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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]fluoroestradiol; estra-1,2,5(10)-triene-3,17-diol, 16-[^{18}F]-fluoro-,(16alpha, 17beta); 16-alpha-[^{18}F]-fluoro-17-beta-estradiol; ([^{18}F]FES)

7.1.3. Chemical Structure

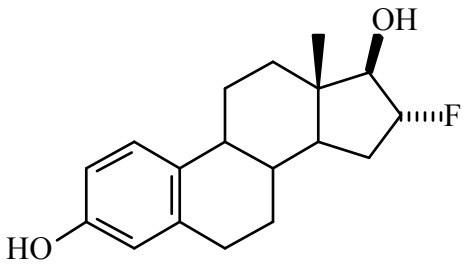

Figure 7.1. [^{18}F]fluoroestradiol

7.1.3. Specifications for the [^{18}F]FES final product

Table 7.1. Specifications for the [^{18}F]FES final product

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):	**FES < 5 µg per injected dose**
	other UV absorbing impurities beyond void volume (280 nm) ≤ 5 µg per injected dose
Radiochemical Purity(TLC):	**R_f > 0.5** **Purity ≥ 95%**
Radionuclidic Purity:	**Measured half-life 100-120 minutes**
Residual Solvent Levels:	**Acetone < 5,000 ppm** **Acetonitrile < 400 ppm**
Bacterial Endotoxin Levels:	**< 175 EU per dose**
Sterility:	**no growth observed in 14 days, also must pass filter integrity test prior to injection**

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

The final product has been characterized and is as follows:

Table 7.2. Components of the final product.

COMPONENTS		Amount in Injectate
[^{18}F]FES, 16 alpha-[^{18}F]-fluoro-17-beta-estradiol	**Same as for [^{19}F]FES**	**nominally 6 mCi (3.0 to 6.0 mCi allowed)**
[^{19}F]FES, 16 alpha-[^{19}F]-fluoro-17-beta-estradiol	**NSC# 743445**	**≤ 5 µg**
Ethanol, absolute	**USP**	**<15% by volume**
Saline for injection	**USP**	**0.075 M**
Sodium phosphate	**USP**	**0.075 M**
Water for Injection	**USP**	**remainder**

Table 7.3. Impurities in the final product.

IMPURITIES	Acceptance Criteria (See Section 7.10.3)	Highest Values (lowest values) in Qualification Runs (n=6)
Kryptofix® [2.2.2]	< 50 µg/mL	< 50 µg/mL*
Acetonitrile	< 400 ppm	<125 ppm*
Acetone	< 5000 ppm	<1,570 ppm*
4.8 min retention M/Z 395 (ES-)	< 5 µg total per patient dose as measured by UV at 280 nm	0.41 (<0.08) µg/mL
5.3 min retention M/Z 253 (ES-)		0.15 (<0.10) µg/mL
6.9 min retention M/Z 315 (ES-)		0.43 (<0.10) µg/mL
7.7 min retention M/Z <250 (ES-)		0.28 (< 0.10) µg/mL

* This was the concentration of the lowest standard

The name of the drug is 16-alpha-[^{18}F]-fluoro-17-beta-estradiol, or [^{18}F]fluoroestradiol, ([^{18}F]FES). FES is the only active ingredient and it is injected in a solution of ≤ 20 mL of 0.15M phosphate buffered saline: <15% 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 **(10)** hours **(note that site specific validation data are needed to support your assigned expiration)**. The injectable dose of [^{18}F]FES for this study will 6 mCi with a specific activity greater than 170 Ci/mmol at the time of injection. The amount of injected drug is ≤ 5 µg (≤ 17 nmol) of FES. [^{18}F]FES is administered to subjects by intravenous injection of ≤ 20 mL. There is no evidence that nonradioactive and radioactive FES molecules display different biochemical behavior.

The radiosynthesis of [^{18}F]FES is shown schematically in Figure 7.2. The synthesis procedure follows that reported by[1] and uses a single reaction vial for the synthesis, similar to that described by[2]. The original method for radiosynthesis of [^{18}F]FES was reported by[3]. There is a difference in the methods we propose for manufacture of [^{18}F]FES from those used for some of the patient data supplied in the toxicity section. The most significant difference

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is that the method we propose involves the nucleophilic displacement of a sulfate group by fluorine atom and the loss of the sulfate from the molecule. The older method[3] used a different organic precursor that gave a lower yield of the [^{18}F]FES. We have chosen to use the cyclic sulfate reaction because it is reliable, yields [^{18}F]FES with a good specific activity, and the nonradioactive precursor is commercially available from a GMP facility (Advanced Biochemical Compounds, ABX).

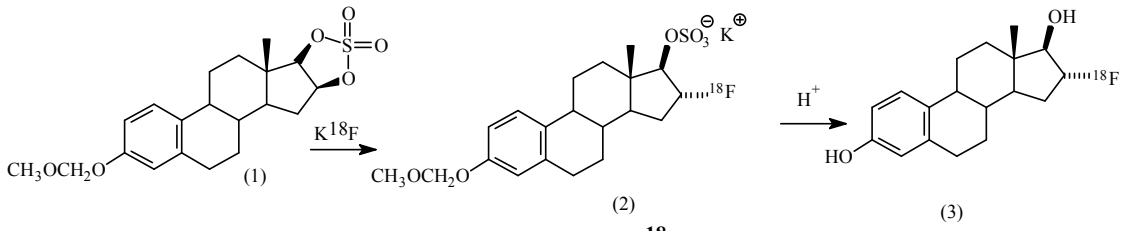

Figure 7.2. Schematic of the radiosynthesis of [^{18}F]fluoroestradiol.

We will use an automated procedure instead of manual radiosynthesis. The use of automation should minimize the variance in the chemical reaction when compared with previously published syntheses of [^{18}F]FES. This is particularly important in the case of a multi-center trial where the manufacturing is performed at the individual sites and not at a central location. 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 list 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]FES is provided 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.

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7.2.1. Organic Substrate Used for the Radiosynthesis

Table 7.4. Organic Substrate Specifications.

REAGENT	CURRENT SUPPLIER	ADDITIONAL TESTING REQUIRED
[^{18}F]FES Precursor; 3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestriol CAS No. [177714-21-5] (**SS-MSE**)	**ABX**	1. HPLC-UV or MS. UV absorbance post void volume is >90% of the precursor with 80% methanol mobile phase. 2. Quality control testing on first batch made to show the correct product is made.

