Template Provided By:
Cancer Imaging Program
National Cancer Institute

Master Production Record

MPR-[^{18}F]FMISO

For Synthesis of [^{18}F]-Fluoromisonidazole: [^{18}F]FMISO Injection

Purpose: This is the Master Production Record for synthesizing [^{18}F]-Fluoromisonidazole using an automated radiochemistry system, to produce this specific fluorine-18 labeled radiopharmaceutical product for investigational use only.

REFERENCES & MATERIALS OVERVIEW

A. REFERENCES

1. **MBR-[^{18}F]FMISO-L**, "Master Batch Record for Synthesis of [^{18}F]-Fluoromisonidazole, [^{18}F]FMISO Injection"
2. **Gen-Q121,** "Chemical Purity by Visual Inspection**"**
3. **Nucleophilic Synthesis Module System Operator Guide, to be provided by Sponsor**
4. **Nucleophilic Synthesis Module System Software Manual**
5. **Nucleophilic Synthesis Module Operator Manual**
6. **Gen-Q211,** "Radiochemical Identification & Purity of [^{18}F] -Fluoromisonidazole ([^{18}F]FMISO) Injection by Radio-Thin Layer Chromatography Analysis"
7. **Gen-Q212**, "Gas Chromatography Analysis of Residual Solvents for [^{18}F]-fluoromisonidazole [^{18}F]FMISO and [^{18}F]-fluoroestradiol [^{18}F]FES"
8. **Gen-Q113**, "Radionuclidic Identification by Half-Life Determination"
9. **Gen-Q114**, "Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test"
10. **Gen-Q114A,** "Alternative procedure for Bacterial Endotoxins by Limulus amoebocyte Lysate (LAL) Test Using the PTS (Portable Test System)"
11. **Gen-Q115**, "pH Testing of Final Product"
12. **Gen-Q117**, "Sterility Test"
13. **Gen-Q117A**, " Alternative Procedure for the Sterility Test"
14. **Gen-Q110**, "Filter Integrity Test by Bubble Point"
15. **Gen-Q118**, "A Color Spot Test for the Detection of Kryptofix® [2.2.2]"
16. **Gen-Q118A**, "An Alternate Test for the Detection of Kryptofix® [2.2.2]"
17. **Gen-Q219**, "Chemical and Radiochemical Purity by HPLC for [^{18}F]FMISO"

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

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18. **Gen-Q012**, "Quality Systems Administration"
19. **Gen-Q015**, "Investigating Out of Specification QC Test Results"
20. **Gen-R004**, "Assigning Batch & Compounding Record Numbers to Production Runs"
21. **Gen-R005**, "Drug Product Complaints"
22. **Gen-R006**, "Product Labeling"
23. **Gen-R008**, "Good Documentation Practices"
24. **Gen-R209**, "QC Testing, Review, & Final Release for [^{18}F]FMISO"
25. **Gen-R502**, "General Equipment Maintenance"
26. **Gen-R503**, "Software Administration and Change Control for the Nucleophilic Synthesis Module"
27. **Form FQC-010**, "[^{18}F]FMISO Final Product QC Test Results" Form
28. **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"
29. **Form MVR-001**, "Manufacturing Variance Report Form"
30. **Form EQ-001**, "General Equipment History Log"
31. **Form R004-L**, "General Log for Assigning Unique Batch or Compounding Record Numbers"
32. **Form S-FMISO**, "Supply Sheet for Production of [^{18}F]-Fluoromisonidazole"
33. **Form DCI-L**, "Drug Product Complaint Investigation Form"
34. **Form DPC-L**, "Drug Product Complaint Log"
35. **W5ETH-L**, "Master Compounding Record for the Preparation of 5% Ethanol in Water, USP"
36. **ETH70-L**, "Master Compounding Record for Preparation of 70% Ethanol"
37. **KCO2-L**, "Master Compounding Record for the Preparation of 0.1M Potassium Carbonate"
38. **KRYP-L**, "Master Compounding Record for the Preparation of Kryptofix [2.2.2] Solution"

B. EQUIPMENT

Equipment Description
Nucleophilic Synthesis Module System with glassware including glass reaction vial and stir bar
Cyclotron with F-18 target
HPLC Pump with Injector
Flow through UV Absorbance Detector
Vacuum Pump
Computer with Nucleophilic Synthesis Module Software installed
Printer
HPLC Preparative Column (Econosphere C_{18} 10 x 250 mm)

