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This is a template; you must substitute your own procedures/data where relevant

7. Chemistry, Manufacturing, and Control

7.1. Product Summary:

[^{18}F]fluoromisonidazole, 1H-1-(3-[^{18}F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole, [^{18}F]FMISO

7.1.1. Chemical Structure

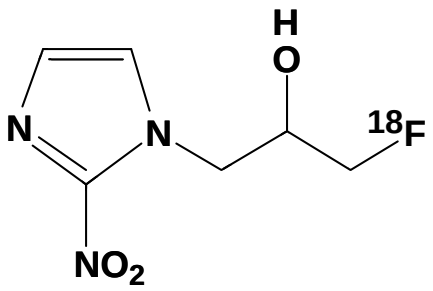

Figure 7.1. [^{18}F]fluoromisonidazole.

7.1.2. Specifications for the [18F]FMISO final product

Table 7.1.

TEST	SPECIFICATION
Chemical Purity (particulates)	Clear and Colorless
pH	6-8
Residual Kryptofix® [2.2.2]	< 50 µg/ mL Kryptofix®
Radiochemical Purity (HPLC)	> 95%
Chemical Purity (HPLC)	FMISO ≤ 15 µg per injected dose
	other UV absorbing impurities beyond void volume (327, 280 or 254 nm) ≤ 35 µg per injected dose Specific Impurity at ~4 min ≤3µg/ml Specific impurity at ~6 min ≤4µg/ml
Radiochemical Purity(TLC)	R_f = >0.5 Purity ≥ 95%
Residual Solvent Levels	Acetone < 5000 ppm Acetonitrile < 400 ppm
Radionuclidic Purity	Measured half-life 110-120 minutes
Bacterial Endotoxin Levels	< 175 EU per dose
Sterility	no growth observed in 14 days , also must pass filter integrity test

7.1.3. [^{18}F]FMISO Drug Product Components and Composition

The final product has been characterized and is as follows:

TABLE 7.2.

COMPONENTS	GRADE	Amount in Injectate
[^{18}F]FMISO, 1H-1-(3-[^{18}F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole	**Same as for [^{19}F]FMISO**	**≤ 10 mCi**
[^{19}F]FMISO, 1H-1-(3-[^{19}F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole	**NCS#292930**	**≤ 15 µg**
Ethanol, absolute	**USP**	**5% by volume**
Saline for injection	**USP**	**0.15 M**

TABLE 7.3.

IMPURITIES	Acceptance Criteria See Section 7.9.3	Highest Values in Qualification Runs (n=9)
Kryptofix® [2.2.2]	< 50 µg/mL	None detected
Acetonitrile	< 400 ppm	< 50 ppm
Acetone	< 5000 ppm	< 313 ppm

The name of the drug is 1H-1-(3-[^{18}F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole, or [^{18}F]fluoromisonidazole, ([^{18}F]FMISO). FMISO is the only active ingredient and it is formulated in a solution of ≤ 10 mL of 95% 0.15 M saline: 5% ethanol (v:v). The drug product solution is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial with an expiration time of **(12)** hours **(note that site specific validation data are needed to support your assigned expiration)**. The injectable dose of [^{18}F]FMISO for this study will be ≤ 0.10 mCi/kg not to exceed 10 mCi with a specific activity greater than 125 Ci/mmol at the time of injection. The amount of injected drug is ≤ 15 µg (≤ 80 nmol) of FMISO. [^{18}F]FMISO is administered to subjects by intravenous injection of ≤ 10 mL. In the dose of [^{18}F]FMISO only a small fraction of the FMISO molecules are radioactive. There is no evidence that nonradioactive and radioactive FMISO molecules display different biochemical behavior.

The synthesis of [^{18}F]FMISO is shown schematically in Figure 7.2.

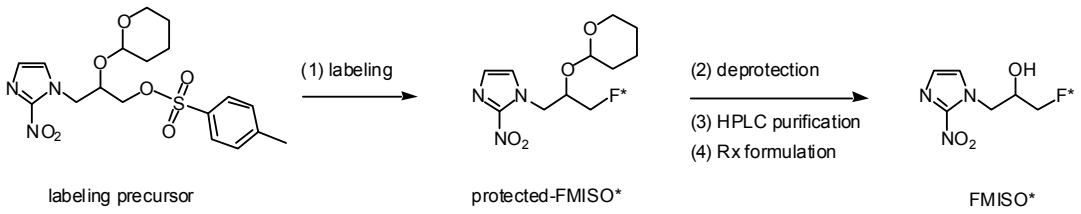

Figure 7.2. Schematic of the radiosynthesis of [^{18}F]fluoromisonidazole.

The synthesis procedure follows that reported by Lim and Berridge (1993). The method in this IND for manufacture of [^{18}F]FMISO differs from some of those used for the

patient data supplied in the toxicity section. The most significant difference is that the proposed method involves the nucleophilic displacement of a tosylate group by a fluorine atom and the removal of a tetrahydropyranyl group protecting the hydroxyl group. The purpose of the protecting group is to prevent ^{18}F from labeling the hydroxyl position. The older method (Grierson et al. (1987) synthesized the molecule by preparing [^{18}F]-epifluorohydrin from the reaction of [^{18}F]-fluoride with glycidyl tosylate. Then the [^{18}F]-epifluorohydrin was reacted with 2-nitroimidazole to form [^{18}F]-fluoromisonidazole. The newer reaction chemistry of Lim et al. is superior because it is reliable, yields [^{18}F]FMISO with a good specific activity, and the nonradioactive precursor is commercially available from a GMP facility (Advanced Biochemical Compounds, ABX).

An automated synthesis procedure is used instead of manual radiosynthesis, which will minimize the variance in the chemical reaction when compared with previously published syntheses of [^{18}F]FMISO. Thus, the use of a semi-closed and automated system should reduce the risk to the subjects.

7.2. Controls for Components and Raw Materials

There is a complete set of forms and standard operating procedures in place to follow the manufacturing, materials and process from ordering to final approval for injection. A list of the documents used in the manufacture and control of [^{18}F]FMISO is in **Appendix 1 (your document list goes here)**. Each reagent or supply has a unique internal specification code of three characters and a unique sequential number, for each lot for each specification code. When an item is ordered it is given a unique internal tracking number for the reagent or supply. The specification sheets list all of the reagent and supply manufacturers' addresses and contact information, including any pertinent certificate of analysis or certificate of quality criteria that these reagents/supplies must meet prior to their release for further use. Any additional testing beyond what may be listed on the certificate of analysis (C of A) or certificate of quality (C of Q) is described in this IND submission.

7.2.1. Organic Substrate Used for the Radiosynthesis of [^{18}F]FMISO.

TABLE 7.4.

REAGENT	CURRENT SUPPLIER	ADDITIONAL TESTING REQUIRED
[^{18}F]FMISO Precursor; 2´-nitro-1´-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulfonylpropanediol; **NITTP** **CAS No.** [150196-34-2]	**ABX**	1. HPLC-UV or MS. UV absorbance post void volume is >90% NITTP 2. Quality control testing on first batch made to show the correct product is made.

The identity and purity of the [^{18}F]FMISO precursor is supported by the certificate of analysis provided by the supplier, ABX. The ABX test for identity is by ^{1}H and ^{13}C NMR and purity by ^{1}H NMR. The investigational site will confirm the purity and identity of each lot of [^{18}F]FMISO precursor by HPLC analysis. The separation is by

reversed-phase HPLC using an Aquasil C18 column (see Table 7.13) with a 50% 1 mM formate: 50% methanol (v:v) mobile phase flowing at 0.30 mL/min. and a column temperature of 25 to 30°C. UV absorbance and or mass detection (Figure 7.3) **(put your data here)** are used. This analytical separation is similar to that for [^{18}F]FMISO except 50% methanol, rather than 5% methanol, is used in the mobile phase to elute the precursor, due to its lipophilicity. Radiosynthesis of [^{18}F]FMISO will validate the lot of precursor because the product will have to meet all of the quality assurance criteria including HPLC radiochemical purity with the peak retention the same as the FMISO standard and / or mass identification prior to release of the product.