The identity and purity of the [^{18}F]FES precursor (SS-MSE) is supported by the certificate of analysis provided by the supplier, Advanced Biochemicals, Inc. (ABX). The ABX test for identity of the MSE 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]FES precursor by HPLC analysis. The separation is by reversed-phase HPLC using a BetaBasic C_{18} column (see Table 7.4) with a 80% 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]FES purification except 80% methanol, rather than 60% methanol, is used in the mobile phase to elute the precursor, due to the greater lipophilicity of the precursor. Radiosynthesis of [^{18}F]FES 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 FES standard and / or mass identification prior to release of the product.

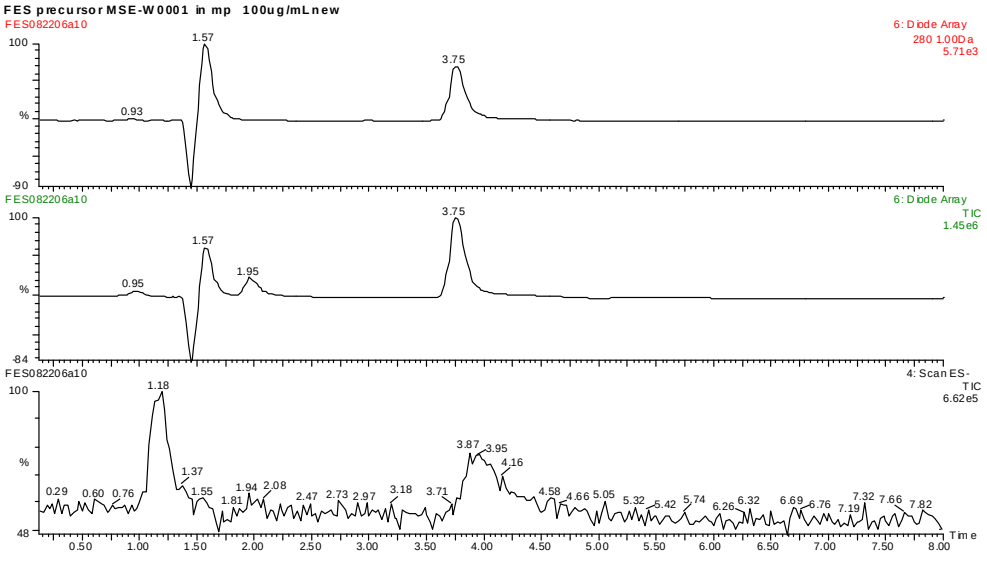

Figure 7.3. This figure shows the elution of the FES precursor from ABX (**SS-MSE**) (10 µL injected of a 100 µg/mL solution), using 20% water: 80% methanol (v:v) and the Betabasic C_{18} column with a flow rate of 0.3 mL /min. The precursor is too lipophilic to elute with the

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60% methanol used for analytical evaluation of the FES product. The traces from the bottom up, respectively, show; a mass scan 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 (i.e. no absorbance above 240 nm). The mass of the peak at 1.18 minutes was 61 and was an impurity in the solvents that were used for the HPLC analysis. The peak at 3.9 minutes is associated with mass 393, the M-H mass of the FES precursor.

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

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

Table 7.5. Target material.

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

7.2.3. Other Ingredients

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

Table 7.6. Formulation ingredients.

REAGENT*	PURPOSE	CURRENT SUPPLIER
Ethanol, absolute, USP	**To solubilize the lipophilic FES, part of preparative HPLC mobile phase**	**Any USP supplier**
0.9% Sodium Chloride Solution, for inj, USP (preservative free)	**To make the formulation isotonic prior to injection.**	**Any USP supplier**
Sodium phosphates, for dilution	**To make the formulation isotonic and buffer pH prior to injection.**	**Any USP supplier**
Water for Injection, sterile, USP	**Other part of preparative HPLC mobile phase**	**Any USP supplier**

* These are USP solutions and no verification is required beyond the certificate of analysis.

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

Table 7.7. Reagents Used in the Radiosynthesis of [^{18}F]FES

REAGENT	GRADE	CURRENT SUPPLIER
Chemicals used in the synthesis:		
Acetone	**99.5 +% ACS grade**	**Sigma-Aldrich or Mallinckrodt Baker**
Acetonitrile,	**99.8%, anhydrous**	**Sigma-Aldrich or Mallinckrodt Baker**
Ethyl Alcohol	**Absolute, USP**	**Any USP supplier**
Kryptofix® [2.2.2], (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane)	**greater than or equal to 99% pure**	**Sigma-Aldrich, EMD (VWR International) or Rotem Industries**
Hydrochloric Acid	**Concentrated USP/NF or diluted 1.0 or 0.5 M EuPh**	**Sigma-Aldrich or Mallinckrodt Baker**
Potassium carbonate, K_2CO_3, high purity	**High purity**	**Sigma-Aldrich or Mallinckrodt Baker**
Sodium Bicarbonate, USP	**2.5 mEq / 5mL, Single Dose Vial, NDC 63323-026-05 2605, sterile, pyrogen free**	**Any USP supplier**
Sterile Water for Injection, SWFI	**Sterile and pyrogen free, USP**	**Any USP supplier**
Sterile, Water for Irrigation	**Sterile and pyrogen free, USP**	**Any USP supplier**
Purification columns used in the synthesis:		
Alumina N Light Sep-Pak Cartridge	**Not applicable**	**Waters Corporation**
[^{18}F] Separation Cartridge, an anion exchange solid phase extraction cartridge	**Not applicable**	**Macherey Nagel supplied by GE Medical Systems**
HPLC Preparative Column (Zorbax C_{18} 10 x 250 mm, with or without guard column)	**Not applicable**	**Agilent Tcchnologies, Inc or any supplier of this column**
Gases and Cryogens:		
Liquid Nitrogen or liquid argon or dry ice / acetone *(Cryogen to cool glass trap used to trap volatile chemicals released from the reaction vessel)*	**check label**	**Any local supplier**
Argon Gas	**Greater than or equal to 99.9% pure**	**Byrne gas (Seattle, WA), AirGas (Radner, PA) Air Liquide (Houston, TX) Matheson Gas, Airco (Murray Hill, NJ), General Welding Supply (Seattle, WA)**
Helium Gas	**greater than or equal to 99% pure**	
Nitrogen Gas	**High purity, supplied as a gas or liquid (cryogen)**	

