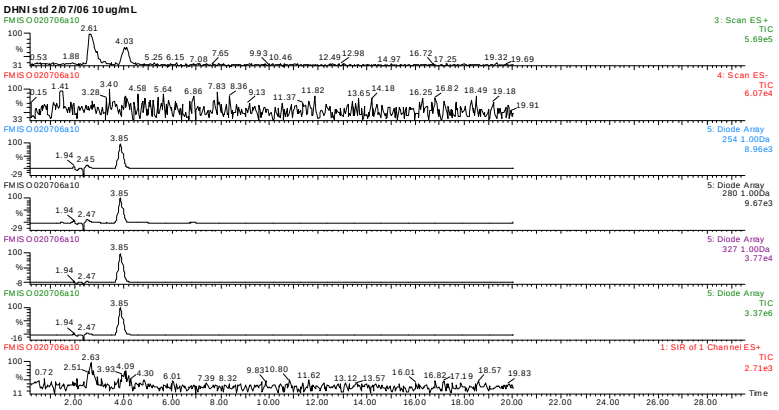

Figure 3 shows a chromatogram of a standard of 1-(2,3-dihydroxypropyl)-2-nitroimidazole with a retention time of 3.8 minutes. The bottom trace shows the mass for FMISO and shows no peak as would be expected. The traces from the second from the bottom going up show the traces for UV absorbance from 210 through 400 nm, 327 nm, 280 nm, 254 nm, all negative ion masses from 200 to 450, and at the top, all positive masses from 200 to 450, respectively. The mass peak at 4.03 is predominantly due to the 179 M/Z of the 1-(2,3-dihydroxypropyl)-2-nitroimidazole. The slight retention time shifts for the UV versus mass are due to tubing in between the detectors.

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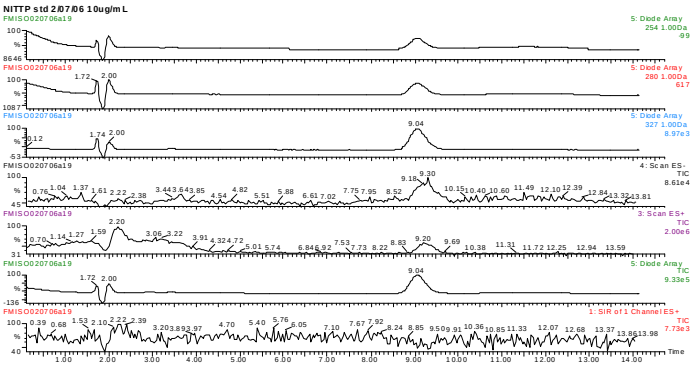

Figure 4 shows the elution of the NITTP FMISO precursor using 50% 1 mM formate: 50% methanol (v:v). The precursor is too lipophilic to elute with the 95% 1 mM formate solution. The traces from the bottom up, respectively, show; the mass of FMISO, a full UV spectrum from 210 to 300 nm, a full scan of positive ion masses from 150 to 450 M/Z, a full spectrum of negative ion masses from 150 to 450 M/Z, UV absorbance at 327 nm, UV absorbance at 280 nm and UV absorbance at 254 nm.

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