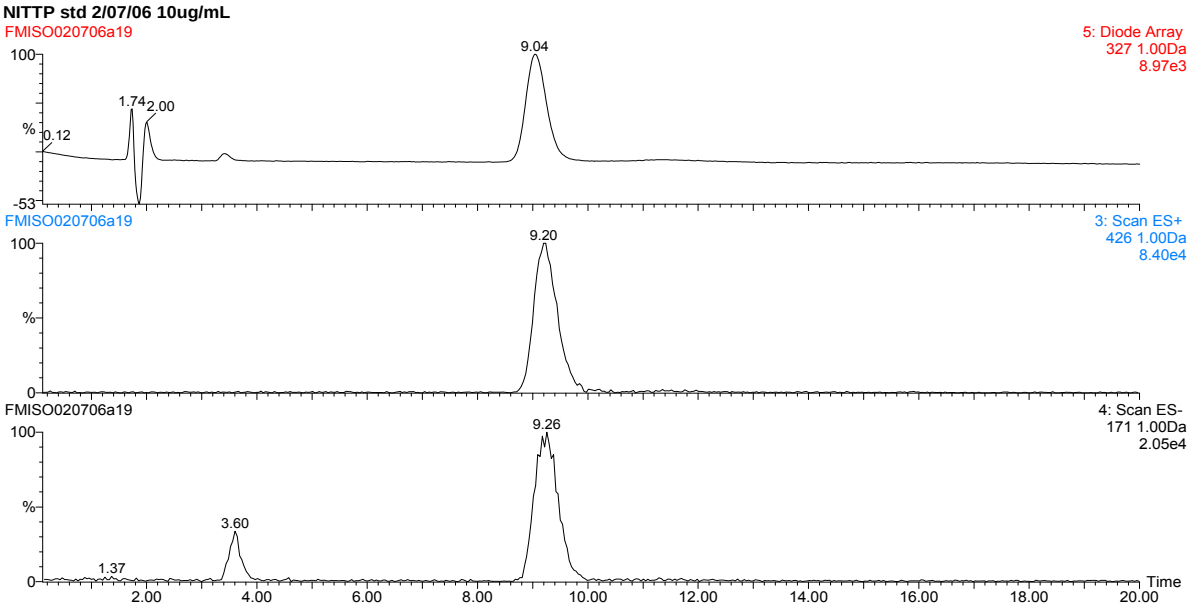

Figure 7.3. Sample chromatograms of HPLC of the nonradioactive [^{18}F]FMISO precursor, NITTP. Top trace shows UV absorbance at 327 nm, the wavelength of maximum absorptivity for FMISO. Middle and bottom traces show masses using positive ion electrospray mass spectrometry. Middle trace is the precursor M+H of 426 M/Z with retention time of 9.2 min (2.7 mL K'~4.2) and bottom trace is a breakdown mass and submass fraction for negative ion at M/Z of 171. The UV detector is upstream of the mass detector so the UV and Mass chromatograms have slightly different retention times.

7.2.2. Target Material for Preparing [^{18}F]-Fluoride Reagent, a Key Intermediate

The radioactive fluoride precursor will be produced on site. The following target material will be used for the production of radioactive fluoride precursor:

TABLE 7.5.

REAGENT	CURRENT SUPPLIER	ADDITIONAL

		TESTING REQUIRED
$H_2{}^{18}O$ target water, > 80% enriched in ^{18}O	**Rotem, Isonics, Isoflex, Isotec, Cambridge Isotope Laboratories, Eastern Isotopes, Medical Isotopes**	**None**

7.2.3. Other Ingredients

The following ingredient(s) are used in the formulation of [^{18}F]fluoromisonidazole ([^{18}F]FMISO) injection.

TABLE 7.6.

REAGENT	PURPOSE	CURRENT SUPPLIER
23.4 % Sodium Chloride Solution, for Dilution, USP (234 mg/mL)	**To make the formulation isotonic prior to injection.**	**Any USP supplier**

7.2.4. Reagents, Solvents, Gases, Purification Columns, Solutions, and Other Auxiliary Materials

TABLE 7.7. Reagents/Supplies Used for Radiosynthesis of [^{18}F]FMISO.

REAGENT/SUPPLY	GRADE	CURRENT SUPPLIER*
Ethyl Alcohol,	Absolute USP	Any USP supplier
Sterile Water for Injection, SWFI	USP	Any USP supplier
Kryptofix® [2.2.2], (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane)	99% purity or greater	Sigma-Aldrich, EMD (VWR International) or Rotem Industries
Potassium carbonate, K_2CO_3	high purity	Sigma-Aldrich or Mallinckrodt Baker
Concentrated Hydrochloric Acid, HCl	36.5-38.0%, NF	Sigma-Aldrich or Mallinckrodt Baker
Hydrochloric Acid Solution	Concentrated USP/NF or 1.0 or 0.5 M EuPh	Sigma-Aldrich or Mallinckrodt Baker
Sodium Bicarbonate 4.2% neutralizing additive solution	2.5 mEq / 5mL, Single Dose Vial, NDC 63323-026-05 2605, sterile, pyrogen free	American Pharmaceutical Partners, Inc or equivalent
Acetonitrile	99.8%, anhydrous	Sigma-Aldrich or Mallinckrodt Baker
Acetone	99.5 +% ACS grade	Sigma-Aldrich or Mallinckrodt Baker
Sterile Vent Filter 0.2 µm Millex SLGV V25 5F	sterile, pyrogen free	Millipore Corporation
[^{18}F] Separation Cartridge, an anion exchange solid phase extraction cartridge	not applicable	Macherey Nagel, supplied by GE Medical Systems
HPLC Preparative Column (Econosphere C^{18} 10 x 250 mm with or without guard column)	not applicable	Alltech Associates, Inc.
Sterile Vented Filter Needle	sterile, pyrogen free	International Medical Industries
Sterilizing Product Filter 0.2 µm	sterile, pyrogen free	Costar Corp. (Cambridge, MA), Millipore Corporation, or VWR International
Empty Sterile Vials, 10 mL, 20 mL or 30 mL borosilicate glass with gray butyl septa	sterile, pyrogen free	Hospira (Lake Forest, IL), SLK Abello, or Miller Analytical
Liquid Nitrogen or liquid argon or dry ice solvent bath	check label	Any local supplier
Argon Gas	> 99.9% pre-purified Certificate of Analysis	Byrne gas (Seattle, WA), AirGas (Radner, PA) Air Liquide (Houston, TX) Matheson Gas, Airco (Murray Hill, NJ), General Welding Supply (Seattle, WA)
Helium Gas	>99% Certificate of Analysis	
Nitrogen Gas, HP from a cylinder	Certificate of Analysis	

* These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

TABLE 7.8. Solutions Used in the Radiosynthesis of [^{18}F]FMISO.

SOLUTIONS	SPECIFICATION	QUANTITY
70% Ethanol by Volume	Compounded from USP reagents	≥ 200 mL
Sterile Water for Injection : Ethanol, absolute (95:5 v:v)	Compounded from USP reagents	≥ 500 mL
0.1 M K_2CO_3 Solution	High purity potassium carbonate in USP Water for Injection	0.5 mL
Kryptofix® [2.2.2] Solution (15 mg in 2 mL acetonitrile)	Anhydrous CH_3CN, reagent grade Kryptofix [2.2.2]	2.0 mL
0.5 M Hydrochloric acid	Compounded from USP/NF reagents or supplied as Eu Ph grade solution	2.0 mL

TABLE 7.9. Disposable Supplies Used in the Radiosynthesis of [^{18}F]FMISO.