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

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

SOLUTIONS	SPECIFICATION	QUANTITY
70% Volume Ethanol Ethanol (30:70 v:v)): (Water for Injection :	**Compounded from sterile water for injection and absolute ethanol as specified in Table 7.7.**	**≥ 500 mL**
50% Volume Ethanol (Water for Injection : Ethanol (50:50 v:v))	**Compounded from sterile water for injection and absolute ethanol as specified in Table 7.7.**	**≥ 500 mL**
0.1 M K_2CO_3 Solution	**Compounded from potassium carbonate and Water for Injection as specified in Table 7.7.**	**0.50 ± 0.05 mL**
Kryptofix® [2.2.2] Solution (15 ± 2 mg in 2 mL acetonitrile)	**Compounded from [2.2.2] and CH_3CN as specified in Table 7.7.**	**2.0 ± 0.2 mL**
0.5N Hydrochloric acid	**Compounded from sterile water for injection and HCl or used as supplied as a 0.5 M EU solution as specified in Table 7.7.**	**1.0 ± 0.05 mL**
Sodium Bicarbonate, solution	**Used as supplied: 2.5 mEq / 5mL, Single Dose Vial, NDC 63323-026-05 2605, sterile, pyrogen free (Table 7.7)**	**1.2 ± 0.1 mL**

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

SUPPLY	DESCRIPTION	SPECIFICATION	SUPPLIER*
Sterile Vented Filter Needle	Vent for final product vial	Sterile, nonpyrogenic	International Medical Industries
Sterilizing Product Filter 0.2 µm	Final sterilization of product before product vial and used as automated system closure	Sterile, nonpyrogenic	Costar Corp. (Cambridge,MA), Millipore Corporation, or VWR International
Sterile Vent Filter 0.2 µm Millex SLGV V25 5F	Used to prevent particles from entering the automated system	Sterile, nonpyrogenic	Millipore Corporation
Empty Sterile Vials 30 mL borosilicate glass with gray butyl septa, sterile, nonpyrogenic.	Final product vial	Sterile, nonpyrogenic	Hospira (Lake Forest, IL), SLK Abello, or Miller Analytical
Sterile Disposable Syringe, 1 mL	Used to transfer reagents	Plastic, no silicone or latex sterile and nonpyrogenic	Sigma Aldrich or GE Medical Systems
Sterile Disposable Syringe, 3 mL	Used to transfer reagents	Plastic, no silicone or latex sterile and nonpyrogenic	Sigma Aldrich or GE Medical Systems
Sterile Disposable Syringe, 10 mL	Used to transfer reagents	Plastic, no silicone or latex sterile and nonpyrogenic	Sigma Aldrich or GE Medical Systems
Disposable Needles 25G x 3½"	Used to transfer reagents	Sterile, nonpyrogenic	Becton Dickinson & Co.
Syringes, various sizes, 0.5 to 60 mL disposable plastic	Used for compounding and to transfer reagents	Sterile, nonpyrogenic	Beckton Dickinson & Co. (Franklin Lakes, NJ)
Disposable Needles 16G, 22G or smaller	Used for compounding and to transfer reagents product	Sterile, nonpyrogenic	Beckton Dickinson & Co (Franklin Lakes, NJ)
Alcohol Swabs	Used to wipe septa on vials	Sterile Individually Wrapped	Various including Cardinal Health
Tubing	Used in the automation system to transfer fluids and gases	Teflon® and PEEK and polyethylene	Alltech Associates (Deerfield, IL), Upchurch Scientific (Oak Harbor, WA) or equivalent
Tube fittings and connectors:	Used in the automation system to transfer fluids and gases	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 or equivalent

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

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7.3. Reference Standards

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

Table 7.10. Reference Standards.

REAGENT/SUPPLY	CHEMICAL SPECIFICATION	CURRENT SUPPLIER*
[^{19}F]fluoroestradiol ([^{19}F]FES)	**As supplied, checked in house for purity by product analytical HPLC UV to be >95% pure and for M/Z (es-) 289**	**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, VWR International 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 that is not specifically referenced here. Quality Systems Administration and Software Administration and Change Control for the Nucleophilic Synthesis Module standard operating procedures are in place to ensure that software changes are qualified prior to implementation. The written documentation will also reflect these changes prior to implementation in both the production and batch records.

The [^{18}F]FES 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 the [^{18}F]FES

The general chemical scheme for the radiochemical synthesis of [^{18}F]FES is

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illustrated in Figure 7.2. The general procedure for the synthesis of [^{18}F]FES 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, 3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestriol, in acetonitrile is added to the reaction vessel. The reaction is heated at 130°C for 7 minutes in a closed reaction vessel to promote the substitution of the ^{18}F for the sulfate at the 16 position of the 5 member carbon ring, then 2 minutes at 130°C under vacuum to evaporate the acetonitrile. Next 0.5 N HCl is added and the reaction is heated at 140°C for 15 minutes to remove the sulfate and methylmethoxy protecting groups from the FES. Aqueous 50% ethanol containing sodium bicarbonate is added to dilute and neutralize the reaction mixture prior to loading it on the preparative HPLC column for purification.

The resulting [^{18}F]FES is separated from the other compounds in the reaction mixture using preparative high performance liquid chromatography (HPLC). A sterile mobile phase consisting of 50% water for injection and 50% ethanol (v:v) is the preparative mobile phase that is used to elute the purified [^{18}F]FES.

The radiosynthesis takes 59 minutes from the time that all of the [^{18}F]-fluoride is delivered to the automation unit to the time the automation program ends and 54 minutes to the end of the FES synthesis. The decay corrected radiochemical yield for the qualification runs was 30.3 ± 3.0% (N=6). The drug substance is not isolated. Instead the drug substance is collected directly through a 0.22 µm sterilizing filter into a vented sterile vial containing 15 mL of saline for injection, preservative free USP, 10 mL of sterile water for injection, USP, and 0.75 mL of sodium phosphate for dilution, USP (150 mmol phosphate, 200 mmol sodium per 50 mL) to reduce the ethanol concentration to <15%, to bring the pH to 7 and to make the final drug product isotonic. Samples are removed for analysis of product quality (see Section 7.6).