C. REAGENTS/MATERIALS

GENERAL SUPPLIES
Appropriate Safety Wear including radiation badges, lab coat, safety glasses, shielding, gloves
2 beakers. One for filter integrity test, one for clean up.
Graduated cylinder to measure HPLC flow with stopwatch
Container for Solvent Waste, 200-2,000 mL bottle
Cryogen: dry ice/solvent mixture, Nitrogen $_{liq}$, or Argon $_{liq}$
Helium, Argon, or Nitrogen Gas 30-150 psig supply on and > 500 psi in tank or sufficient cryogen
Compressed Air 70-150 psig supply on with sufficient tank or compressor capacity

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Reagents / Solutions	Quantity
FMISO supplies kit	1
FMISO Precursor, 1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonyl-propanediol	5 ± 1 mg
Acetonitrile, USP	1.0 ± 0.05 mL
Acetone, spectrophotometric grade	qs to clean unit
$H_2{}^{18}O$, Target material	1.0 – 5.0 mL
Compounded Solutions (Compounded from USP reagents)	**Quantity**
70% Vol. Ethanol	500 mL
(95:5) Water for injection: Ethanol (absolute)	~ 1 L

D. SPECIAL NOTES AND POLICIES

- Refer to the appropriate section of the Nucleophilic Synthesis Module Operator's Manual for Health and Safety Procedures prior to operating this equipment.
- Always use non-lubricated syringes for any reagent that enters the reaction vessel.
- For loading the Nucleophilic Synthesis Module, use fresh, sterile syringes and needles for each solution.
- To extend the lifetime of the reagent vials you should use **only long, thin needles that reach down to the bottom of the reagent vial.** This prevents air bubbles in the reagent vial neck; the recommendation is 23G x 22/g inch or 0.60 x 60 mm. Additions can be made by unscrewing the caps and then recapping the vials.

E. RESPONSIBILITIES

- Each site should ensure that their operators have all of the required credentials and prerequisite training required to synthesize radiopharmaceuticals. Document all manufacturing control information on the corresponding Master Batch Record, **MBR-[^{18}F]FMISO-L**.

I. Maintenance Cleaning and Preparation of the Nucleophilic Synthesis Module

I.1. Condition the Preparative HPLC Column as Follows:

I.1.1. Using aseptic technique, add ≥ 200 mL of 70% ethanol (**ETH70-L**), USP to eluent bottle number 2 and ≥ 500 mL of fresh preparative mobile phase (**W5ETH-L**, 5% ethanol abs, USP: 95% water for injection, USP (v:v) to eluent bottle number 1.

I.1.2. Make certain that the Econosphere preparative HPLC column, **(SS-ECO**), is correctly installed.

I.1.3. On the Nucleophilic Synthesis Module software page, select "Manual" operation. Switch the injector to the inject position.

I.1.4. In manual operation, start the mobile phase purge gas. Check that the gas is flowing at the flasks. Open value to eluent 2, (70% Ethanol), Set the pump flow to 5 mL/min and run 70% ethanol (**ETH70-L**) through the column for at least 10 minutes to rinse the HPLC column and injector. Do not let the HPLC column go

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dry and check for leaks. If there are any leaks tighten the fittings and flow an additional 10 minutes with no leaks.

I.1.5. After the 70% ethanol has flowed through the column, set the HPLC pump flow to 0 mL/min. The value to eluent 1, (**W5ETH-L**, 95% water for injection: 5% ethanol).

I.1.6. Set the pump flow to 5 mL/min. Let the HPLC pump run for at least 10 minutes. Then measure the flow rate out of the HPLC column to assure that the system is operating correctly. The measured flow rate must be between 4.8 and 5.2 mL/min. If the flow rate is incorrect purge the pump, check the fittings, and recheck flow. Once the correct flow is established, set the pump flow to 0 mL/min and close the HPLC gas purge. The HPLC column will be rinsed further during the automated synthesis.

I.1.7. Stop manual run and return to automatic operation.

Perform Maintenance on the Nucleophilic Synthesis Module Box as follows:

I.2.1. If necessary, remove ^{18}F separation column and purification column from synthesizer, and store the used columns in a shielded waste container to decay as per the institutional radiation waste policy.