SUPPLY	DESCRIPTION	SUPPLIER*
Sterile Syringe, 1 mL	No silicone or latex (for vial 1-6 additions and ^{18}F separation cartridge wash)	Sigma Aldrich or GE Medical Systems
Disposable Needles 25G x 3½	To fill Reagent Vials 1-6.	Becton Dickinson & Co.
Sterile Disposable Syringe, 10 mL	No silicone or latex (for cleaning with acetone)	Sigma Aldrich or GE Medical Systems
Syringes, various sizes, disposable plastic	Sterile, nonpyrogenic	Beckton Dickinson & Co. (Franklin Lakes, NJ)
Disposable Needles 22G or smaller	Sterile, nonpyrogenic	Beckton Dickinson & Co (Franklin Lakes, NJ)
Alcohol Swabs	Sterile Individually Wrapped	Various including Cardinal Health
Tubing	Teflon® and PEEK and polyethylene	Alltech Associates (Deerfield, IL), Upchurch Scientific (Oak Harbor, WA)
Tube fittings and connectors:	Miscellaneous made of Delrin, Kel-F, PEEK, polypropylene, nylon or stainless steel. Brass for gas lines only	Alltech Associates (Deerfield, IL), Upchurch Scientific (Oak Harbor, WA) or equivalent Swagelok

* These are current suppliers. If the equivalent grade of supply can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

7.3. Reference Standards

The following reference standards are used in the quality control methods of [^{18}F]fluoromisonidazole ([^{18}F]FMISO) injection:

TABLE 7.10.

REAGENT/SUPPLY	CHEMICAL SPECIFICATION	CURRENT SUPPLIER*
[^{19}F]fluoromisonidazole ([^{19}F]FMISO)	as supplied	ABX
Kryptofix® [2.2.2], (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane)	99% purity or greater	Sigma-Aldrich, EMD or Rotem Industries
Acetone, 99.5+%, ACS, spectrophotometric grade	99.5 +% ACS grade	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Acetonitrile (CH_3CN), anhydrous 99.8%	99.8%, anhydrous	Sigma-Aldrich or Mallinckrodt Baker, Inc.

* These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

7.4. Manufacturing & Testing Facilities:

The Sponsor will maintain responsibility for oversight of quality assurance and will work closely with the principal chemistry investigator to ensure correct implementation of the written SOPs, procedures, Master Production Record, Master Batch Record, Master Compounding Records, Specification Sheets, Forms, and all other written documentation. Quality Systems Administration and Software Administration and Change Control for the nucleophilic synthesis box, standard operating procedures are in place to ensure that software changes are qualified prior to implementation.

The [^{18}F]FMISO will be manufactured and tested at the following site

Name: **Insert name and address of facility with responsible person**

7.5. Manufacture of the Drug Substance

7.5.1. General Description of the Method for Preparation of [^{18}F]FMISO

The general chemical scheme for the radiochemical synthesis of [^{18}F]FMISO is illustrated in Figure 7.2. The general procedure for the synthesis of [^{18}F]FMISO is shown as a flow chart in Figure 7.4. Briefly, ^{18}F is made by irradiating enriched [^{18}O]-water with protons. The ^{18}F is separated from the [^{18}O]-water by trapping the ^{18}F on an anion exchange cartridge. Then aqueous potassium carbonate is used to elute the [^{18}F]-fluoride into the reaction vessel. Acetonitrile and the phase transfer reagent, Kryptofix® [2.2.2], are added to the reaction and the reaction is heated through two temperature steps, 95°C and 120°C, to azeotropically remove the water and the acetonitrile. Next the organic precursor for the reaction, 2´-nitro-1´-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulfonylpropanediol, in acetonitrile is added to the reaction vessel. The reaction is heated at 95°C for 9.0 minutes in a closed reaction vessel to promote the substitution of the ^{18}F for the tosyl group, then ~ 1 minutes\ at 120°C under vacuum to evaporate the acetonitrile. Next 2.0 mL of 0.5 M HCl is added and the reaction is heated at 120°C for 3 minutes to remove the THP protecting group. The reaction is cooled to 50°C and aqueous 4.2% sodium bicarbonate, 2.5 mL, is added to neutralize the reaction mixture prior to loading it on the preparative HPLC column for purification.

The resulting [^{18}F]FMISO is separated from the other compounds in the reaction mixture using preparative high performance liquid chromatography (HPLC). A sterile mobile phase consisting of 95% water for injection and 5% ethanol is used to elute the purified [^{18}F]FMISO.

The typical decay corrected chemical yield is 36 ± 9 % (n=9). The drug substance is not isolated. Instead the drug substance is collected directly through a 0.2 µm sterilizing filter into a vented sterile vial containing 0.40 mL of concentrated NaCl (23.4% USP) to make the final drug product isotonic. Samples are aseptically removed for analysis of product quality (see section 7.6).

The radiosynthesis of [^{18}F]FMISO will be conducted at the Sponsor's Sites using an automated synthesis procedure that is described in greater detail in Appendix 1 in [^{18}F]FMISO: Master Production Record **MPR-[^{18}F]FMISO**. The control information for each batch is captured in a copy of the Master Batch Record that is assigned a unique batch number for every production run. The automation unit that will be used for the manufacturing of [^{18}F]FMISO is a **(Insert your system name here)** nucleophilic synthesis box.