The radiosynthesis of [^{18}F]FES will be conducted at the contract site using an automated synthesis procedure that is described in greater detail in Appendix 1 in [^{18}F]FES: Master Production Record **MPR-[^{18}F]FES**. 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]FES is a **(Insert your system name here).**

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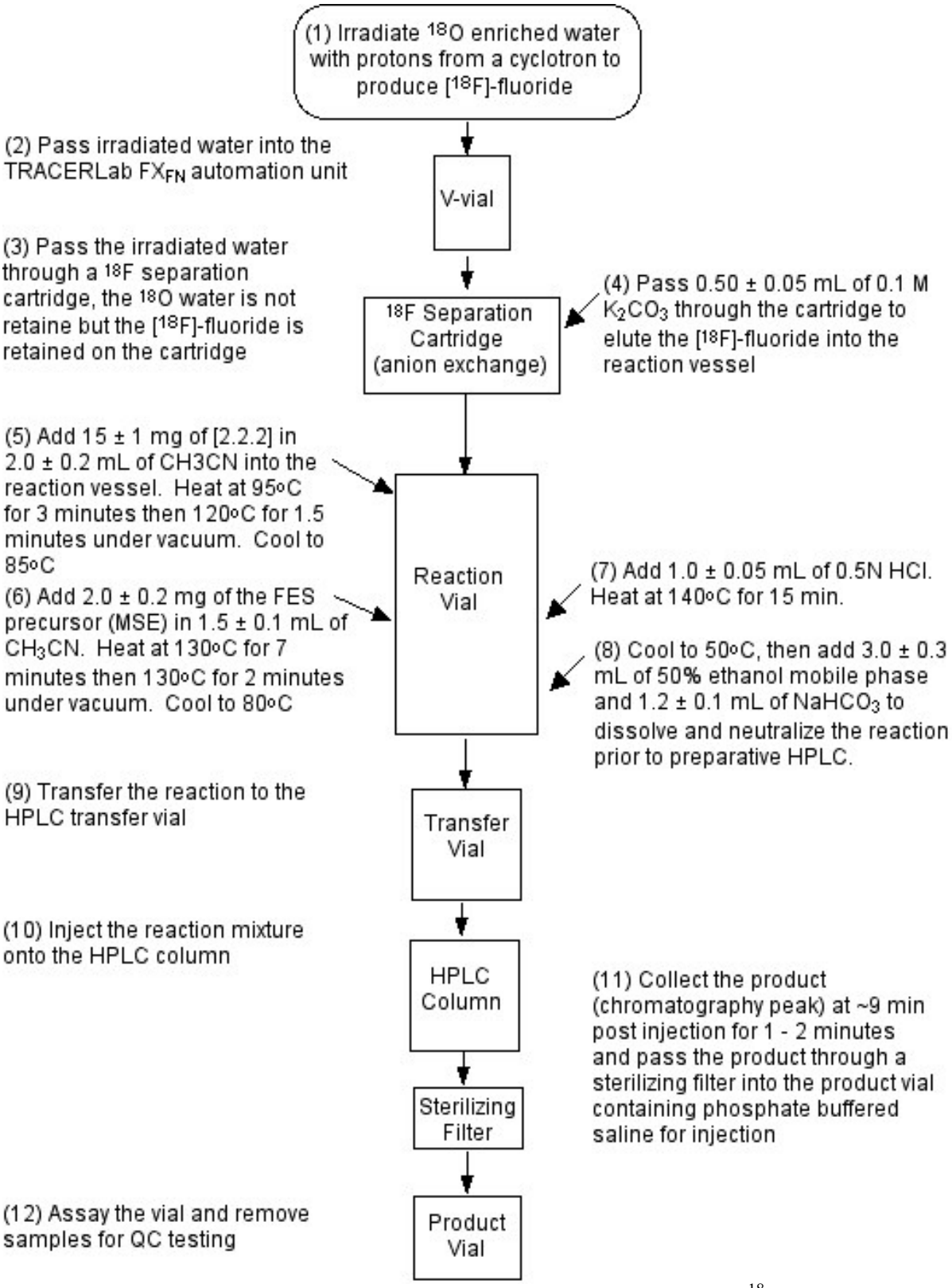

Figure 7.4. Schematic Flow Chart of the Process for Radiosynthesis of [^{18}F]FES.

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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]FES. The measured yield of [^{18}F]fluoride is approximately 18 mCi/µA for a 30 minute irradiation at the University of Washington. Yield is approximately linear with the beam current but can be different for different cyclotrons and targets.

The information below describes the typical targets and irradiation conditions for production of ^{18}F at the investigative site. However none of these parameters are critical variables for the quality of the final [^{18}F]FES 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 10 mL.
- The target is made of silver, titanium, niobium or tantalum.
- The target windows are made of 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 ~5 minutes to remove the water.

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

Anhydrous acetonitrile containing 2.0 ± 0.2 mg of 3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestriol (1.5 ± 0.1 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 sulfate group and forms a bond at the 16 alpha-position. The fluoride addition reaction is performed at 130°C under pressure for 7.0 minutes and then under vacuum for 2 minutes at 130°C to evaporate the acetonitrile. Then the reaction is cooled to 80°C.

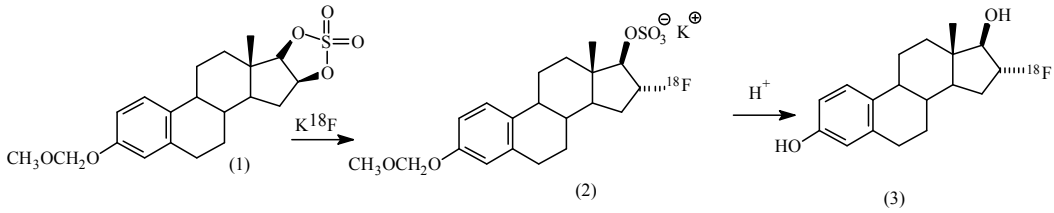

Figure 7.2 (repeat). Schematic of Radiosynthetic Method for [^{18}F]fluoroestradiol

Once the fluorination reaction is finished, the sulfate and methyl/methoxy protecting groups are removed by acid hydrolysis. Specifically, 1.0 ± 0.05 mL of 0.5N hydrochloric acid is added to the reaction and the reaction is heated at 140°C for 15 minutes. The reaction is diluted and neutralized by adding 3.0 ± 0.3 mL of the HPLC preparative mobile phase, 50% ethanol, and 1.2 ± 0.1 mL of 2.5 meq / 5 mL $NaHCO_3$ and then the reaction mixture is passed from the reaction vessel through an aluminum oxide cartridge to remove unreacted fluoride to a HPLC transfer vessel using gas pressure.