I.2.2. Empty the contents of the ^{18}O water vial into a clean storage vial. Keep that storage vial in a shielded container to decay. Dry the ^{18}O water vial with a clean low particulate wipe and reattach it to the synthesizer.

I.2.3. Remove the cold trap from the dry ice/solvent mixture, liquid nitrogen or argon dewar. Empty the liquid from the cold trap into an approved solvent waste container. Allow the cold trap to air dry before placing it in the drying oven, if oven drying the cold trap.

I.2.4 Using appropriate safety gear fill the dewar halfway with new dry ice/solvent mixture, liquid nitrogen or liquid argon. Replace the dewar in the synthesizer. If using a clean cold trap from the drying oven; allow it to cool before use. Reattach the cold trap lines and carefully lower it into the dewar.

I.2.5. Empty the v-vial into an approved solvent waste container. Dry the vial with a low particulate wipe and reattach it to the synthesizer.

I.2.6. Empty the waste bottle into an approved solvent waste container and reattach the 250 – 2,000 mL plastic waste bottle to the 3 waste lines from the synthesizer. The waste lines must be near the top of the bottle to prevent back-flow into the synthesizer.

I.2.7. Inspect or replace the Reaction Vessel: (*Sponsor should provide these procedures here by referring to the appropriate maintenance manual for the Nucleophilic Synthesis Module.*)

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I.2.8. Record any change in the reaction vessel or the magnetic stir bar in the Equipment History Log Book for the Nucleophilic Synthesis Module, on **Form EQ-001**.

I.3. Cleaning the Nucleophilic Synthesis Module (the Sponsor is responsible for providing the remainder of the cleaning procedure for the corresponding synthesis module as appropriate.)

I.2.1. Turn on the compressed air and nitrogen or helium gas supplies. Verify that the gas supplies are sufficient and within at the specified pressure ranges.

I.2.2. Turn on the computer to the nucleophilic synthesis module and the power to the synthesizer's electronics unit. Initialize the module's software program.

I.2.3. The program will prompt the user to enter a password and username. Please note that all operations performed on the system should require the use of a password and username.

I.2.4. Document the equipment cleaning as per **GEN-R502** in the equipment history log, **Form EQ-001**.

II. Pre-Synthesis Setup for Manufacturing [^{18}F]FMISO Using A Nucleophilic Synthesis Module (Note: These instructions may vary with different modules)

II.1. Prepare the ^{18}F Separation Column as follows:

II.1.1. Wash the ^{18}F Separation column (**SS-FSC**) with 1 mL of ethanol, absolute, USP, (**SS-ETH**) using a 1 mL, non-lubricated syringe.

II.1.2. Wash the ^{18}F Separation column with 1 mL of sterile water for injection (**SS-WFI**) using a 1 mL, non-lubricated syringe. Wait 5 minutes.

II.1.3. Expel the residual water from the column using 1 mL of air in the syringe. Do not dry the column any further.

II.2. Prepare the Sterile Product Vial Assembly as Follows:

II.2.1. Label the empty product sterile vial (**SS-SBV**) with the [^{18}F]FMISO Master Batch Number (**MBR-[^{18}F]FMISO-XXXXX**). Swab the top and sides of the vial. Place the vial in a laminar flow hood. Reswab the top of the vial using an alcohol swab.

II.2.2. Insert a venting filter needle (**SS-VFN**) into the empty sterile vial (**SS-SBV**).

II.2.3. Add 0.40 mL of concentrated saline, for dilution, USP (**SS-NCC**) to the vial.

II.2.4. Use aseptic technique, in a laminar flow hood, to insert a sterilizing filter (**SS-SPF**) with a ≤ 22 G needle into the final product empty sterile vial.

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II.3. Attach the [^{18}F]-fluoride line from the cyclotron target to the [^{18}F] inlet line of the synthesis box.

II.4. Add 0.1 M K_2CO_3 Solution as follows:

II.4.1. Draw up 0.50 ± 0.05 mL of 0.1 M K_2CO_3 (**KCO2-L**) solution using a 1.0 mL, non-lubricated syringe and long needle and transfer it to vial 1 of the synthesis box. The needles can be pushed through the reagent vial septa or the reagent vials can be uncapped (preferred) for reagent addition. Make certain the caps on the glass vials are tight after the additions.