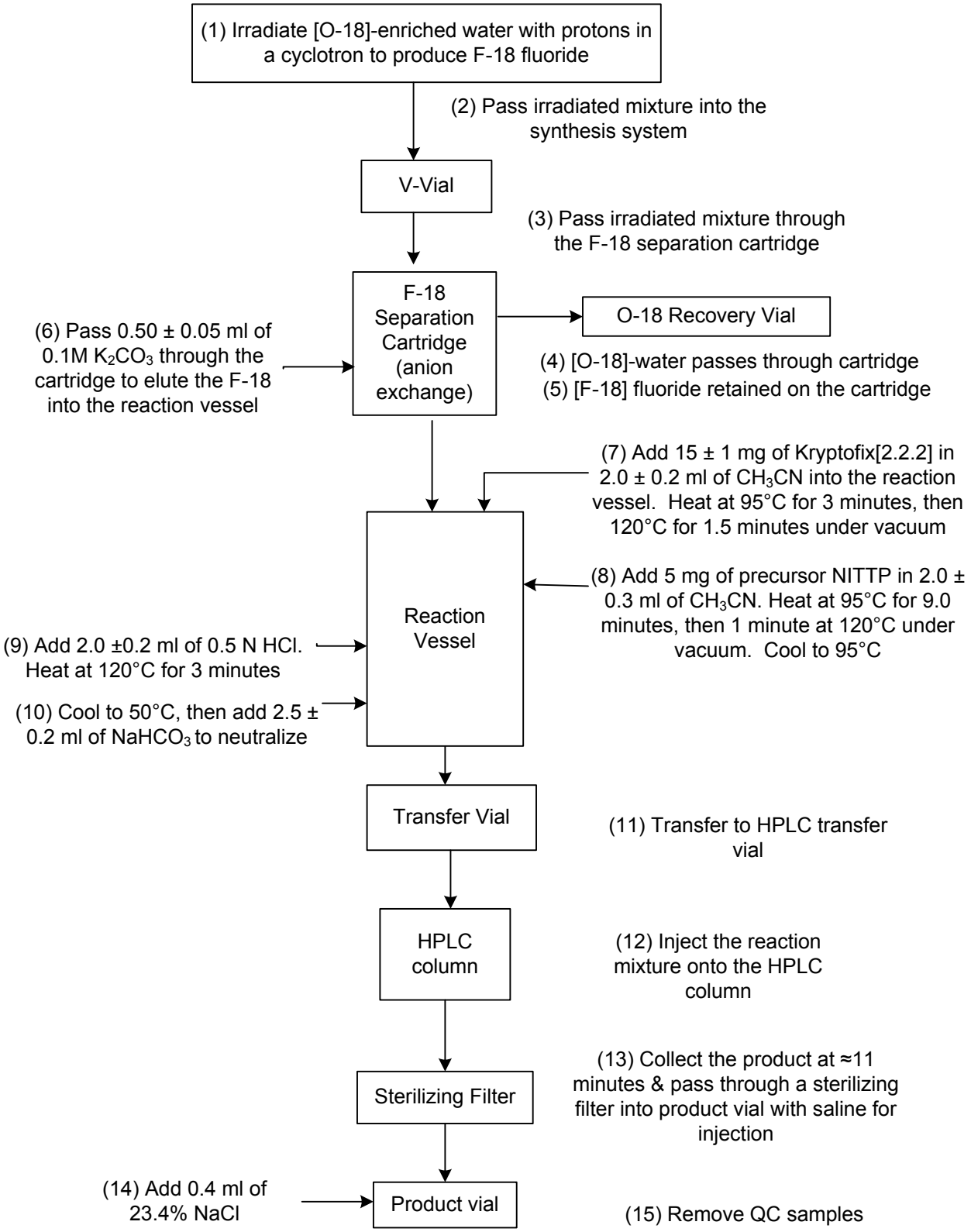

Figure 7.4. Schematic Flow Chart of the Steps for Radiosynthesis of [^{18}F]FMISO.

7.5.2. Cyclotron Production of [^{18}F] Fluoride

[^{18}F]fluoride is produced by irradiating [^{18}O] enriched water with protons in a silver, titanium, niobium or tantalum target. The target entrance window is made of Havar, Arnavar, titanium, or tantalum. One to two targets are irradiated for 10-120 min with 10-80 µA of proton beam for one batch synthesis of [^{18}F]FMISO. The measured yield of [^{18}F]fluoride is approximately 18 mCi/µA for a 30 minute irradiation. Yield is approximately linear with the beam current but can vary due to different cyclotron focusing.

The information below describes the typical targets and irradiation conditions for production of ^{18}F. None of these parameters are critical variables for the quality of the final [^{18}F]FMISO drug product. **Insert your here parameters if different**

Particle Accelerator (Cyclotron) Used:

Make: **Insert here**

Model: **Insert here**

Operating Parameters (verify with your system).

- During irradiation a beam current of 10 µA to 80 µA is used on one to two targets.
- Irradiation times of 10 minutes to 120 minutes are used. High-pressure targets are used, irradiations are performed under approximately 400-500 psi of pressure.

Specifications for Target Body (verify with your system).

- Volume of the target(s): between 1 and 5 mL.
- The target material: silver, titanium, niobium or tantalum.
- The target windows: Havar, Arnavar, titanium, or tantalum.

7.5.3. [^{18}F] Fluoride.

The [^{18}F] fluoride is transferred from the target to the nucleophilic synthesis box using inert gas pressure. After the transfer to the synthesis box is complete, the fluoride is passed through an anion exchange cartridge to retain the [^{18}F] fluoride and to separate it from the [^{18}O] water. The [^{18}F] fluoride is eluted from the anion exchange cartridge using 0.5 ± 0.05 mL of 0.1 M potassium carbonate (K_2CO_3) and transferred to a glass reaction vessel that is supplied with the synthesis box. A solution of anhydrous acetonitrile (CH_3CN) 2.0 ± 0.2 mL containing 15 ± 2 mg of Kryptofix® [2.2.2], a phase transfer reagent, is then added to the reaction. This fluoride / Kryptofix solution is dried at 95-120°C for ~10 minutes to remove the water.

7.5.4. Radiosynthesis of [^{18}F]FMISO

Anhydrous acetonitrile containing 5 ± 1 mg of 1-(2'-nitro-1'-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulfonyl-propanediol (2.0 ± 0.2 mL) is added to the reaction vessel. The [^{18}F]-fluoride is transferred into the organic phase, presumably carried as an anionic counterion to the potassium ions in the Kryptofix® [2.2.2]. The fluoride displaces the toluenesulfonyl group and forms a bond at the 3-propyl position. The fluoride addition reaction is reacted at 95°C under pressure for 9.0 minutes and then under vacuum for ~ 1 minute at 120°C to evaporate the acetonitrile.

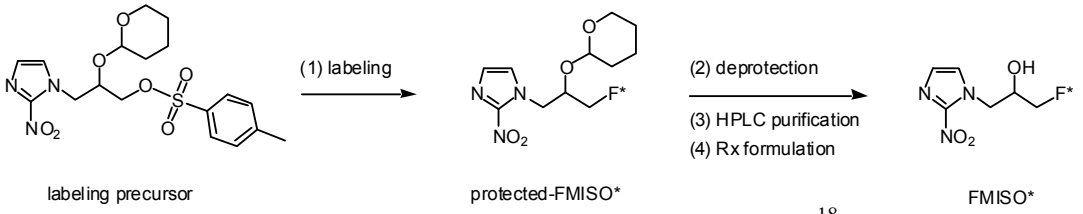

Figure 7.2(repeat): Schematic of Radiosynthetic Method for [^{18}F]fluoromisonidazole

Once the fluorination reaction is complete, the THP protecting group is removed by acid hydrolysis. Specifically, 2.0 ± 0.2 mL of 0.5 M hydrochloric acid is added to the reaction and it is heated at 120°C for 3 minutes. The reaction is then cooled to 50°C and neutralized by adding 2.5 ± 0.2 mL of 4.2% $NaHCO_3$ solution and finally the reaction mixture is passed from the reaction vessel to a HPLC transfer vessel using gas pressure.

7.5.5. Purification of [^{18}F]FMISO

The resulting [^{18}F]FMISO is separated from the other compounds in the reaction mixture using preparative high performance liquid chromatography (HPLC). A sterile mobile phase consisting of 95% water for injection, USP and 5% ethanol, absolute USP is used to elute the purified [^{18}F]FMISO from a C18 HPLC column (Econosphere C18, 10 µm, 250 x 10 mm from Alltech Associates, Inc.). The mobile phase is pumped at a flow rate of 5 mL/min at room temperature. The product, [^{18}F]FMISO, is typically eluted at approximately 9.2 min (46 mL, k´= 2.5) with a peak full width half maximum (FWHM) of 1.8 mL. A typical preparative chromatogram is shown below in Figure 7.5. The [^{18}F]FMISO is baseline separated from the other radioactive products and from the other UV absorbing (254 nm) compounds except for the tailing edge of 1-(2,3-dihydroxy)propyl-2-nitroimidazole misonidazole peak that can be in the product and the leading edge of another impurity from some batches of the precursor that is eluted just after the FMISO. The concentration of both of these impurities and any other aromatic UV absorbing compounds in the final product must be less than or equal to 35 µg, assuming the same UV absorbance as FMISO, before the product is released (see section 7.9.3).

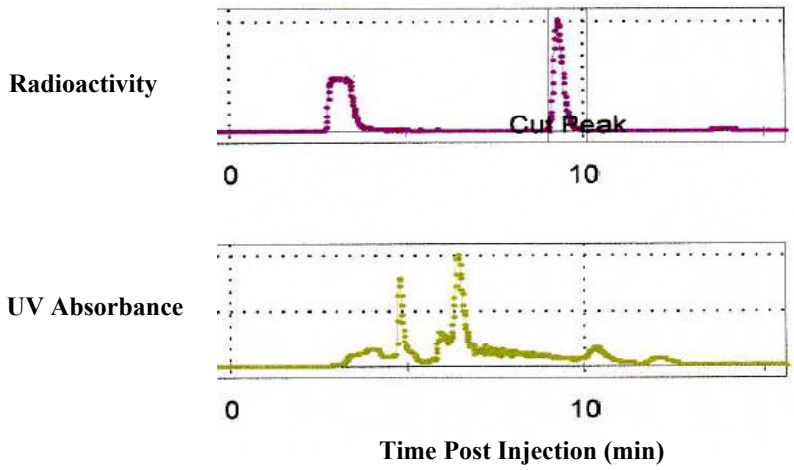

Figure 7.5 Chromatogram of preparative HPLC separation of [^{18}F]FMISO reaction mixture. Top trace is radioactivity and bottom trace is UV absorbance. The two vertical solid lines in the top trace show the start (leftmost) and end (right) of the product collection.