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

The resulting [^{18}F]FES is separated from the other compounds in the reaction mixture using preparative high performance liquid chromatography (HPLC). A sterile mobile phase consisting of 50% water for injection, USP and 50% ethanol, absolute USP is used to elute the purified [^{18}F]FES from a reversed-phase HPLC column (Zorbax phenyl, 10 µm, 250 x 10 mm from Phenomenex, Inc.). The mobile phase is pumped at a flow rate of 4 mL/min at room temperature. The product, [^{18}F]FES, is typically eluted at 9 min (36 mL, k′= 3) with a peak full width at half maximum peak height (FWHM) of 1.3 mL. A typical preparative chromatogram is presented below in Figure 7.6. The [^{18}F]FES is baseline separated from the other radioactive products and from the other UV absorbing compounds. The concentration of any other aromatic UV absorbing compounds in the final product must be less than or equal to 5 µg, assuming the same UV absorbance as FES, before the product is released (see

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Section 7.9.3). For the qualification run shown in figure 7.5, the product [^{18}F]FES was collected slightly longer than necessary and gave the highest UV impurity for the analytical HPLC trace (retention 6.9 min 0.4 µg/mL) showing that we can reduce the impurities by collecting the radioactive peak narrowly without the tail.

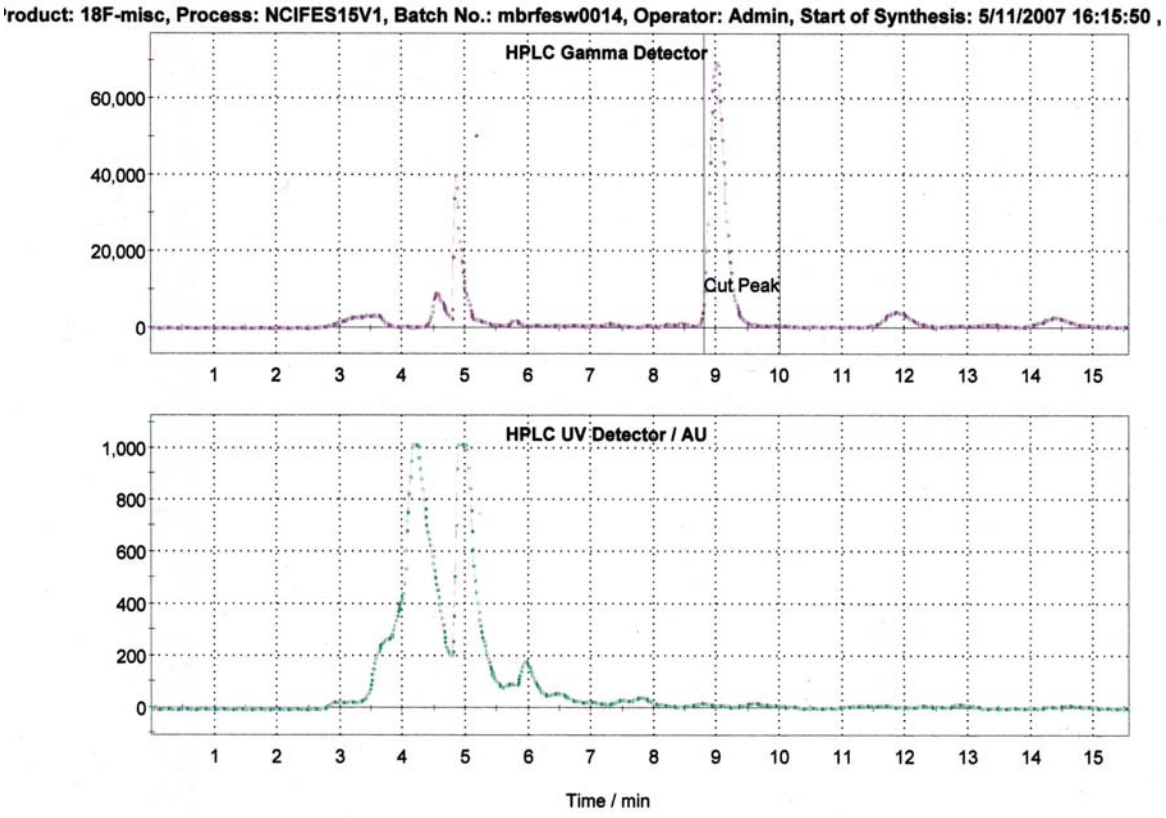

Figure 7.6. Chromatogram of preparative HPLC separation of [^{18}F]FES reaction mixture. Top trace is radioactivity and bottom trace is UV absorbance at 254 nm with 1000 = 1 AU full scale. The two vertical solid lines in the top trace show the start (leftmost) and end (right) of the product collection.

The decay corrected radiochemical yield for the qualification runs was 30 ± 3% (n=6). The [^{18}F]FES solution is collected through a 0.22 αm sterilizing filter into a vented sterile vial containing 15 mL of saline for injection, preservative free USP, 10 mL of sterile water for injection, USP, and 0.75 mL of sodium phosphate for dilution, USP (150 mmol phosphate, 200 mmol sodium per 50 mL) to reduce the ethanol concentration to <15%, to bring the pH to 7 and to make the final drug product isotonic. Samples are removed for analysis of product.

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At the end of synthesis, a HPLC cleaning program is run first to clean with water for injection to remove salts, then with absolute ethanol, USP to remove highly lipophilic compounds and last 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 >200 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 automation 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]FES: Master Production Record **MPR-[^{18}F]FES**.

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

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 at least 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. At least 0.5 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, after the sample has decayed to a background radiation level.

7.6.2. Master Production and Master Batch Records

The University of Washington conducted six qualification runs. The information collected from these qualification runs was used to optimize the language of the Master Production and Master Batch Records but no substantial changes were required. See Section 7.10 for more information on the qualification runs. These optimized procedures will be used at the University of Washington for production of [^{18}F]FES for human use.