II.5. Add Kryptofix [2.2.2] solution as follows:

II.5.1. Draw up 2.0 ± 0.2 mL of 15 mg/ 2 mL Kryptofix [2.2.2] (**KRYP-L**) in anhydrous acetonitrile using a 3.0 mL, non-lubricated syringe with long needle. Transfer the solution to vial 2 of the synthesis box.

II.6. Add 0.5 M HCl Solution as follows:

II.6.1. Draw up 2.0 ± 0.2 mL of 0.5 M HCl (**HCL-L**) using a 3.0 mL, non-lubricated syringe and long needle. Transfer the solution to vial 4 of the synthesis box.

II.7. Add Sodium Bicarbonate Solution as follows:

II.7.1. Draw up 2.5 ± 0.2 mL of USP sodium bicarbonate solution (**SS-BIC**) using a 3.0 mL, non-lubricated syringe and long needle. Transfer the solution to vial 5 of the synthesis box.

II.8. Prepare and add the FMISO precursor Solution as follows:

II.8.1. Draw up 2.0 ± 0.2 mL of anhydrous acetonitrile (**SS-ACN**) using a 3.0 mL, non-lubricated syringe and long needle.

II.8.2. Add the acetonitrile to the vial containing 2 mg of the FMISO precursor, 1-(2´-nitro-1´-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulfonylpropanediol; NITTP (**SS-NIT**), through the septum and shake the vial to dissolve.

II.8.3. Leave the syringe in the vial and the vial as it is until it is time to transfer the dissolved contents into reagent Vial 6 using the 3 mL non-lubricated syringe and long needle.

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II.9. Run the FMISO Program as follows: *This section is the list of prompts for the specific computer program and the adding of reagents prior to starting the synthesis—the sponsor should provide this depending on which nucleophilic synthesis module they choose to utilize.*

III. Manufacturing [^{18}F]FMISO Using A Nucleophilic Synthesis Module

III.1. Synthesize [^{18}F]FMISO

III.1.1. After the requested inputs are made, press the start the synthesis.

III.1.2. From the cyclotron control station, deliver the $^{18}F^-$ activity to the synthesis box. Observe the accumulation of $^{18}F^-$ activity in the v-vial. Allow activity to transfer from the target until the v-vial activity ceases to increase. Record the times that $^{18}F^-$ collection started and stopped.

III.1.3. Verify that the cyclotron transfer process is complete on the cyclotron workstation. Then on the synthesizer computer, initiate the synthesis. The synthesis program will take approximately 30 minutes to complete. Record synthesis start and stop times, and the amount of ^{18}F delivered in the corresponding master batch record.

III.1.4. Once the synthesis is complete, the product is injected on the HPLC column (**SS-ECO**) for purification. The software display should show the HPLC detector output screens once the product has been injected onto the HPLC system. Both the UV and Radioactivity traces are displayed. The FMISO should be visible on the Radioactivity display at approximately 9-11 minutes. Begin collection when the FMISO peak appears on the Radioisotope display. When the peak is no longer visible end the collection (> 1 minute later). Record the start and stop chromatographic retention times.

III.1.5. Once the FMISO program remove the [^{18}F]FMISO product vial assembly from the hot cell if necessary and transfer it behind the shield.

III.1.6. Remove the sterilizing filter from the [^{18}F]FMISO product vial.

IV. Post Synthesis

IV.1. Withdraw Samples for Quality Control Testing

IV.1.1. Assay the collection vial using the calibrated ion chamber. Record the assay time as the End of Synthesis Time in the corresponding batch record for this batch of product. Place the collection vial into the dose-drawing shield.

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IV.1.2. Visually inspect the product [^{18}F]FMISO as per **Gen-Q121** looking for particulates, describe clarity and color. If the sample is not free of particulates and colorless, the product cannot be used. Document the color and particulate state, i.e. free of particulates or not, on **Form FQC-010**.

IV.1.3. Detach the sterilizing filter from the delivery line, and place it in a lead shield for the filter integrity test. Perform the integrity test as per SOP **Gen-Q110** and record the bubble point pressure on the batch record.

IV.1.4. Swab the product [^{18}F]FMISO vial with an alcohol swab. Using a 0.5 or 1 mL tuberculin syringe, aseptically withdraw an accurate 0.5 mL sample for Quality Control Testing. Replace the sheath on the needle. Assay the sample and then place the syringe in a lead shield. Re-swab the product vial. Transfer the shielded syringe to Quality Control for testing with **Form FQC-010**.