The [^{18}F]FMISO solution is collected through a 0.22 µm sterilizing filter into a vented sterile vial containing 0.40 mL of concentrated NaCl (23.4% USP) to make the final drug product isotonic. Samples are removed for analysis of product quality (see section 7.6).

At the end of synthesis, a HPLC cleaning program is run with a mobile phase of 70% ethanol, USP, so that the preparative HPLC column and injector are disinfected. The column and injector are stored in the 70% ethanol, USP. The product output luer fitting is sealed with a 0.2 µm sterilizing filter between uses. The HPLC column is prepared for use by passing >300 mL of the preparative mobile phase through the column and injector less than 3 hours prior to injection of the ^{18}F product reaction mixture onto the column. The vessels and tubing for the nucleophilic synthesis box are rinsed with acetone using a clean-up program after the synthesis. All of the clean-up procedures are also detailed in Appendix 1 in the [^{18}F]FMISO: Master Production Record **MPR-[^{18}F]FMISO**.

7.6. Post Synthesis Processing and Quality Assurance of the [^{18}F]FMISO

7.6.1. Sampling for Quality Assurance and Storage

The drug product is assayed for total radioactivity and is examined for particulates. The integrity of the sterilizing filter is tested. Two samples totaling nominally 1.5 mL are removed for measurement of pH, residual levels of Kryptofix®[2.2.2], analytical HPLC measurements of specific activity, radiochemical and chemical purity, GC measures of residual solvents, radionuclidic purity by half-life determination, apyrogenicity, and sterility of the product. Approximately 1.0 ml of the sample is retained for further testing, if necessary. The product dose is drawn, labeled, and once all but the sterility tests have been passed, the product dose is released for injection. If the PTS chromogenic

endotoxin test is used then the endotoxin test must be completed before release of the product. If the Limulus ameobocyte lysate gel clotting method is used to determine endotoxin level, then a 20 minute endotoxin test may be used to release the dose but a 60 minute endotoxin test must be completed. The sample for sterility testing is inoculated within 48 hours but after the sample has decayed to a background radiation level.

7.6.2. Reprocessing of PET Drug Product

The final [^{18}F]FMISO drug product will be reprocessed for only one reason. The final drug product may be resterilized in the event of an integrity test failure.

7.7. Container/Closure

The drug substance is manufactured in a semi-closed automated system, **(Insert your system name here)** nucleophilic substitution box using in-process controls such as high purity solvents to synthesize the radiopharmaceutical. Automated software controls the temperature and timing parameters as well as recording the information from the production run. Sterile water for injection and absolute ethanol, USP are used to compound the solutions for the preparative HPLC purification. The drug substance is then sterile filtered directly into the final drug product vial, the sterile USP Type I, Glass, Gray Butyl Rubber Stopper, Vial. An integrity bubble point test is performed on the sterilizing filter, in addition to performing an endotoxin and a sterility test on the final drug product.

7.8. Controls For Finished Drug Product

The quality control oversight is at the manufacturing site. Several quality control tests are run on the [^{18}F]FMISO product prior to release for human administration, to assure the quality of the final product. The quality control tests that are run are summarized in Table 7.11 and are consistent with the guidelines in USP<823> Radiopharmaceuticals for Positron Emission Tomography-Compounding.

TABLE 7.11: Quality Control Tests for the [^{18}F]FMISO Product

QUALITY CONTROL TEST	DESCRIPTION	REQUIREMENTS FOR PASS	REQUIRES TEST PASS PRIOR TO PRODUCT RELEASE
Chemical Purity	Visual inspection for color and particulates	**Clear and Colorless**	yes
Filter Integrity	Test bubble point	**Meet pressure specified by manufacturer**	yes
pH	pH as per USP<791> pH	**pH must be between 6 and 8**	yes
Residual Kryptofix® [2.2.2]	Color spot test	**< 50 µg/mL Kryptofix[2.2.2] by comparison with standard**	yes
Chemical and Radiochemical Purity **(specific activity is calculated from dose assay and chemical purity)**	HPLC consistent with guidelines of USP<621> Chromatography subsection High-Pressure Liquid Chromatography	**Radiochemical Purity > 95%** **Chemical purity:** **FMISO ≤ 15 µg** **other UV (254, 280 or 327 nm) peaks ≤ 35 µg in final product** **Specific Impurity at ~4 min ≤3µg/ml** **Specific impurity at ~6 min ≤4µg/ml**	yes
Radiochemical Purity	TLC	**Rf > 0.5 and** **Purity ≥ 95%**	yes
Residual Solvent Levels	Gas Chromatography	**Acetone < 5,000 ppm** **Acetonitrile < 400 ppm**	yes
Radionuclidic Purity	Half-life Determination	**100 – 120 minutes**	yes
Bacterial Endotoxin Levels	Limulus Amoebocyte Lysate (LAL) by gel clot or PTS	**< 175 EU per dose**	yes (20 min gel clot test acceptable for release)
Sterility	USP sterility test (USP <71>)	**No growth observed in 14 days**	no

All of the quality control tests will be completed prior to product release except for the USP sterility test. In the event of a positive sterility test result, action will be taken within 24 hours. The treating physician will be notified with a summary from the principal physician investigator at the investigational site and a report will be sent to the IND sponsor.

If all tests, with the exception of the pending sterility test, are within acceptable limits, the product will be released for administration to the subject. If one or more of the quality assurance tests do not meet the required specifications listed above, then one of two actions will be taken. For all tests with the exception of the filter integrity and half-life tests, if the test does not meet the specifications listed above, the product will be failed and will not be released. As a result of this failure an Out of Specification Investigation will be conducted to determine the cause of the aberrant test result. If the 0.22 µm filter used to filter the final product fails the integrity test both initially and upon

rewetting of the filter then the final product will be resterilized using a new sterile filter. If the half-life determination does not meet specifications, it may be due to insufficient time points. In this case, the dose is not to be released and the half-life determination may be repeated or more time points may be collected for the determination to improve the counting statistics. If the half-life determination passes after the second set of counts, then the dose may be released and an Out of Specification Investigation will be conducted as well. If the half-life determination does not pass after the second set of counts, the product will be failed and will not be released.

7.9. Analytical Test Procedures

7.9.1. Chemicals for Quality Control Analyses (QC)

TABLE 7.12. Reagents Used for Quality Control Analyses (QC).

REAGENT	CHEMICAL GRADE	CURRENT SUPPLIER*
Potassium Iodide	Granular, USP	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
Iodine	USP	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Methanol	HPLC grade	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Acetonitrile	99.8%, anhydrous	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Acetone	spectrophotometric grade, 99.5%	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Formic acid	>88%	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Water	HPLC Grade	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
Chloroplatinic Acid	ACS Reagent grade	Sigma-Aldrich
Limulus Amoebocyte Lysate (LAL) single test vials	as supplied	Associates of Cape Cod, Charles River or Cambrex
Limulus Amoebocyte Lysate (LAL) portable test system cartridges (PTS)	as supplied	Charles River
Control Standard Endotoxin (CSE)	as supplied	Associates of Cape Cod, Charles River or Cambrex
Water for injection (WFI)	USP	Any USP supplier
LAL Reagent Water	as supplied according to USP/NF	Associates of Cape Cod, Charles River or Cambrex
Ammonium Hydroxide Solution	28% in water ≥ 99.99%	Sigma-Aldrich and Mallinckrodt Baker
Hydrochloric Acid	36.5-38.0%, NF	Sigma-Aldrich or Mallinckrodt Baker, Inc.

* These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

TABLE 7.13. Solutions Used for Quality Control Analyses (QC).