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7.6.3. Reprocessing of PET Drug Product

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

7.7. Container/Closure

The drug substance is manufactured in a semi-closed automated system, **(Insert your system name here)** a Nucleophilic Synthesis Module 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 used 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.

7.8. Controls For Finished Drug Product

The quality control oversight will remain at the University of Washington. Several quality control tests are run on the [^{18}F]FES 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 for USP<823> Radiopharmaceuticals for Positron Emission Tomography-Compounding.

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Table 7.11. Quality Control Tests for the [^{18}F]FES 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 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: FES ≤ 5 µg other UV (280 nm) peaks ≤ 5 µg in final product**	**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

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integrity test both initially and upon re-wetting of the filter then the final product will be re-sterilized 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.

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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*
Reagents used for more than 1 test:		
Ethanol	Absolute, USP	any USP supplier
Water for injection (WFI)	Sterile, pyrogen free, USP	Any USP supplier
Water	HPLC Grade	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
Methanol	HPLC Grade	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
pH:		
pH buffers	any NIST traceable buffer	VWR
Kryptofix [2.2.2]:		
Ammonium Hydroxide Solution	28% in water ≥ 99.99%	Sigma-Aldrich and Mallinckrodt Baker
Chloroplatinic Acid	ACS Reagent grade	Sigma-Aldrich
Hydrochloric Acid	36.5-38.0%, NF	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Iodine	USP	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Potassium Iodide	Granular, USP	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
Chemical and radiochemical Purity (HPLC and TLC) methanol and water (above):		
GC for solvents:		
Acetonitrile	99.8%, anhydrous	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Acetone	spectrophotometric grade, 99.5%	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Bacterial Endotoxin Test:		
Control Standard Endotoxin (CSE)	as supplied	Associates of Cape Cod, Charles River or Cambrex
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
LAL Reagent Water	as supplied according to USP/NF	Associates of Cape Cod, Charles River or Cambrex

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

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

Solutions	Grade of Chemicals
HPLC Analytical Mobile Phase, 60% Methanol, 40% HPLC Grade Water (v:v)	HPLC grade water and methanol as listed above
The preparation of GC Standard Solutions uses: 50% Ethanol: 50% water (v:v)	The preparative mobile phase is the diluent for the acetonitrile and the acetone
The preparation of FES Standard Solutions for analytical HPLC uses: 50% Ethanol: 50% water (v:v)	The preparative mobile phase is the solvent for the FES standard

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
Analytical Column: BetaBasic C_{18} 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)		
GC Column: ATWAX 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 [^{18}F]FES 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]FES product solution sterility cannot be assured prior to injection. In

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addition to working in an ISO class 5 environment when preparing the solutions used in manufacture of the [^{18}F]FES product and the final product vial, the [^{18}F]FES is passed through a 0.22 µm sterilizing filter into the final product vial. After the product [^{18}F]FES 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]FES may only be resterilized once. This test must be passed for the [^{18}F]FES 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]FES is pipeted 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]FES to be released.

Kryptofix® [2.2.2]. The qualified Kryptofix test is based on the method by Mock[4] 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]FES 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[4]. 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]FES 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]FES 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]FES product dose cannot be released

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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 and Identity. HPLC chromatography analysis will be used to determine purity and identity for this drug product. The final radiochemical purity is proposed to be greater than or equal to 95%, and the nonradioactive FES must be less than or equal to 5 µg in the final product dose. The amount of nonradioactive FES is measured by UV absorbance at 280 nm of the FES eluted from a BetaBasic C_{18} reversed-phase HPLC column with a mobile phase of 60% methanol: 40% water (v:v). The column flow rate is 0.3 mL / min and is kept at approximately room temperature, 25 to 30°C. The typical retention of FES is 5.7 min for the UV absorbance (the radioactivity and mass detectors are ~0.1 min and 0.2 min, respectively, further downstream from the UV detector. The mass of FES (in µg/mL) is quantified with a calibration curve of a minimum of five FES standards. The standard concentrations must 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 FES drug product is fit on the calibration curve to determine the FES concentration in the drug product sample.

For identity, either the mass of fluoroestradiol must be observed at the retention of the radioactive peak or an FES standard coinjected with a sample of the FES product must show that the radioactive peak coelutes with the FES standard.

Typically the final concentration of the [^{19}F]FES in the [^{18}F]FES product to be tested will be below 1 µg/mL. The total allowable, column retained (post 3.0 minutes) UV absorbing contaminants (peaks on the chromatogram) in the [^{18}F]FES product must be $\leq$ 5 µg in the injected dose, which assumes that impurities have the same molar absorption coefficient for quantifying the impurity peaks. In the qualification runs, four impurity peaks were seen with retentions (M/Z (es-)) of 4.8 min (395 M - H), 5.3 min (253 M - H), 6.9 min (315 M - H) and 7.7 min (M - H < 250). Not all four of the impurities were detected in each qualification run. The peak areas for the impurities were very small, from not detected to 0.4 µg/mL estimated by UV, so a larger scale synthesis using 20 mg of FES precursor, was done to identify the impurities. The peak at 6.9 minutes has a mass of 316 (M/Z ES- = 315). A mass of 316 is consistent with estradiol with the methylmethoxy protecting group still on the 3 position of the estradiol ring structure. The peak at 5.3 minutes with a mass of 254 (M/Z ES- = 253) is consistent with the estrogen ring containing one OH group and missing two hydrogens. This may be the 3 hydroxyl-estrogen with a double bond between positions 16 and 17. The peak at 4.8 was not observed with the larger mass labeling but had a mass of 396 (M/Z 395 (ES-)) in the qualification runs where it was observed, and is consistent with the FES precursor (3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestriol) but with the

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sulfate detached at the 16-position. The amount of impurities obtained, even with a 20 mg labeling, was < 1 µg each and too small to do further structural analysis. The peak at 7.7 min had masses that were less than that for the estrogen 4-ring structure (mass 240) and has been observed in mobile phase injections and is not thought to be a result of the FES reaction.