IV.1.5. In the batch record, calculate the specific concentration in mCi/mL, at the End of Synthesis (EOS).

IV.1.6. Aseptically use a (21G or 22G) x 1½" needle on a 3-mL syringe to withdraw a 2.0 mL sample into the syringe for sterility tests (1.0 mL) and reserve sample (1.0 mL) and place the syringe in a lead shield. Transfer the shielded syringe to Quality Control for preparing the sterility samples and saving the reserve.

NOTE: The sterility sample must be inoculated within 48 hours of EOS. Remove and discard the vent filters.

IV.1.7. Record the results of the QC release tests on **Form FQC-010**. The tests to be conducted should include the following:

- "Radiochemical Identification & Purity of [^{18}F]-Fluoromisonidazole ([^{18}F]FMISO) Injection by Radio-Thin Layer Chromatography Analysis," per SOP **Gen-Q211**
- "Gas Chromatography Analysis of Residual Solvents for [^{18}F]-fluoromisonidazole [^{18}F]FMISO and [^{18}F]-fluoroestradiol [^{18}F]FES," per SOP **Gen-Q212**
- "Radionuclidic Identification by Half-Life Determination," per SOP **Gen-Q113**
- "Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test," per SOP **Gen-Q114 or** "Alternative Procedure for Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test Using the PTS (Portable Test System), " per SOP **Gen-Q114A.**
- "pH Testing of Final Product," per SOP **Gen-Q115**

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- "A Color Spot Test for the Detection of Kryptofix® [2.2.2]," per SOP **Gen-Q118** or "An Alternative Test for the Detection of Kryptofix® [2.2.2] per SOP **Gen-Q118A**.
- "Chemical and Radiochemical Purity by HPLC for [^{18}F]FMISO," per SOP **Gen-Q219**
- "Chemical Purity by Visual Inspection," per SOP **Gen-Q121**

IV.1.8. Record any manufacturing variances as per SOP **Gen-M120**, "Documentation of Manufacturing Variances," using **Form MVR-001**, and attach them to the corresponding batch record for this batch of product. Record the number of variances reported for this batch.

IV.1.9. Attach appropriate labels to the [^{18}F]FMISO product vial and in the batch production record where indicated.

IV.1.10. Perform final review and release of QC test results as per **Gen-R209**, "QC Testing, Review, & Final Release for [^{18}F]FMISO."

IV.1.11. Record final sterility results on **Form FQC-010** once they are complete and available. The sterility testing should be completed as per "Sterility Test," per SOP **Gen-Q117 or** "Alternative Procedure for the Sterility Test," as per SOP **Gen-Q117A.**

V. Post-Synthesis Clean-Up of the Nucleophilic Synthesis Module System after [^{18}F]FMISO Synthesis

V.1. Clean the HPLC Injector & Column with Ethanol

V.1.1. Attach the lines from the system to the waste bottle.

V.1.2. Add ethanol (**SS-ETH**, 4 mL) to the system HPLC loading vial by passing the alcohol.

V.1.3. Change out the eluent bottle 1 containing the preparative mobile phase (**W5ETH-L**), 95% water for injection and 5% absolute ethanol to a new eluent bottle 1 containing >200 mL of 70% ethanol (**ETH70-L**).

V.1.4. Select the Nucleophilic Synthesis Module HPLC ethanol cleaning Program.

V.1.5. Start the FMISO HPLC cleaning program.

V.1.6. Verify the cleaning program is complete.

V.1.7. Sign and date print out, and attach it to the batch record. Document any work on the equipment in the equipment history log book on **Form EQ-001**.

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V.2. Clean the Nucleophilic Synthesis Module Box (the Sponsor is responsible for providing the remainder of the cleaning procedure for the corresponding synthesis module as appropriate.)

I.2.1. Turn on the compressed air and nitrogen or helium gas supplies. Verify that the gas supplies are sufficient and within at the specified pressure ranges.

I.2.2. Turn on the computer to the nucleophilic synthesis module and the power to the synthesizer's electronics unit. Initialize the module's software program.

I.2.3. The program will prompt the user to enter a password and username. Please note that all operations performed on the system should require the use of a password and username.

I.2.4. Document the equipment cleaning as per **GEN-R502** in the equipment history log, **Form EQ-001**.

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