Solutions	grade of chemicals
HPLC Aquasil Column Mobile Phase, 5% Methanol, 95% 1 mM formate in HPLC Water (v:v)	HPLC grade water, methanol and formate as listed above
Preparation of GC Standard Solution	The preparative mobile phase is the diluent for the acetonitrile and the acetone
Preparation of FMISO Standard Solution for HPLC	The preparative mobile phase is the solvent

7.9.2. Materials for Quality Control Analyses (QC)

TABLE 7.14: Supplies Used for Quality Control Analyses (QC)

SUPPLY	WHAT USED FOR	CURRENT SUPPLIER*
pH strips, Panpeha® pH Indicator Strips (0-14)	assay of pH	Sigma-Aldrich
TLC plates, Silica Gel 60 F_{254} Aluminum Backed	TLC analysis	Alltech Associates and VWR Scientific
TLC plates, Silica Gel 60 F_{254} Plastic Backed	Kryptofix [2.2.2] assay	Alltech Associates and VWR Scientific
Column analytical Aquasil C18 2.1 X 150 mm (5µm)	HPLC analysis for chemical and radiochemical purity	Thermo-hypersil Keystone
Helium (UHP)	GC analysis for solvents	Byrne gas (Seattle, WA), AirGas (Radner, PA) Air Liquide (Houston, TX) Matheson Gas, Airco (Murray Hill, NJ), General Welding Supply (Seattle, WA)
Hydrogen (UHP if use FID)		
ATWAX column 30m long 0.25 mm ID 0.25µm	GC analysis for solvents	Alltech Associates

* These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

7.9.3. Validated Standard Test Procedures

All of the analytical test procedures are performed using high-quality solvents and reagents, which have been carefully logged in, controlled, and verified in the same manner as the reagents for the manufacturing process. Testing forms have been developed to ensure consistency for documentation of the collected test information. **(Validate these assays in your lab).**

Particulates. The [18F]FMISO product solution is examined visually. The chemical purity by visual inspection is straightforward, the final drug product in the vial should be clear and colorless without any visible particulates as per USP <823> and USP <631> Color and achromicity. The product must pass this test in order to be released for injection.

Filter Integrity. Because the USP sterility test requires 14 days to complete, the [^{18}F]FMISO product solution sterility cannot be assured prior to injection. In addition to working in an ISO class 5 environment when preparing the solutions used in manufacture of the [^{18}F]FMISO product and the final product vial, the [^{18}F]FMISO] is passed through

a 0.2 µm sterilizing filter into the final product vial. After the product [^{18}F]FMISO is collected, the sterilizing filter is tested for filter integrity to give an indication of likelihood of product sterility. Filter integrity is tested in a bubble point procedure, whereby the sterilizing filter is placed on a gas line with a pressure gauge and the outlet of the filter is placed under water. The gas pressure on the inlet to the filter is increased slowly until a steady stream of bubbles is observed at the filter outlet. The pressure when the bubble stream begins is recorded and compared with the manufacturer's pressure rating for the filter, from the certificate of quality. If the observed bubble point pressure exceeds the manufacturer's specifications, the filter integrity test is passed. If the bubble point pressure is too low, the filter may be rewetted and tested again. If the bubble point pressure is greater than the manufacturer's specifications, the test is passed, if not then the product must be resterilized by passing through a second sterilizing filter. That filter must then pass the bubble point test. The product [^{18}F]FMISO may only be resterilized once. This test must be passed for the [^{18}F]FMISO dose to be released.

pH. Because the product volume is small and because the product is radioactive, pH is measured using an appropriate variation of USP <791> pH. Instead of a pH meter, pH test strips are used. The pH test strips are checked by pipeting pH 5 and pH 7 calibrated commercial pH standards onto individual strips. The color on the strips must match the pH 5 and pH 7 on the color key supplied with the test strips. Then the [^{18}F]FMISO is pipetted onto another test strip and the color checked against the color key. The result is written down and the measured pH must be between pH 6 and pH 8 for the product [^{18}F]FMISO to be released.

Kryptofix® [2.2.2]. The test, is based on the method by Mock *et al.* (1997) using a color spot test for the detection of residual Kryptofix® [2.2.2] in the final drug product for this IND. The FDA has proposed a maximum permissible level of 50 µg/mL of Kryptofix® [2.2.2] in 2-[^{18}F]FDG, therefore this maximum permissible level is appropriate for the [^{18}F]FMISO final product. For this IND, there are two SOPs to test for residual Kryptofix® [2.2.2] that can be used for testing the final drug product. The USP test is the less preferred method. The preferred method is based on the method by Mock using a color spot test for the detection of Kryptofix® [2.2.2], which is more sensitive but slightly less selective than the standard method listed in the USP. For this test, a strip of plastic backed silica TLC plate is soaked in an acidic iodoplatinate solution, dried overnight or longer before use and stored in a closed chamber. The iodoplatinate solution is that described by Mock et al. (1997). The Kryptofix® [2.2.2]] test is done by pipeting 1 to 2 µL each of saline, 50 µg/mL Kryptofix® [2.2.2] standard in preparative mobile phase and [^{18}F]FMISO product in three spots on the plate. The Kryptofix standard should show a spot with a blue ring around the edge. The saline should have no blue ring. The [^{18}F]FMISO must have no blue ring or a ring that is clearly paler than the blue ring of the standard to pass the test for Kryptofix® [2.2.2]. The [^{18}F]FMISO product dose cannot be released unless this test passes. The silica test strip is color photocopied and the copy is maintained in the batch record to verify that the test passed.

Chemical and Radiochemical Purity. HPLC chromatography analysis will be used to determine purity for this drug product. The final radiochemical purity is greater than or equal to 95%, and the nonradioactive FMISO must be less than or equal to 15 µg in the final product dose. The amount of nonradioactive FMISO is measured by UV absorbance at 327, 280 or 254 nm of the FMISO eluted from a C18 Aquasil column with a mobile

phase of 5% methanol: 95% 1 mM formate in water. The column flow rate is 0.3 mL / min and is kept at approximately room temperature, 25 to 30°C. The typical retention of FMISO is 5.9 min for the UV absorbance (the radioactivity and mass detectors are 0.05 and 0.1 min further downstream from the UV detector. The UV absorbance at 4.0 min is from the impurity 1-(2,3-dihydroxy)propyl-2-nitroimidazole and an unknown impurity is at 6.0 min for UV absorbance. The mass of FMISO (in µg/mL) is quantified by measuring the peak area response of a minimum of five FMISO standards to bracket the sample or bracket the minimum acceptable mass limit. All standards must be baseline resolved (resolution >1.5) for a valid analysis. A linear regression is determined for UV absorbance peak areas of the standards. This constitutes the calibration curve. Then the peak area of the FMISO drug product is fit on the calibration curve to determine the FMISO concentration in the drug product sample. Typically the final concentration of the [^{19}F]FMISO in the [^{18}F]FMISO product to be tested will be equal to or below 1 µg/mL. The total allowable, column retained (post 3.0 minutes) UV absorbing contaminants (peaks on the chromatogram) in the [^{18}F]FMISO product are ≤ 35 µg, which assumes that impurities have the same molar absorption coefficient for quantifying the impurity peaks. The impurity at eluting at approximately 4.0 minutes, 1-(2,3-dihydroxy)propyl-2-nitroimidazole , must be ≤3 µg/mL and the impurity eluting at approximately 6.0 minutes must be ≤4 µg/mL. Usually the FMISO peak area is so small that it is only a shoulder on the 6 min impurity peak and cannot be quantitated accurately (See Table 7.17).