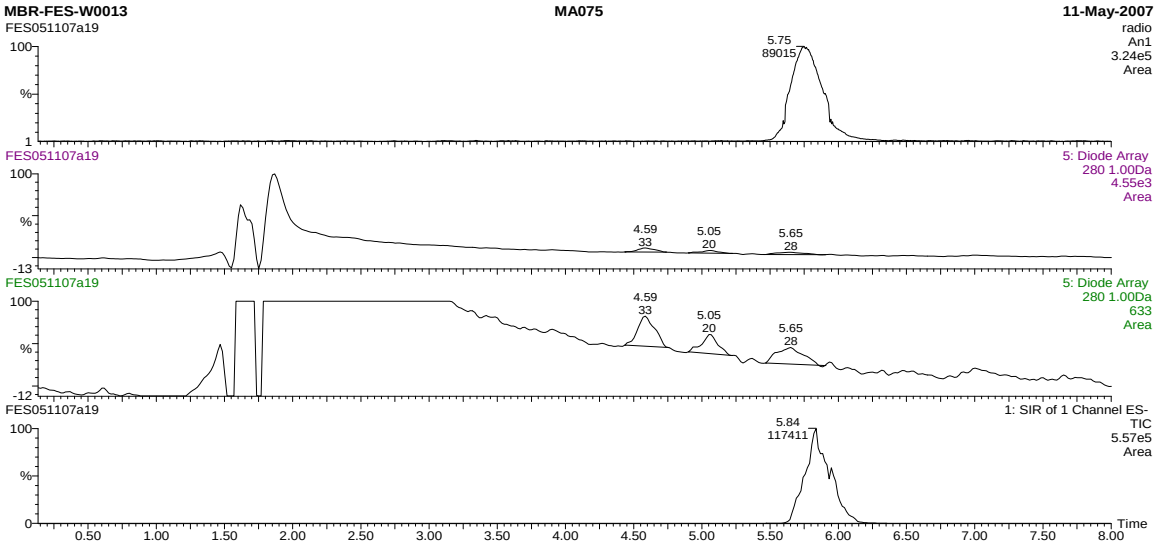

Figure 7.6. Typical HPLC chromatogram of [^{18}F]FES product. Bottom trace shows the mass of FES (M/Z 289) (ES^-) confirming identity. Top trace is the radioactivity. UV absorbance at 280 nm is shown in the two middle traces. The trace second from bottom is expanded so that the FES mass peak (0.1 µg/mL 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 **(verify with your system)**.. 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.

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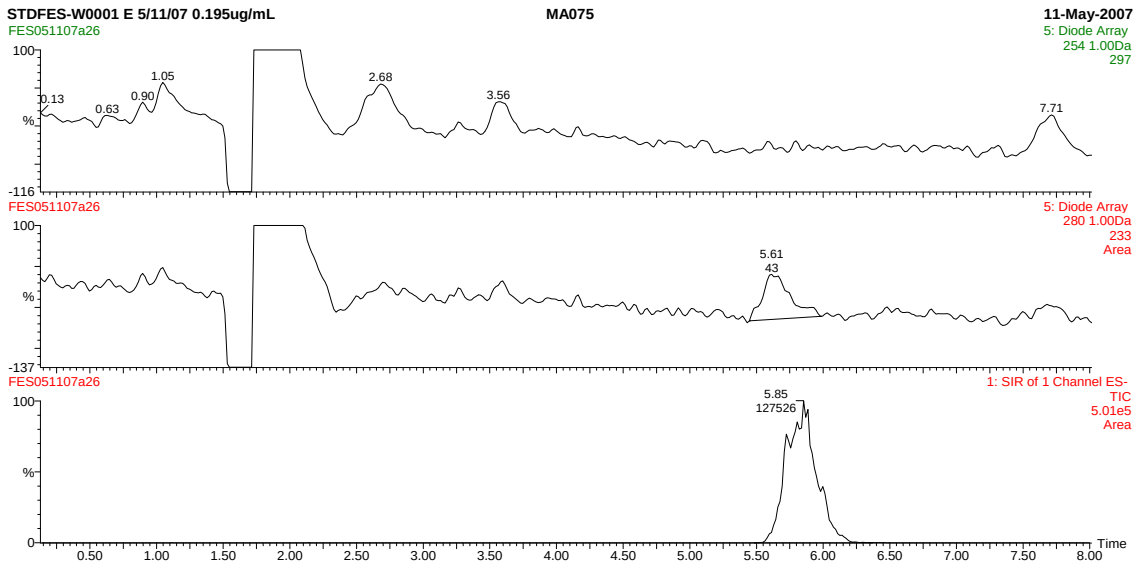

Figure 7.7. HPLC chromatograms for a 10 µL injection of a 0.195 µg/mL FES standard. Top trace is an expanded scale of the UV absorbance at 254 nm showing poorer FES sensitivity and mobile phase impurities at this wavelength. The middle trace is an expanded scale of the UV absorbance at 280 nm (near the 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 typical retention time of the FES is 5.7 minutes for UV and 5.9 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 quantification 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.

The [^{18}F]FES organic precursor does not elute from the HPLC column with the analytical mobile phase of 60% methanol, but elutes at ~3.9 minutes from the FES analytical column when the mobile phase is 80% methanol : 20% water (see Figure 7.3). No precursor is expected in the final product. The acid hydrolysis removes the sulfate and the methylmethoxy protecting groups and leaves the major synthetic byproduct, estradiol. Any residual precursor, or sulfate and methylmethoxy protecting groups that were left would not elute with the FES product when using the 50% ethanol preparative mobile phase. Other impurities that can be expected are Kryptofix [2.2.2], other reaction products and impurities in the USP solutions. Kryptofix [2.2.2] does not absorb UV light at 280 nm. Kryptofix is assayed using a chemical spot test (see Section on Kryptofix® [2.2.2] above). Other chemical impurities must be ≤ 5 µg total for

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any retention time between 3 and 10 minutes, assuming the same UV molar absorption coefficient as FES. The maximum amount of sulfate (the leaving group) possible in the final reaction is less than or equal to 500 µg, a nontoxic amount since sodium sulfate can be compounded by pharmacists and injected intravenously at a concentration of 1.6% (USP28 NF23). The other leaving group, methylmethoxy (dimethylether) is not stable as methylmethoxyalcohol. It is possible that it can be lost as dimethylether, which is used commonly in bonding agents and hair spray. Dimethylether is a narcotic, similar to diethylether when inhaled and a single acute inhalation can be toxic from lack of oxygen (MSDS Praxair). We were unable to find any LD_{50} or information on the toxicity of injected dimethylether. However, dimethylether is similar to diethylether in solvent behavior. Diethylether is a Class 3 solvent that the FDA recommends be limited to less than 50 mg/day in pharmaceuticals. (FDA Guidance for Industry ICH Q3C) The maximum possible amount of dimethylether that is in the reaction and that could be administered is 0.23 mg (or 110 ppm) in a 20 mL FES dose. This is far below the limit of 5,000 ppm for diethylether, as a class 3 solvent, and is not assayed in the final product.

Radiochemical Purity. The use of Radio-Thin Layer Chromatography to determine the radiochemical purity was validated using HPLC grade methanol as the mobile-phase. The R_f values obtained from these studies were between 0.78 and 0.80 for the 6 qualification runs **(insert your values here)**. The limit has been set for R_f >0.5 for the final [^{18}F]FES 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 FES 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 FES precursor and is used to dissolve the Kryptofix® [2.2.2]. Acetone is used to clean the Nucleophilic Synthesis Module 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 3 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,[5] 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 initial qualification syntheses (n=9).

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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]FES.

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 150°C	
Oven temperature	Initial Temperature 38°C, hold for 2 minutes.	Temperature ramp 1: 38° to 56°C @ 10°C/min, hold at 56°C for 1.0 min.	Temperature ramp 2: 76° to 120°C @ 20°C/min, hold at 120°C for 3 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 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]FES 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.

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

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

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variation we use 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 might only provide somewhat better counting statistics. Furthermore, the need for precise 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 make FES.

Bacterial Endotoxin Levels are tested and qualified using one of two procedural methods; both are based on the USP recommendations, 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). All of the bacterial endotoxin levels were <175 EU per batch for the initial qualification syntheses.

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]FES product and the USP sterility method is presented in (Appendix 3).

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

All of the six completed qualification [^{18}F]FES 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]-FES from the University of Washington qualification runs.

Insert your data here

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

*Manufacturer specified pressure rating was >40 psi.

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

Batch Number MBR-FES	TLC		HPLC				Cyclotron Irradiation time (min @ 32-33 µA)
	R_f	Radio-chemical Purity %)	Radio-chemical Purity (%)	FES µg per 6 mCi dose*	FES (µg/mL)	Other** µg/mL (µg/dose)	

*time of dose calculation is 1 hour post end of synthesis.
** The value in parentheses is the mass of impurities for a 6 mCi dose 1 hour after the end of synthesis.

As shown in Table 7.17, the mass of impurities by UV at 280 nm are not correlated with the amount of radioactivity that was used. The radioactivity produced is essentially proportional to the time irradiated and the exact relation of production to time follows the equation: 1 – exp (-ln(2) * time irradiated /110 minutes). For the shortest run, batch W0009, the 5 minute irradiation was insufficient to provide enough radioactivity to inject a 6 mCi dose and still have the unknown impurities sum to less than 5 µg in the dose an hour after the end of synthesis, with longer irradiation times the masses of impurities would be smaller.

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

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

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*All solvent peaks were below the lowest standard concentration, and so the lowest standard concentration that was measured is listed.

** A 1/40 dilution is used to avoid interference from the salts so < 2 is equal to 40 X < 0.05 EU/mL, the lowest sensitivity for the PTS cartridges that were used.

7.10. Information to Support the Stability of the Drug Substance

[^{18}F]FES was made according to the procedures described in this IND application. The final drug product was stored at room temperature for up to 23 hours. At periodic times over the 23 hours, the product was measured for radiochemical purity using analytical HPLC and TLC. In addition, the FES product was examined for changes in UV absorbance of the product peak with time. There was no detectable breakdown of the product by UV over that time period. These results are summarized in the Table 7.19.

Table 7.19. [^{18}F]FES stability test results.

Insert your data there

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

Expiration dating: **(This must be based on your data)** the expiration time is **xx** hours for the product stored at room temperature. This is slightly less than 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 but TLC

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measures after 16 hours show that the [^{18}F]FES product may be losing the label by as much as 12% between xx hours after the end of synthesis. The uncertainties given in Table 7.19 are the 1 standard deviation counting error for the propagated counts to provide and idea of the accuracy of the measurements. At the late time points, there was very little remaining activity. In the HPLC, there were no other radioactive peaks detected but there could be an impurity that is not detectable. In addition to the counting error, the standard deviation of the analog background peaks as a percent of the [^{18}F]FES are listed in parentheses for the HPLC data. The data in bold are those where the 3 standard deviations of the background were less than 5%. We are using 3 standard deviations of the background taken as peak areas (our software does not allow a ruler determination of peak to peak noise) as the limit of detection. These data provide good evidence that the product is stable to xx hours after synthesis but do not support a longer expiration time at this point. Our proposed xx hour expiration time is based upon the amount of impurities that would be found in a dose xx hours after the end of synthesis based upon our qualification runs (see Section 7.10.4).

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7.11. Drug Product Vial Labeling

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

UW PET RADIOCHEMISTRY LABORATORY
[^{18}F]Fluoroestradiol
in Phosphate Buffered Saline, for injection, containing < 15% Ethanol
MBR-[^{18}F]FES-W_____

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 8 hours after calibration. Calculate injection dose from date & time of calibration. $T_{½}$ is 109.8 minutes.

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

7.12. Environmental Assessment

We request a categorical exclusion 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]FES 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 20 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.

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7.13 Bibliographic Citations:

[1] Lim, JL, Zheng, L, et al: The use of 3-methoxymethyl-16 beta, 17 beta-epiestriol-O-cyclic sulfone as the precursor in the synthesis of F-18 16 alpha-fluoroestradiol. Nucl Med Biol 23:911-5, 1996

[2] Romer J, Fuchtner F, Steinbach J, Johannsen B. Automated production of 16alpha-[^{18}F]fluoroestradiol for breast cancer imaging. *Nuclear Medicine and Biology*. 26:473-9, 1999

[3] Kiesewetter, DO, Kilbourn, MR, et al. Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immature rats. J Nucl Med 25:1212-21, 1984

[4] 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 and Biology.* 1997, 24:193-195.

[5] FDA. Guidance for Industry ICH Q3C – Tables and List (November 2003 Revision 1)

Appendices:

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

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