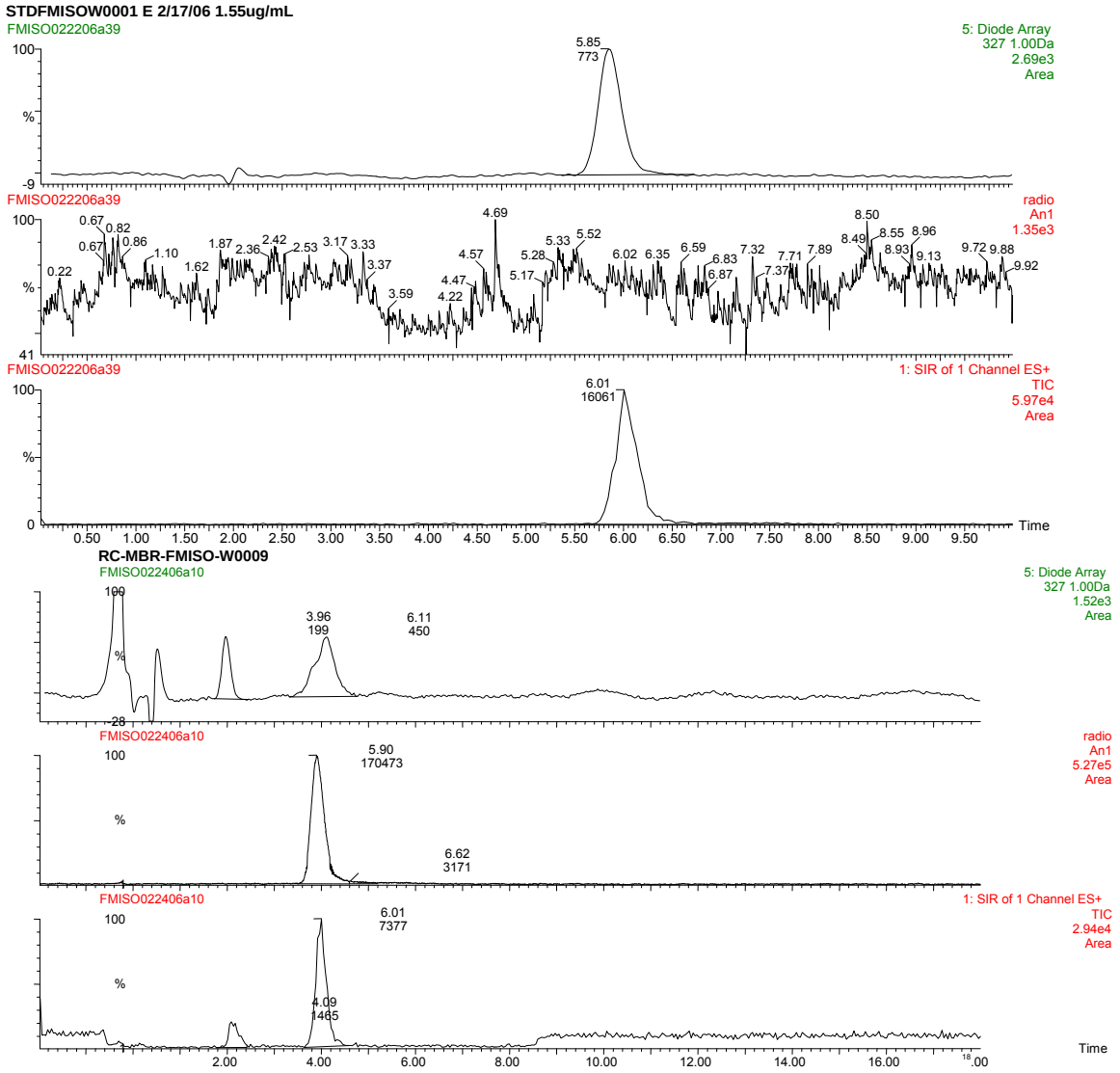

Figure 7.6. Typical analytical HPLC chromatograms for FMISO standard (top chromatograms) and [^{18}F]FMISO product (bottom). The chromatograms show the UV absorbance at 327 nm in the top trace, radioactivity (none) in the middle trace and the electrospray + H peaks from a mass spectrometer on the bottom trace. The FMISO is the peak eluted at 5.9 to 6.0 min. **(verify with your system).**

The [^{18}F]FMISO organic precursor does not elute from the HPLC column with the analytical mobile phase, but elutes after 9 minutes from the FMISO analytical column when the mobile phase is 50% methanol : 50% 1 mM formate (v:v) (see Figure 7.3). No precursor is expected in the final product. The acid hydrolysis should remove any protecting groups and leave the major synthetic byproduct, 1-(2,3-dihydroxypropyl)-2-nitroimidazole. Any residual precursor, or toluenesulfonate and tetrahydropyranyl (THP) protecting groups that were left would not elute with the FMISO product when using the 5% ethanol preparative mobile phase. After synthesis the preparative HPLC column is washed with 70% ethanol to remove any retained organic material. Other chemical impurities must be $\leq$ 35 µg total for any retention time between 3 and 15 minutes,

assuming the same UV molar absorption coefficient as FMISO. The impurity at eluting at approximately 4.0 minutes, 1-(2,3-dihydroxy)propyl-2-nitroimidazole, must be ≤3 µg/mL and the impurity eluting at approximately 6.0 minutes must be ≤4 µg/mL. The maximum amount of toluenesulfonate or THP (the leaving groups) possible in the final reaction is less than or equal to 35 µg, a nontoxic amount.

Other impurities that can be expected to absorb light in the UV region are Kryptofix [2.2.2], which elutes at ~ 2 minutes, but is assayed using a chemical spot test, and impurities in the USP solutions.

Radiochemical Purity And Identity. The use of Radio-Thin Layer Chromatography to determine the radiochemical purity and identity were validated using 99% acetonitrile: 1% water (v:v) as the mobile-phase. The R_f values obtained from these studies were between 0.76 and 0.84 for the qualification runs **(insert your values here)**. The limit has been set for R_f >0.5 for the final [^{18}F]FMISO because the TLC test is included only to separate unbound fluoride (origin) from the product. The specification for the purity is greater than or equal to 95% using this methodology. This is primarily a test for free fluoride. If present, unlabeled ^{18}F will remain at the origin with an R_f value equal to 0 and so is an adjunct test to the analytical HPLC. The identity is confirmed by running the nonradioactive FMISO standard side by side with the drug product to confirm the R_f values are consistent.

Residual Solvent Levels. Acetonitrile is the solvent for the FMISO precursor and is used to dissolve the Kryptofix® [2.2.2]. Acetone is used to clean the nucleophilic synthesis system. The permissible level of acetonitrile in the final product is less than or equal to 400 ppm. This value is the same as that for the USP permissible level of acetonitrile in 2-[^{18}F]FDG which may not exceed 400 ppm and is consistent with USP <467> Organic Volatile Impurities, which limits acetonitrile to 410 ppm. The allowable levels for acetone is less than 5,000 ppm. Acetone is a Class three solvent; this class of solvents includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. Therefore this limit is based upon the FDA's Guidance for Industry ICH Q3C-Tables and List (November 2003 Revision 1), page 7, where it considers 5,000 ppm in 10 mL i.e., 50 mg or less per day, of these Class 3 residual solvents as an acceptable limit, without additional justification. This 5,000 ppm limit is also recommended by USP <467> for acetone. All of the residual solvent levels met these acceptance criteria in the qualification syntheses.

Residual Solvent Levels are determined using the methods listed in Table 7.15, below, using a method similar to that reported by Channing et al. (2001). The detector signal to noise must be greater than 10:1 for the maximum allowable concentration for each solvent when injecting 0.5 µL of the analyte. The methods to determine acetone and acetonitrile levels are consistent with or more stringent than USP <467> Organic Volatile Impurities. Five prepared concentrations of the external standard are analyzed using the GC method to obtain values for calculating the standard curve.

The retention times are approximately 2.3 minutes for acetone (k' ~0.5), 3.4 minutes for ethanol (k' ~1.2), 4.5 minutes for acetonitrile (k' ~1.9), 4.7 minutes for water (k' ~ 2.0). All of the peaks for these compounds are baseline resolved.

Table 7.15. Gas Chromatographic method for analysis of residual solvents in [^{18}F]FMISO.

General conditions	GC with FID or MS	ATWAX column	30 m long, 0.25mm ID, 0.25 µm film thickness
Carrier gas	Helium ultra high purity (UHP)	flow 1 mL/min (linear velocity ~ 34 cm/sec)	Split ratio of 100:1 (shunted:on column injection)
Injection Conditions	0.5 to 1 µL injected	Injector at 225°C	
Oven temperature	Initial Temperature 40°C, hold for 1 minute.	Temperature ramp 1: 40° to 76°C @ 40°C/min, hold at 76°C for 1.0 min.	Temperature ramp 2: 76° to 150°C @ 20°C/min, hold at 150°C for 1.5 min.
Detection	**FID**: use UHP H_2	**MS**: electron impact @ 70eV with a scan range of 15 – 100 M/Z	**MS (cont.):** ion source temperature: 200°C transfer line temperature: 275°C

Radionuclidic Purity. The method of half-life determination used is a variation (see below) of the test listed in the USP. This test is used to determine the identity of the radioactive nuclide and should not vary with the compound being tested. For the test, an aliquot of the product [^{18}F]FMISO is counted in an ion chamber or gamma counter at least five times. The half-life of the radioactivity is determined for each activity measurement using the following equation.

$$T_{½} = \frac{[\ln(2)*(t_x-t_0)]}{(\ln A_0-\ln At_x)}$$

Where $\mathbf{A_t}$ is the activity (background subtracted) measured at each time-point (other than time zero).
$\mathbf{A_0}$ is the activity (background subtracted) measured at time-zero.
t is the time in units of minutes
$\mathbf{t_{½}}$ is the calculated half-life in units of minute

The half-life test result for ^{18}F must be between 100 and 120 minutes for the dose to pass.

The USP radioactivity general chapter <821> states that the half-life can be "readily determined by successive counting of a given source of a radionuclide over a period of time that is long compared to its half-life". The variation is to count for only a fraction of the half-life of 110 minutes. To count a sample for over 2 hours would reduce the radiopharmaceutical dose by over half and would only provide somewhat better counting statistics. The need for half-life measurement is questionable since irradiation of water in a < 15 MeV proton cyclotron can only make N-13 and F-18 and trace contaminants. N-13 will not be converted to FMISO.

Bacterial Endotoxin Levels are tested and qualified using one of two procedural methods; both are based on the USP recommendations, but using control standard endotoxin referenced to USP RSE. Either a gel clot method is used with single test vials or the portable test system from Charles River Laboratories (Appendix 2). Bacterial endotoxin levels must be <175 EU per batch.

Sterility. Sterility is tested using the direct inoculation method as is required by the USP. A report of the validation tests for the [^{18}F]FMISO product and the USP sterility method is presented in Appendix 3.

7.9.4. Results of [^{18}F]FMISO qualification runs

All of the completed qualification [^{18}F]FMISO radiosyntheses passed all the acceptance criteria. The results are summarized in Tables 7.16, 7.17 and 7.18 below.

Table 7.16. Summary of the measured particulates, filter integrity, pH, and residual Kryptofix [2.2.2] for [^{18}F]FMISO from the qualification runs.
Insert your data here

Batch Number MBR-FMISO-	Particulate Test	Filter Integrity (psi)*	pH	Kryptofix [2.2.2] µg/mL

*Manufacturer specified pressure rating was >40 psi.

Table 7.17. Summary of the measured [^{18}F]FMISO radiochemical and chemical purity from the qualification runs.
Insert your data here

Batch Number MBR-FMISO	TLC		HPLC				
	R_f	Radio-chemical Purity (%)	Radio-chemical Purity (%)	FMISO** µg/10 mCi dose*(µg/mL)	Individual impurities (µg/mL)		Other*** µg/mL (µg/dose)
					4 min	6min	

*time of dose calculation is 1 hour post end of synthesis.
**note that the UV FMISO peak was a shoulder on one impurity peak so was determined by mass spectrometry. The combined FMISO / impurity mass concentration from UV is given in parentheses as a maximum for the FMISO mass. The mass from all UV impurities is included in the other category.
*** The value in parentheses is the mass of impurities for a 10 mCi dose 1 hour post end of synthesis

Table 7.18. Summary of the residual solvents, half-life, endotoxin and USP sterility test results for [^{18}F]FMISO product from qualification runs.
Insert your data there

Batch Number MBR-FMISO	Residual Solvent Acetone (ppm)	Residual Solvent Acetonitrile (ppm)	Half-life (min)	Measured Endotoxin (EU/mL)	USP Sterility Test (14 day)

7.10. Information to Support the Stability of the Drug Substance

[^{18}F]FMISO was made according to the procedures described in this IND application. The final drug product was left at room temperature for up to 24 hours. At periodic times over the 24 hours, the product was measured for radiochemical purity using analytical HPLC and TLC. In addition, the FMISO product has been examined for changes in UV absorbance of the product peak with time. There was no detectable breakdown of the product over that time period. These results are summarized in the Table 7.19.

Expiration dating: **(This must be based on your data)** The expiration time is **xx** hours for the product stored at room temperature. This is comparable to the 12 hours now allowed for [^{18}F]FDG. The measurements shown in Table 7.19 suggest there is no detectable loss of radiolabel over **xx** hours at room temperature in ambient light.

Table 7.19. [^{18}F]FMISO stability test results.
Insert your data there

Batch MBR-FMISO-	Time post synthesis(hr)	% pure by HPLC	% pure by TLC

7.11. Drug Product Vial Labeling

Labels are attached to the patient dose and examples are shown below:

Dose Labels

CAUTION: New Drug – Limited by Federal (or United States) law to investigational use.

PET RADIOCHEMISTRY LABORATORY
^{18}F-Fluoromisonidazole in 95: 5 Water for injection: Ethanol
MBR-[^{18}F]FMISO-_____

Caution

Radioactive Material

Patient:_____
Physician: _____
Activity: _____ mCi in _____ mL
Calibration Date: _____ Time: _____
This radiopharmaceutical is for intravenous injection. Do not use if cloudy or if it contains particulate matter. Expires 12 hours after calibration. Calculate injection dose from date & time of calibration. $T_{½}$ is 109.8 minutes.

7.12. Environmental Assessment

A categorical exclusion is requested for the Environmental Impact Statement requirement in section 21 CFR part 25 because this radiopharmaceutical meets the conditions stipulated in Section 25.24(c)(4). Namely [^{18}F]FMISO involves a short-lived radionuclide ($T_{1/2}$ = 109.8 min) prepared in unit dose and resulting in a total mass of active drug that does not exceed 15 µg, and the ^{18}F decays to a stable nuclide. This procedure will be done no more then 100 times per year. Thus the amount of the waste that is expected to enter the environment may be reasonably expected to be nontoxic.

Bibliographic Citations:

Channing M.A., Huang B.X., Eckelman W.C.: Analysis of residual solvents in 2-[^{18}F] FDG by GC, Nuclear Medicine and Biology; 28:469-471, 2001

FDA Guidance for Industry ICH Q3C—Tables and List (November 2003 Revision1)

Grierson J.R., Link J.M., Mathis C.A., Rasey J.S. Krohn K.A., (1987), A radiosynthesis of [F-18]fluoromisonidazole. J. Nucl. Med. 30, 343-350.

Lim J-L., Berridge M.S., (1993), An efficient radiosynthesis of [^{18}F]fluoromisonidazole. Appl. Radiat. Isot. 44, 1085-1091.

Mock BH, Winkle W, Vavrek MT, Technical Note: A Color Spot Test for the Detection of Kryptofix® [2.2.2] in [^{18}F]FDG Preparations, Nuclear Medicine & Biology; 24:193-195, 1997

Appendices

1. Documents
    a. List of SOPs
    b. MBR
    c. MPR
2. Endotoxin test validation
3. Sterility validation